

Research Article

Hygiene Quality and Consumer Safety of Traditional Short- and Long-Ripened Cheeses from Poland

Renata Pyz-Łukasik , Przemysław Knysz, and Michał Gondek

Department of Food Hygiene of Animal Origin, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland

Correspondence should be addressed to Renata Pyz-Łukasik; renata.pyz@up.lublin.pl

Received 24 November 2017; Revised 6 June 2018; Accepted 14 June 2018; Published 9 August 2018

Academic Editor: Jordi Rovira

Copyright © 2018 Renata Pyz-Łukasik et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The aim of this study was the assessment of the microbiological quality of three types of traditional cheeses which are produced from raw and pasteurized cow's milk. Two types of cheeses were of the short-ripened type, and one cheese was long-ripened. A microbial examination was conducted for the presence of *Salmonella* spp. and *Listeria monocytogenes* microorganisms and the count of aerobic, psychrotrophic, lactic acid bacteria, and coliform bacteria, as well as *Escherichia coli*, *Enterobacteriaceae*, *Enterococcus* spp., *Staphylococcus* spp., and yeasts. The examined cheeses did not fulfill the microbial criteria for food safety (presence of *L. monocytogenes*) and process hygiene (exceeded allowable levels of *E. coli* and coagulase-positive *Staphylococcus*). The levels of coliform bacteria, *E. coli*, and *Enterobacteriaceae* and the presence of *Enterococcus faecalis* determined in the three examined cheese types indicated that insufficient hygiene procedures were used during the production process. The results of the study indicate that the examined cheeses did not fulfill the microbial criteria for food safety and process hygiene according to the legislation. It is necessary to introduce correction procedures as indicated in the current report.

1. Introduction

Microbiological quality of traditional homemade cheeses and their safety for the potential consumers depend on the microbiological quality of the raw material used in their production, maintaining standards of hygiene in the production environment and by cheese production workers, as well as on the possibility of postprocessing contamination [1–5].

Some results of the microbiological study of regional cheeses produced using traditional methods indicate an unsatisfactory quality of the production process and the need to introduce correction procedures [6–10]. Moreover, the data on the occurrence of pathogenic bacteria in these kinds of products indicate a real threat that can affect the health of consumers [9, 11–13]. These products should fulfill microbiological requirements in terms of food safety criteria (irrespective of the material type, *Salmonella* and *Listeria monocytogenes* should be absent in 25 g) and the criteria for the process hygiene (for cheeses produced from heat-treated

milk, no more than 10^3 cfu/g (number of colony-forming units) of *E. coli* and coagulase-positive *Staphylococcus* in 2 of the 5 examined samples, and for cheeses made of raw milk, no more than 10^5 cfu/g of coagulase-positive *Staphylococcus* in 2 of the 5 tested samples) [14].

The aim of the study was the assessment of the microbial quality and consumer safety of traditional cheeses produced in southern and eastern Poland.

2. Materials and Methods

2.1. Data Collection. The study material consisted of three types of cheese, produced using traditional methods in small farms located in southern and eastern Poland. In terms of the ripening time, 2 types of cheeses were classified as short-ripened (ripening time of 7–14 days) and one cheese type was classified as long-ripened (ripening time of 3 months). Among the short-ripened cheeses, one group consisted of smoked cheeses and the other group of natural cheeses

(commonly referred to as “oscypek,” although in legal terms the name is reserved only for cheeses produced from sheep’s milk or from cow’s and sheep’s milk mixed in adequate proportions). Both short-ripened cheeses were produced in one manufacture, and long-ripened cheeses were produced in another manufacture. Twelve samples of each type of cheese were collected in the same season (winter). Cheeses ($n = 36$) were purchased in the retail stores of the manufacturers. The samples were delivered to the microbiological laboratory within one hour of collection and maintained at a temperature between 0°C and +4°C during transport.

In the direct conversation with the manufacturers of the examined cheeses, the following information was obtained: all three cheese types are listed as traditional products. The material for the production of short-ripened cheeses was pasteurized cow’s milk obtained from dairies, used for the production within the period of 48 h from the purchase. The material for the long-ripened cheeses was raw cow’s milk from the farm of the cheese manufacturer, used for the production within 2 h after collection (mechanical milking). Commercial dairy cultures were used in the production of the three examined cheese types, and production and ripening room temperatures were 18–20°C and 14–20°C, respectively.

