COMPARATIVE STUDY ON THE MICROBIOLOGY AND MORPHOLOGY OF MILK AND CHEESE MICROBIOTA

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Abstract

Over time, many studies have been developed to describe microbial communities and to understand the dynamics and the role of these organisms during milk processing and during production of different types of cheese. For an evaluation of the microbiology of milk and cheese, a comparative study was carried out on 5 samples of whole cow's milk and 5 samples of Telemea-type cheeses in terms of identification and morphological characterization of milk microorganisms as raw material and cheese, as a finished product obtained, by correlating their microbiological characteristics in relation to the identified physico-chemical characteristics. The results obtained for the milk and cheese samples corresponded to those mentioned in the product-specific standards. Microbiologically, for the milk samples, the median value was 6.1 x 10^3 CFU/mL for milk and for the telemea cheese the CFU of samples was 4.3 x 10^3 CFU/g product. Microscopic analysis revealed a total of 9 colonies: 7 colonies of yeast and 2 colonies of bacteria, to which was added a mold (*Fusarium* spp.). The final results showed that there may be a certain degree of contamination, due to factors such as sanitary-veterinary hygiene and resistance increasing degree of microorganisms.

Key words: yeast, bacteria, microorganism, milk, cheese

In the human diet, milk is an essential component, compared to other foods being one of the most valuable sources of nutrients. Regardless of the species from which it comes, milk can be consumed as such, but it is also an excellent raw material for obtaining various assortments of milk products, such as cheeses, dietary products or butter (Usturoi M.G., 2012).

Obtained from milk, as a raw material, cheeses include the most dynamic and complete category of dairy products, the complexity of their particularities being related to the specific process of deep transformation of milk components, by maturation or fermentation, but is also related to the nutritional content especially (Richter R.L., Vedamuthu E.R., 2001).

Being important sources of nutrients, milk and cheese can be an excellent substrate and a favorable and complete medium for the growth and development of many microorganisms such as bacteria, yeasts or molds, non-pathogenic, such as the initial microbiota of milk or pathogenic, such as the contamination microbiota (Ray B., 2001).

Initially, the microorganisms that predominate in the milk microflora are few as number, but, regardless of the degree of compliance with the hygienic-sanitary conditions during milking, the milk cannot be sterile microbiologically, the internal microbiota counting up to 10^3–$15x10^3$ cells/mL (Tofan C., 2004). Specifically, milk is initially populated microbiologically by saprophytic germs such as lactic streptococci, *Lactococcus* and lactic bacilli, *Lactobacillus* (Lipșa F.D., Ulea E., 2017), and their presence in milk is given by their presence in the galactophores channels, in the udder of animals or may be a consequence of various treatments applied to animals, the administration of contaminated feed or the existence of any pathological condition of the animal from which it is harvested.

With a complex and varied chemical composition, milk can also acquire an external microbial intake, with various pathogenic microorganisms (*Bacillus cereus, Salmonella* spp., *Shigella* spp., *Bacillus anthracis*, *Clostridium* spp., *Alcaligenes* spp., *Proteus* spp., *Streptomyces, Enterobacter* spp., *Escherichia* spp., *Citrobacter* spp.) which may come from either the milk collection and storage equipment, the operator's equipment and hands or the atmosphere in the animal shelter. As a raw material for different products, the knowledge of the characteristics of milk from the viewpoint of the development of microorganisms finds its applicability mainly in the practice of production, in the conditioning, transport or processing of milk. In this sense, dairy products

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such as cheese have been considered, similar to milk, common vectors of useful microbial agents, but also of pathogens (Borges M.F., 2006).

Microbial populations are an indispensable component of all cheese varieties and play an important role in the manufacture of cheese, especially in terms of the ripening process (Beresford T. *et al*, 2001). The cheese microbiota consists of bacteria, yeasts or molds, some of which are useful, such as lactic acid bacteria, which contribute to the coagulation and ripening processes, with implications in milk acidification and flavor development, but also unwanted populations and potential pathogens or spoilers (Perry K.S.P., 2004).

In cheeses, at the beginning of production, the most common microorganisms are the initial bacteria from milk, their complex and specific microbial communities developing during their ripening (Delgado S. *et al*, 2002). As a source of origin, the cheese microbiota may be derived from natural contamination of milk, the environment or deliberate inoculation as part of the original culture. The groups of microorganisms used to make cheese are lactic acid bacteria, alkalizing bacteria, propionic bacteria, filamentous caps and yeasts (Bockelmann W., 2002).

Over time, many studies have been developed to describe microbial communities and to understand the dynamics and role of these organisms during milk processing and the production of cheese (Ray B., 2001). The individual and associated effects of physicochemical factors on harmful and useful bacterial populations, as well as the interaction between the most important microorganisms, have been established. The study and application over time of various research methods in the field of milk and cheese microbiology have led to new perspectives in this complex microbial ecosystem and it has been concluded that the role of milk or cheese microflora is essential for defining the characteristics and quality traits of milk and cheese products (Richter R.L., Vedamuthu E.R., 2001).