2.2. Microbiological Analysis. Microbiological counts of aerobic bacteria, psychrotrophic bacteria, lactic acid bacteria, coliform bacteria, *E. coli*, *Enterobacteriaceae*, *Enterococcus* spp., *Staphylococcus* spp., and yeasts were performed by placing a 10 g of a sample and 90 mL of saline peptone water in a stomacher bag and subsequently homogenizing it in a stomacher for 2 min at normal speed (230 rpm). From this dilution, other decimal dilutions were prepared (PN-EN ISO 6887) and plated on the appropriate media according to the International Organization for Standardization standards (ISO) and Polish Standard (PN-ISO 4833-2, PN-ISO 17410, PN-ISO 15214, PN-ISO 4832, PN-ISO 16649-2, PN-ISO 21528-2, PN-A-82055-7, PN-EN ISO 6888-1, and PN-ISO 21527-1). To determine the count of aerobic and psychrotrophic bacteria, plates with nutrient agar medium were used, incubated at 30°C for 72 h and at 4°C for 10 days, respectively: for lactic acid bacteria, plates with MRS medium, incubated at 30°C for 72 h; for coliform bacteria, plates with VRBL medium, incubated at 37°C for 24 h; for *E. coli*, plates with TBX medium, incubated at 44°C for 18 h; for *Enterobacteriaceae*, plates with VRBG medium, incubated at 37°C for 24 h; for *Enterococcus* spp., plates with Slanetz–Bartley medium, incubated at 37°C for 48 h; for *Staphylococcus* spp., plates with Baird Parker medium, incubated at 37°C for 48 h; and for yeasts, plates with Sabouraud dextrose medium with chloramphenicol, incubated at 25°C for 5 days. For the identification of *Staphylococcus*, *Enterococcus*, and *E. coli*, nonreagent Erba Lachema tests (Brno, Czech Republic) were used: Staphyttest 24, Streptotest 24, and Enterotest 24 new, as well as Multiscan EX test reader. The number of microorganisms is provided in log cfu/g.

In order to determine the presence of *Salmonella* spp., an examination was performed according to PN-EN ISO 6785. Twenty-five grams of a sample in 225 mL of buffered

peptone water was homogenized in a laboratory blender (Stomacher 400) and incubated at 37°C for 18 h. The media used subsequently were Rappaport-Vassiliadis incubated at 41.5°C for 24 h, and then, simultaneously, XLD and BGA were incubated at 37°C for 24 h (in the case of the absence of typical colonies on XLD and BGA media, the test was discontinued according to the procedure).

In order to determine the presence of *Listeria* spp., an examination was performed according to PN-EN ISO 11290-2. Twenty-five grams of a sample in 225 mL half Fraser broth was homogenized in a laboratory blender (Stomacher 400) and incubated at 30°C for 24 h. Then, the Fraser broth was used, incubated at 37°C for 48 h, and, subsequently, Ottaviani and Agosti medium (ALOA) along with Oxford medium was used, incubated at 37°C for 24–48 h. Confirmatory tests were performed (Gram staining, mobility capacity, sugar fermentation, and hemolysis test). The presence of *Listeria monocytogenes* was confirmed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker, Germany).

All microbial media used in the study were purchased from Biocorp (Poland).

2.3. Statistical Analysis. All the data are presented as means and \pm SD. The statistical analysis of the data was performed using the Statistica software (StatSoft, Poland, version 13.1) and were determined by the one-way ANOVA and Tukey’s multiple comparison *post hoc* test; $P < 0.05$ was considered as statistically significant. Correlations were determined by Pearson’s correlation coefficient (r).

3. Results

The levels of particular microorganism groups demonstrated in three types of examined cheeses are presented in Table 1. In long-ripened cheeses, a significantly higher ($P < 0.001$) level of total aerobic, psychrotrophic, lactic acid bacteria, *Enterococcus* spp., and yeasts was determined, as well as a significantly higher ($P < 0.05$) level of coliform bacteria and *Enterobacteriaceae*, than in the short-ripened cheeses (smoked and natural). In long-ripened cheeses, a significantly higher number ($P < 0.05$) of *E. coli* was determined than in short-ripened natural cheeses. Short-ripened natural and smoked cheeses differed significantly ($P < 0.05$) in terms of the levels of two microorganism groups, that is, coliform bacteria and *Enterobacteriaceae*, the count of which was higher in natural cheeses. No significant differences were determined between the three examined cheese types in terms of *Staphylococcus* spp. contamination. The presence of *Salmonella* was not detected in any of the samples of three types of the examined cheeses. *Listeria monocytogenes* was confirmed in one sample of short-ripened natural cheese. *Staphylococcus aureus* was confirmed in three samples of long-ripened cheeses.

3.1. Correlation. In the analysis of the correlation between the counts of microorganisms indicating the hygienic conditions of the production of all examined cheeses, significant correlations between the counts of coliform bacteria,

TABLE 1: Microbiological counts (log cfu/g) of different microbial groups for three types of cheese (mean \pm SD).

	Short-ripened natural cheese ($n = 12$)	Short-ripened smoked cheese ($n = 12$)	Long-ripened cheese ($n = 12$)
Total aerobic count*	7.97 ^a \pm 0.30	7.97 ^a \pm 0.52	10.19 ^b \pm 0.51
Psychrotrophic bacteria*	7.28 ^a \pm 0.54	6.92 ^a \pm 0.37	9.40 ^b \pm 0.81
Lactic acid bacteria*	8.45 ^a \pm 0.46	8.30 ^a \pm 0.64	10.38 ^b \pm 0.99
Coliforms	6.29 ^a \pm 0.56	5.35 ^b \pm 0.69	8.36 ^c \pm 1.28
<i>Escherichia coli</i>	3.30 ^a \pm 1.29	3.78 ^{ab} \pm 0.64	4.78 ^b \pm 1.71
Enterobacteriaceae	6.23 ^a \pm 0.51	5.53 ^b \pm 0.62	8.28 ^c \pm 0.87
<i>Enterococcus</i> *	6.15 ^a \pm 0.37	6.44 ^a \pm 0.62	8.22 ^b \pm 1.17
<i>Staphylococcus</i>	6.32 ^a \pm 0.63	6.26 ^a \pm 0.51	6.46 ^a \pm 0.58
Yeasts*	5.23 ^a \pm 0.33	4.99 ^a \pm 0.82	7.74 ^b \pm 0.64

^{a,b,c}Mean values within a row marked with different letters differ significantly at $P < 0.05$. * $P < 0.001$.

TABLE 2: Pearson correlation coefficients (R) between the microbial groups of three types of cheese.

	Type of cheese	Enterobacteriaceae	<i>Enterococcus</i>	Psychrotrophic bacteria	Lactic acid bacteria
Total aerobic count	a	-0.24	-0.06	0.84*	0.73*
	b	-0.66*	0.85*	0.79*	0.85*
	c	0.15	0.23	0.66*	0.36
Coliforms	a	0.77*	0.69*	-0.05	-0.27
	b	0.97*	-0.78*	-0.41	-0.64*
	c	0.90*	0.93*	0.82*	0.07
<i>Enterococcus</i>	a	0.79*		0.17	-0.25
	b	-0.71*		0.78*	0.77*
	c	0.74*		0.79*	-0.05
Yeasts	a	-0.74*	-0.47	0.70*	0.88*
	b	-0.75*	0.88*	0.59	0.83*
	c	-0.43	-0.64*	-0.37	0.67*

*Statistically significant correlation at $P < 0.05$. a: short-ripened natural cheese; b: short-ripened smoked cheese; c: long-ripened cheese.

Enterobacteriaceae, and *Enterococcus* spp. were found (Table 2). The values of the coefficients for short-ripened natural cheeses and long-ripened cheeses ranged from $r = 0.69$ to $r = 0.93$ and for short-ripened smoked cheeses from $r = -0.78$ to $r = 0.97$. For all types of the examined cheeses, significant correlations between the number of lactic acid bacteria count and the yeast count ($r = 0.67$ – 0.88) and between the number of psychrotrophic bacteria count and total aerobic count were found ($r = 0.66$ – 0.84).

4. Discussion

The total bacterial count was similar in smoked and natural short-ripened cheeses (log 7.97 cfu/g) and lower by approximately 2 log than in long-ripened cheeses (log 10.19 cfu/g). The total bacterial count of the examined long-ripened cheeses was higher than that in long-ripened cheeses produced using traditional methods from raw cow's milk in Spain and Italy, for which the total aerobic count, depending on the ripening stage, was from 8 to 9.06 log cfu/g [6, 15]. The total bacteria count in short-ripened cheeses produced regionally using traditional methods, that is, smoked and natural oscypek cheeses from sheep's, cow's, and mixed milk and golka cheeses from mixed (sheep's and cow's) milk was at the level of 10^8 – 10^{10} cfu/g (see [39], cited in [7], and [16]). In the presented study, the values of total microbiological count of both short-ripened cheese types (smoked and natural) were similar to the lower levels of the range (approximately 10^8 cfu/g). Smoked and natural oscypek cheeses

produced using the traditional method from sheep's milk differed in the total bacteria count, which was higher in natural cheeses (resp.: 10^5 – 10^8 and 10^7 – 10^9 cfu/g) [7]. In the presented study, no significant differences in the level of the total bacteria count were determined between smoked and natural cheeses, and their total bacteria count remained in the ranges of values determined by Berthold-Pluta et al. [7]. Three types of ripened goat cheeses had similar total bacteria count of about 3.2×10^7 cfu/g, which was not affected by the milk type (raw, pasteurized, and high-pressure-treated). According to the authors of this study, the starter bacteria inoculated into the cheese milk affected the total bacterial count [17].