In order to develop a correct view on the microbiology of milk and cheese, the aim of this paper is to carry out an applied and comparative study regarding the identification and morphological characterization of microorganisms in milk, as raw material and in cheese, as finished product, by correlating their microbiological characteristics in relation to the identified physico-chemical characteristics.

MATERIAL AND METHOD

In this study, 5 samples of whole cow's milk and 5 samples of telemea-type cheeses were analyzed from a microbiological and physico-chemical part for the morphological characterization of the constituent microorganisms.

The supporting material, the milk samples, taken from the Agricultural Research and Development Station, Secuieni farm and the cheese samples, taken from the Milk Processing Center, part of the same station, were analyzed from a physico-chemical part to determine the density, titratable acidity and fat content (milk samples), determination of acidity, dry matter and fat content (cheese samples) and microbiologically to determine the total bacteria count and morphological characterization of the identified micro-organisms.

Microbiologically, the total bacteria count in whole milk and Telemea cheese was determined according to the method of successive dilutions, the principle of which is based on the identification of the total number of live aerobic mesophilic bacteria capable of it is developed on specific culture media, at temperatures of 30–37^{o}C, performing in this sense different simple Gram stains. To highlight the shape, grouping and identification of microorganisms developed after inoculation and incubation, 5 Petri plate were chosen on which a wider development of microorganisms was observed, after drying and prior staining. From the selected plates, 9 microorganisms were chosen for Gram staining procedure, analyzed under a microscope at the immersion objective.

The physico-chemical characteristics of the studied samples were highlighted by classical working methods, in accordance with the standards and regulations applicable to each category of analyzes performed.

RESULTS AND DISCUSSIONS

In the physico-chemical analysis of whole milk samples and Telemea cheese samples, the identified parameters, related to the minimum quality conditions provided in the specific standards, generally indicated a uniformity in terms of the values obtained (*table 1*).

Analyzes performed on whole milk, as a raw material for obtaining cheeses, showed an average density value of 1.0029 g/cm^3, without deviations of the empirical arithmetic mean from the theoretical average, with a coefficient of variation <10% (CV% = 0.03), which highlighted a uniformity of the analyzed indicator. The lowest density values were determined for the WM_1 and WM_4 samples (1.0289 g/cm^3), and the maximum density value was recorded in the WM_5 sample (1.0295 g/cm^3). Given that according to STAS 6347–73, the density of raw milk for industrial processing must have a minimum value of 1.029 g/cm^3, it was estimated that the milk samples

studied had a compliant density, which may show that the milk analyzed did not contain other added components in its mass.

Table 1

Physico-chemical characteristics of whole milk and Telemea cheese

Sample	Density	Acidity	Fat	Sample	Acidity	DM	Fat
	g/cm^3	^{o}Thörner	%		^{o}Thörner	%	% DM
Whole Milk (WM)				**Telemea Cheese (TC)**			
WM_1	1.0289	16	4.20	TC_1	21	38.06	40.73
WM_2	1.0291	17	4.10	TC_2	21.5	37.99	40.7
WM_3	1.0293	17	4.15	TC_3	21.5	38.1	40.65
WM_4	1.0289	16	4.10	TC_4	21	38.2	40.75
WM_5	1.0295	17	4.12	TC_5	21	38.13	40.7
n	5			***n***	5		
$\bar{x} \pm s_{\bar{x}}$	1.029±0.00	16.60±0.24	4.134±0.019	$\bar{x} \pm s_{\bar{x}}$	21.20±0.12	38.10±0.04	40.706±0.017
CV	0.03	3.30	1.021	CV	1.29	0.21	0.093
Min.	1.0289	16	4.1	Min.	21	37.99	40.65
Max.	1.0295	17	4.2	Max.	21.5	38.2	40.75
	STAS 6347-73	STAS 6353-61	STAS 6352-61		STAS 6353-61	STAS 6344-64	SR 1981/2008
	Min. 1.029	15-19	Min. 3.2		Max. 120	Min. 43	42-45

The determination of milk acidity indicated an average value of the analyzed samples of 16.60 ±0.24 ^{o}T, with 3.30 coefficient of relative variability of the samples compared to the mean (CV%). However, the values obtained were on average uniform, from minimum values of 16^{o}T for samples WM_1 and WM_4 and maximum values of 17^{o}T for samples WM_2, WM_3, WM_5.

Given that the normal acidity of fresh cow's milk is between 15 and 19^{o}T (STAS 6353–61), the raw milk samples analyzed were according to parameters, which, in relation to the microbiological content, does not indicate an abundant multiplication of the existing microbiota.

The analysis of the fat content of whole milk samples indicated an average value of 4.134 ±0.019, with 1.021 CV% coefficient of variability. The values obtained for the analyzed samples were higher than those mentioned by STAS 6352–61, the lowest value being recorded for the WM_2 and WM_4 samples (4.10%), and the highest value being recorded for the WM_1 sample (4.20%). Microbiologically, the presence of a high fat content can prevent the entry of air into the product mass, thus facilitating favorable conditions for the development of anaerobic and optional anaerobic microorganisms.