The count of psychrotrophic bacteria was higher in long-ripened cheeses (log 9.40 cfu/g) than in short-ripened natural and smoked cheeses (log 7.28 and log 6.92 cfu/g, resp.). The number of these bacteria in the product depends, for example, on their level in the raw milk and its storage temperature. Psychrotrophic bacteria are naturally present in milk, where they can reach counts of up to 10^5 cfu/mL [18]. Raw milk is usually refrigerated, especially when it is not subjected to processing directly at the dairy farm. The increase of the population of psychrotrophic bacteria in the milk can be inhibited by storing it at 4°C and by thermization. The psychrotrophic bacteria count was reduced approximately to log 2 cfu/ml and was stable during storage at 4°C for about 7 days [19]. The use of higher temperatures, that is, pasteurization or pressure treatment, reduced the number of psychrotrophic bacteria in raw milk from 7×10^5 cfu/mL to < 10

and 10 cfu/mL, respectively [17]. In the current study, lower psychrotrophic bacteria count in the short-ripened cheeses in comparison to the long-ripened cheeses may stem from heat-treated (pasteurized) milk used in the production of short-ripened cheeses. The total psychrotrophic count in traditional goat cheeses ranged from log 8.6 (in cheeses produced from milk which was cooled after milking) to log 9.1 cfu/g (in cheeses produced from milk which was not cooled after milking) [9]. The examination of the refrigeration chain was not the objective of the present study; therefore, it was not possible to determine the impact of temperature on the count of these bacteria in the final products.

Two fundamental groups of microbiota are found in cheeses: the starter microbiota, which are added at various stages of cheese production, and nonstarter (native) microbiota, which can originate from, for instance, milk, equipment, or the production environment. The main group of cheese microbiota consists of lactic acid bacteria (LAB), which are included in both starter and nonstarter microbiota [20]. LAB are the dominant microbial group; they usually reach levels above 9 log cfu/g within the first day of cheesemaking and remain dominant until the end of the process of ripening, although there occur certain variations in the species balance in the course of this process [3]. The lactic acid bacteria count in long-ripened cheeses was significantly higher (log 10.38 cfu/g) than that in short-ripened cheeses (log 8.30–8.45 cfu/g). This ensued from a longer ripening time. Lactic acid bacteria were the dominant bacteria group in all the examined cheeses and other traditional short- and long-ripened cheeses from Northern Morocco and Italy [9, 15]. In long-ripened cheeses produced using traditional methods in Italy and Argentina, the lactic acid bacteria count was, depending on the ripening stage, log 7–log 9.07 cfu/g for cow's milk cheeses [15, 21] and log 9.8 cfu/g for short-ripened cheeses of the golka type (mixed sheep's and cow's milk) from Poland [16].

Microorganisms such as coliform bacteria, *E. coli*, *Enterobacteriaceae*, and *Enterococcus faecalis* determined the production hygiene of the examined cheeses. The counts of coliform bacteria under the conditions of good production practice should not exceed 10^3 cfu/g (see [40], cited in [22]). Contamination with coliform bacteria in each of the examined cheeses exceeded this level (log 5.35–8.36 cfu/g). The lowest count of these microorganisms was determined in short-ripened smoked cheeses, significantly higher in natural cheeses, and the highest in long-ripened cheeses. The results obtained by other authors demonstrated high divergence in the level of coliform contamination in different types of cheese produced using traditional methods from cow's, goat's, sheep's, or mixed milk, which ranged from under 1 to 7.89 log cfu/g [6, 9, 15, 23–25]. The level of coliform bacteria in ripening cheeses changed during the ripening time. In a traditional mountain cheese from Italy, the highest coliform count (log 4.7 cfu/g) was observed 24 h after production, which was probably due to the fact that the pH reduction by the autochthonous starters took 24 h. At the end of ripening, very low concentrations of coliform bacteria were detected [23]. Similarly, in traditional Greek cheeses, the number of coliform bacteria was reduced during ripening, and usually they were not detected in mature cheeses

[25]. Coliform bacteria count was reduced even during curd draining, which was probably due to unfavorable conditions created by the increasing population of LAB and the decreasing pH [25]. In contrast to the results of the above-mentioned study, an increase of coliform bacteria until the end of ripening was determined for traditional, starter-free cheese made from cow's milk [6].

The number of *E. coli* in the three examined cheese types ranged from 3.30 to 4.78 log cfu/g and remained within the ranges of values determined by other authors. The level of *E. coli* in long-ripened cheeses produced using traditional methods in Italy, Brazil, and Argentina was from under 1 to 5 log cfu/g or log MPN (most probable number)/g [8, 21, 24, 26] and in short-ripened cheeses from Poland, that is, smoked and natural oscypek type cheeses, from 0 to over 10^2 MPN/g. Oscypek smoking did not result in a reduction of the number of *E. coli* [7], but the ripening period at room temperature had a significant impact on the reduction of their count in cheeses [24]. The higher coliform bacteria and *E. coli* counts at the beginning of ripening were connected with raw milk which was collected manually and coagulated at low temperature. The cheese ripening process caused a decrease in the bacteria count or their elimination as a result of increased lactic microflora, lowered pH, and increased dry matter and chloride content of the cheese [8].

Three types of cheeses in the present study differed significantly in terms of the counts of *Enterobacteriaceae*, which ranged from log 5.53 to 8.28 cfu/g. Numbers of microorganisms in this group for other ripened cheeses remained within broad ranges and amounted from 2.49 to 7.25 log cfu/g for cheeses produced in Greece and the Island of Tenerife from sheep's and goat's milk [25, 27], whereas from 1.0×10 to 1.24×10^8 cfu/g for those produced in Spain from cow's milk [10]. Environmental contamination and handling during cheesemaking may be the reason behind the presence of *Enterobacteriaceae* in the cheeses [17]. The level of *Enterobacteriaceae* depended on the time of cheese ripening; as the ripening time was extended, the levels of these bacteria decreased (about log 0.6–1 cfu/g) [25]. Alegria et al. [30] reported that the smoking process increases the quality and safety of the traditional oscypek cheese; the number of *Enterobacteriaceae* in smoked cheeses was lower (about log 0.4–2 cfu/g) than that in cheeses before smoking. This is corroborated by the present study, as the count of *Enterobacteriaceae* was significantly lower (about log 1 cfu/g) in smoked cheeses in comparison to natural cheeses.

Enterococci occur as nonstarter lactic acid bacteria in various cheeses, and particularly in artisan cheeses. They can be found in cheeses which are made from both raw and pasteurized milk. Their levels depend on various factors, including the extent of milk contamination, the type of cheese, the starter used, and the technology applied, such as their survival and growth under the specific conditions of cheese manufacture and ripening [28]. The count of *Enterococcus* spp. was similar in short-ripened natural and smoked cheeses (log 6.15 and log 6.44 cfu/g, resp.) and lower by about 2 log than in long-ripened cheeses (log 8.22 cfu/g). Levels of enterococci in a fully ripened cheese may range from 10^5 to 10^7 cfu/g [28]. In comparison to these levels, the

number of these microorganisms in short-ripened cheeses remained within this range, while it was higher in long-ripened cheeses. The presence of high numbers of *Enterococcus* spp. is typical for cheeses obtained from raw milk [6]. On the contrary, the presence of enterococci in pasteurized milk can be the result of contamination during cheesemaking [17]. This explains the differences in the amounts of these bacteria in the examined cheeses. *Enterococcus* spp. count in traditional cheeses made of milk which was not heat-treated was, depending on the ripening stage, from under 1 to 6.97 log cfu/g [6, 25–27, 31]. Compared to these data, the number of *Enterococcus* spp. in the studied short-ripened cheeses was similar to the upper level of the range, and in long-ripened cheeses it was considerably higher. The presence of a high number of *Enterococcus* spp. could result from poor hygienic practices during the manufacturing process and the resistance of *Enterococcus* spp. to adverse conditions (high temperature, high salt concentration, and high acid levels) [27]. *E. faecalis* was determined in all samples of cheese examined in the present study, which presented a risk to potential consumers. On the other hand, *Enterococcus* spp. is considered as emerging pathogen for humans. Around 80–90% of human enterococcal infections are caused by *Enterococcus faecalis*, while a majority of the remainder is accounted for by *Enterococcus faecium* (see [41], cited in [29]).

In the cheese ripening process, besides the role of the starter lactic acid bacteria, an important contribution is made by the secondary microbiota, which includes, among others, yeasts [32]. Yeasts can play a significant role in sensory and functional properties of cheese ripening, based on the interactions between yeasts and starter cultures, proteolytic and lipolytic activities, aroma compound formation, and other metabolic activities [33]. However, yeasts can also be responsible for some of the major defects in cheesemaking (see [42], cited in [34]). The growth of yeast is intensive on the cheese surface, and yeast counts range from around log 6 to log 8 cfu/cm² within 2–7 days; then, they remain fairly constant until the end of ripening, with variations in species balance [3]. In the examined short-ripened natural and smoked cheeses, the number of yeasts was comparable (log 5.23 and log 4.99 cfu/g resp.) and significantly lower than that in long-ripened cheeses (log 7.74 cfu/g). The differences between the amounts of yeasts in the examined short- and long-ripened cheeses could be the consequence of different ripening times. The number of yeasts in the examined short-ripened cheeses remained in the range determined for yeasts and moulds in traditional cheeses with a similar ripening time, that is, oscypek cheeses produced from sheep's milk (log 5.11–6.56 cfu/g) [30]. On the contrary, the number of yeasts in the examined long-ripened cheeses was higher than that in other traditional long-ripened cheeses produced from cow's milk (log 2.18–7.21 cfu/g), goat's milk (log 3.89–4.66 cfu/g), and sheep's milk (log 4.43–6.37 cfu/g) [21, 25–27].

Staphylococcus spp. were found in all three examined cheese types and they were at a similar level (log 6.26–6.46 cfu/g). The number of these microorganisms in traditional ripened cheeses produced from raw sheep's or cow's milk was from log 3.54 to log 6.50 cfu/g, depending on the ripening stage [6, 25]. In the current study, the presence of

Staphylococcus aureus was confirmed in three samples of long-ripened cheeses. *S. aureus* was recorded at the level of about log 1–6.41 cfu/g in ripened cheeses made from goat's and cow's milk [21, 24, 35]. These microorganisms could be inhibited by highly competitive lactic acid bacteria which can survive ripening conditions. Factors such as the type of the starter used, reduction of water activity, increased NaCl concentration, ripening time, and higher ripening temperature could affect the rate of reduction in the pathogen counts [24, 35]. *S. aureus* was not detected in traditional ripening cheeses produced in Italy and Poland [16, 23]. *S. aureus* at levels over log 5 cfu/g can produce enterotoxins in an amount which can be dangerous for human health [36].

In the examined three types of cheeses, no presence of *Salmonella* microorganisms was detected. Similarly, these microorganisms were not revealed in other short- and long-ripened traditional cheeses produced in Poland, Brazil, Northern Morocco, and Spain [9, 10, 16, 24]. The presence of *Salmonella* species was confirmed in 2.4% of samples of Tulum cheeses from Turkey, which could be related to the use of raw milk, unhygienic production process, and storage conditions [37]. According to these authors, it is essential that high safety standards are ensured, such as raw milk quality, effective pasteurization process, hygienic production and storage conditions, and proper cleaning and sanitation processes in production places [37].

In 3 samples of the examined short-ripened natural cheeses, *Listeria* was revealed, including *Listeria monocytogenes* in 1 sample (2.8%). The presence of this pathogen in this type of cheese, which is produced from pasteurized milk, can be explained by the fact that *Listeria* spp. can be isolated from milk heated at 60–67.5°C, and *L. monocytogenes* survived in high-temperature short-time pasteurization (see [43] and [44], cited in [2]). The source of the contamination of cheeses with *L. monocytogenes* may be both raw milk used in cheese production and cheese-processing environment, that is, the conditions of manufacture, ripening, and storage [38]. *L. monocytogenes* was also found in traditional homemade cheeses from Northern Morocco and Western Turkey in 4 (14%) and 13 (9.2%) samples, respectively [9, 11]. The results obtained by Fox et al. [1] demonstrated that the production environment may be the source of *L. monocytogenes* contamination of cheeses (75% of samples from cheesemaking facilities were found to have been contaminated with *L. monocytogenes* in the processing environment).

Listeria spp. was not found in traditional long-ripened cheeses produced in Italy and Brazil or in short-ripened cheeses produced in Poland [16, 23, 24]. The lack of pathogens can be related to the existence of lactic acid bacteria strains able to inhibit cheese pathogens, including *L. monocytogenes* [3]. Ceugniz et al. [34] reported that yeast (*Kluyveromyces lactis* and *Kluyveromyces marxianus*) could inhibit the growth of pathogens (e.g., *L. monocytogenes*) in a traditional handmade cheese.

5. Conclusions

Short-ripened natural cheeses did not fulfill the microbiological criteria in terms of food safety (the presence of

L. monocytogenes) and process hygiene (exceeded maximum levels of *E. coli*). Short-ripened smoked cheeses and long-ripened cheeses did not fulfill microbiological criteria in terms of process hygiene (exceeded maximum levels of *E. coli* and coagulase-positive *Staphylococcus*).

The levels of coliform bacteria, *Enterobacteriaceae* and *E. faecalis*, which were found in the three examined cheese types, indicated insufficient hygiene of the production process. It is necessary to introduce correction procedures in terms of compliance with the rules of GMP (Good Manufacturing Practice) and GHP (Good Hygienic Practice) at the stage of milking (in the case of long-ripened cheeses, which are made of raw milk from the producer's farm), milk storage (in the case of short-ripened cheeses, smoked and natural, due to the possibility of storing the material originating from dairy and used for their production), cheesemaking, cheese storage, and transport, as well as in retail stores and in hygiene training for the employees working at these stages. In addition, microbial control on the particular stages of production and trade would make it possible to determine the points at which microbial contamination of the material, intermediate product, or final product is increased and to develop correction procedures. Considering the fact that *Listeria monocytogenes* and *Staphylococcus aureus* were found in two of the examined cheese types, these cheeses are unsuitable to be commercially available due to the threat they pose to the potential consumers.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

- [1] E. Fox, K. Hunt, M. O'Brien et al., "Listeria monocytogenes in Irish Farmhouse cheese processing environments," *International Journal of Food Microbiology*, vol. 145, no. 1, pp. S39–S45, 2011.
- [2] M. Kousta, M. Mataragas, P. Skandamis, and E. H. Drosinos, "Prevalence and sources of cheese contamination with pathogens at farm and processing levels," *Food Control*, vol. 21, no. 6, pp. 805–815, 2010.
- [3] M.-Ch. Montel, S. Buchin, A. Mallet et al., "Traditional cheeses: rich and diverse microbiota with associated benefits," *International Journal of Food Microbiology*, vol. 177, pp. 136–154, 2014.
- [4] M. Rudolf and S. Scherer, "High incidence of *Listeria monocytogenes* in European red smear cheese," *International Journal of Food Microbiology*, vol. 63, no. 1-2, pp. 91–98, 2001.
- [5] Y. Yoon, S. Lee, and K.-H. Choi, "Microbial benefits and risks of raw milk cheese," *Food Control*, vol. 63, pp. 201–215, 2016.
- [6] Á. Alegría, P. Álvarez-Martín, N. Sacristán, E. Fernández, S. Delgado, and B. Mayo, "Diversity and evolution of the microbial populations during manufacture and ripening of Casín, a traditional Spanish, starter-free cheese made from cow's milk," *International Journal of Food Microbiology*, vol. 136, no. 1, pp. 44–51, 2009.
- [7] A. Berthold-Pluta, A. Pluta, and M. Zaniecka, "Microbiological quality of oscypek cheeses," *Medycyna Weterynaryjna*, vol. 67, no. 5, pp. 335–338, 2011.
- [8] A. Caridi, P. Micari, P. Caparra et al., "Ripening and seasonal changes in microbial groups and in physico-chemical properties of the ewes' cheese Pecorino del Poro," *International Dairy Journal*, vol. 13, no. 2-3, pp. 191–200, 2003.
- [9] O. El Galiou, S. Zantar, M. Bakkali et al., "Chemical and microbiological characteristics of traditional homemade fresh goat cheeses from Northern Morocco," *Small Ruminant Research*, vol. 129, pp. 108–113, 2015.
- [10] M. E. Tornadijo, M. C. García, J. M. Fresno et al., "Study of *Enterobacteriaceae* during the manufacture and ripening of San Simón cheese," *Food Microbiology*, vol. 18, no. 5, pp. 499–509, 2001.
- [11] S. Arslan and F. Özdemir, "Prevalence and antimicrobial resistance of *Listeria* spp. in homemade white cheese," *Food Control*, vol. 19, no. 4, pp. 360–363, 2008.
- [12] A. Margolles, A. Rodriguez, and C. G. de Los Reyes-Gavilan, "Some chemical and bacteriological characteristics of regional cheeses from Asturias, Spain," *Journal of Food Protection*, vol. 59, no. 5, pp. 509–515, 1996.
- [13] D. K. Papageorgiou, A. Abraham, M. Bori et al., "Chemical and bacteriological characteristics of Pichtogalo Chanion cheese and mesophilic starter cultures for its production," *Journal of Food Protection*, vol. 61, no. 6, pp. 688–692, 1998.
- [14] "Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs," *Official Journal of the European Union*, vol. L338, pp. 1–26, 2005.
- [15] P. Dolci, V. Alessandria, G. Zeppa et al., "Microbiological characterization of artisanal Raschera PDO cheese: Analysis of its indigenous lactic acid bacteria," *Food Microbiology*, vol. 25, no. 2, pp. 392–399, 2008.
- [16] A. Sip, A. Olejnik-Schmidt, M. Wieckowicz, and W. Grajek, "Analysis of microflora associated with regional golka cheeses," *Biotechnologia-Acta Scientiarum Polonorum Acta Scientiarum Polonorum-Biotechnologia*, vol. 9, pp. 25–38, 2010.
- [17] M. Buffa, B. Guamis, C. Royo, and A. J. Trujillo, "Microbiological changes throughout ripening of goat cheese made from raw, pasteurized and high-pressure-treated milk," *Food Microbiology*, vol. 18, no. 1, pp. 45–51, 2001.
- [18] D. Ercolini, F. Russo, I. Ferrocino et al., "Molecular identification of mesophilic and psychrotrophic bacteria from raw cow's milk," *Food Microbiology*, vol. 26, no. 2, pp. 228–231, 2009.
- [19] E. A. Rasolofoa, D. St-Gelais, G. LaPointe et al., "Molecular analysis of bacterial population structure and dynamics during cold storage of untreated and treated milk," *International Journal of Food Microbiology*, vol. 138, no. 1-2, pp. 108–118, 2010.
- [20] P. Kołakowski, M. Kowalska, and J. Sędrowska-Ćwiek, "Microbiota of ripening cheese," *Innowacyjne Mleczarstwo*, vol. 1, no. 1, pp. 6–13, 2013.
- [21] O. M. Vasek, S. M. Mazza, and G. S. de Giori, "Physico-chemical and microbiological evaluation of corrientes artisanal cheese during ripening," *Food Science and Technology*, vol. 33, no. 1, pp. 151–160, 2013.
- [22] A. Pluta, A. Bertold-Pluta, M. Olkowski et al., "Effects of modified atmosphere packaging (MAP) on the chosen quality parameters of sliced Swiss-type cheeses," *Zeszyty Problemowe Postępów Nauk Rolniczych*, vol. 572, pp. 53–64, 2013.
- [23] I. Carafa, F. Clementi, K. Tuohy et al., "Microbial evolution of traditional mountain cheese and characterization of early fermentation cocci for selection of autochthonous dairy starter strains," *Food Microbiology*, vol. 53, pp. 94–103, 2016.

- [24] M. T. Dores, J. E. Nobrega, and C. L. L. F. Ferreira, "Room temperature aging to guarantee microbiological safety of brazilian artisan Canastra cheese," *Food Science and Technology*, vol. 33, no. 1, pp. 180–185, 2013.
- [25] E. Litopoulou-Tzanetaki and N. Tzanetakis, "Microbiological characteristics of Greek traditional cheeses," *Small Ruminant Research*, vol. 101, no. 1–3, pp. 17–32, 2011.
- [26] M. Giello, A. La Stora, F. Masucci et al., "Dynamics of bacterial communities during manufacture and ripening of traditional Caciocavallo of Castelfranco cheese in relations to cows' feeding," *Food Microbiology*, vol. 63, pp. 170–177, 2017.
- [27] V. Zárate, F. Belda, C. Pérez et al., "Changes in the microbial flora of Tenerife goats' milk cheese during ripening," *International Dairy Journal*, vol. 7, no. 10, pp. 635–641, 1997.
- [28] G. Giraffa, "Functionality of enterococci in dairy products," *International Journal of Food Microbiology*, vol. 88, no. 2–3, pp. 215–222, 2003.
- [29] B. D. Jett, M. M. Huycke, and M. S. Gilmore, "Virulence of Enterococci," *Clinical Microbiology Reviews*, vol. 7, no. 4, pp. 462–478, 1994.
- [30] A. Alegría, P. Szczesny, B. Mayo, J. Bardowski, and M. Kowalczyk, "Biodiversity in oscypek, a traditional Polish cheese, determined by culture-dependent and-independent approaches," *Applied and Environmental Microbiology*, vol. 78, no. 6, pp. 1890–1898, 2012.
- [31] O. Aydemir, H. Harth, S. Weckx, and M. Dervişoğlu, "Microbial communities involved in Kaşar cheese ripening," *Food Microbiology*, vol. 46, pp. 587–595, 2015.
- [32] T. Beresford, N. Fitzsimons, N. Brennan et al., "Recent advances in cheese microbiology," *International Dairy Journal*, vol. 11, no. 4–7, pp. 259–274, 2001.
- [33] A. Binetti, M. Carrasco, J. Reinheimer et al., "Yeasts from autochthonal cheese starters: technological and functional properties," *Journal of Applied Microbiology*, vol. 115, no. 2, pp. 434–444, 2013.
- [34] A. Ceugniet, D. Drider, P. Jacques et al., "Yeast diversity in traditional French cheese "Tommed'orchies" reveals infrequent species with associated benefits," *Food Microbiology*, vol. 52, pp. 177–184, 2015.
- [35] C. Olarte, S. Sanz, E. Gonzalez-Fandos et al., "The effect of a commercial starter culture addition on the ripening of an artisanal goat's cheese (Cameros cheese)," *Journal of Applied Microbiology*, vol. 88, no. 3, pp. 421–429, 2000.
- [36] A. Morar, K. Imre, O. Onci, and C. C. Sala, "The quality of some cheeses manufactured by traditional methods," *Journal of Biotechnology*, vol. 231, p. S82, 2016.
- [37] H. Colak, H. Hampikyan, E. B. Bingol, and B. Ulusoy, "Prevalence of *L. monocytogenes* and *Salmonella* spp. in Tulum cheese," *Food Control*, vol. 18, no. 5, pp. 576–579, 2007.
- [38] J. Melo, P. W. Andrew, and M. L. Faleiro, "*Listeria monocytogenes* in cheese and the dairy environment remains a food safety challenge: The role of stress responses," *Food Research International*, vol. 67, pp. 75–90, 2015.



Hindawi

Submit your manuscripts at
www.hindawi.com