Regarding the determination of the physico-chemical indicators of the Telemea cheese samples, the acidity analysis showed an average of the values obtained of 21.20±0.12^{o}T, with 1.29 CV% coefficient of variation. Compared to STAS 6353–61, the identified average value is in accordance with the reported one, respectively minimum 21^{o}T, which indicates a normal acidity of the analyzed samples. The minimum values were obtained for samples TC_1, TC_4 and TC_5, and the maximum value was recorded for samples TC_2 and TC_3.

The determination of the dry matter content of the cheese samples reflected an average of the values obtained of 38.10±0.04, with 0.21 coefficient of variability CV%, which reflects the uniformity of the identified values. The minimum dry matter content was obtained for the TC_2 sample, respectively 37.99%, while the maximum dry matter content was obtained for the TC_4 sample, respectively 38.2%. According to SR 1981/1980, the minimum dry matter content must be 38%, thus indicating a consistent value of the result obtained against the standard.

The analysis of the fat content showed a value of this parameter of 40.706±0.017% (compared to the DM), with 0.0093 CV%. The lowest value obtained was recorded in the TC_3 sample (40.65% of DM), while the highest value of the fat content was identified for the TC_4 sample (40.75% of DM). Given that the provisions of SR 1981/2008 mention a fat content of Telemea cheese compared to the DM between 42–45%, the value obtained was slightly lower than reported.

Following the analyzes to determine the total number of germs in whole milk and Telemea cheese, it was found that the values obtained, namely 6059 TBC/mL for whole milk and 4301 colonies/g for Telemea cheese, corresponded to those listed in EC Regulation 853/2004, which showed that the analyzed milk is suitable for processing, and Telemea cheese does not pose a danger to the health of consumers.

The identification and morphological characterization of the microorganisms, presented in Table 2, showed in general that the predominant profile of the identified colonies was a curved one, with a round shape, glossy surface and white color (Figure 1).

Table 2

CFU and the morphological characteristics of the milk and cheese microbiota

Total Bacteria Count (TBC)					
Integral Milk (CFU/ml)		6059	Telemea Cheese (CFU/g)		4301
Morphological characteristics					
Specification		**Shape**	**Profile**	**Surface**	**Colour**
P_1	M_1 (Yeast)	Round	Curved	Smooth	Cream-colored
P_2	M_2 (Bacteria)	Irregular	Curved	Glossy	Cream-colored
	M_3 (Yeast)	Irregular	Hilly	Wrinkled	White
	M_4 (Yeast)	Irregular	Hilly	Wrinkled	White
P_3	M_5 (Bacteria)	Round	Curved	Glossy	Orange
P_4	M_6 (Yeast)	Round	Curved	Glossy	White-pink
	M_7 (Yeast)	Round	Curved	Glossy	White-pink
P_5	M_8 (Yeast)	Round	Hilly	Wrinkled	White
	M_9 (Yeast)	Round	Hilly	Wrinkled	White

Colony M_1, developed on PDA (*Potato Dextrose Agar)* medium, at dilution 10^{-3}, was identified as a yeast, round in shape, with a curved profile, smooth surface and cream-white color.

M_2 was represented by bacilli, presented macroscopically in the form of a colony of irregular shape, with a curved profile, glossy surface and cream-white color, while, from a microscopic viewpoint, the bacilli were identified as cylindrical, rounded at the ends.

M_3 and M_4 were two yeast colonies, which, from a macroscopic viewpoint, showed similar characters, namely irregular shape, hilly profile and white corrugated surface; these colonies developed on the PDA medium at dilution 10^{-2}.

In the third inoculated plate, on the nutrient agar medium, at dilution 10^{-2}, the M_5 colony developed, which, from a macroscopic viewpoint, had a round shape, a curved profile and a glossy, orange surface. Microscopically, it has been identified as consisting of simple, cylindrical, rounded bacilli at the ends.

From plate 4, colonies M_6 and M_7 were analyzed, identified as yeasts, with a round shape, curved profile and glossy white-pink surface. The colonies thus identified developed on the YMA *(Yeast Malt Agar)* medium, at a dilution of 10^{-1}.

From the last inoculated plate, the M_8 and M_9 colonies, developed on PDA medium, were examined at dilution 10^{-1}, identified as yeasts, with a round colony shape, a hilly profile, with a dry, white surface.

It should be noted, however, that from the third plate, a mold was analyzed, identified as *Fusarium* spp. The colony was characterized by an abundant mycelium, white-pink, with the reverse of the pink colony and the lobed edges. Under the microscope, macroconidia were observed, curved and slightly fusoid in shape.

CONCLUSIONS

Physico-chemically, for the milk and cheese, the parameters relevant for the microbiological study of the samples were analyzed, the results obtained being corresponding to those included in the standards specific to each product.

Microbiologically, the total bacteria count was determined in order to highlight the possible contamination to which the products could be exposed. In general, for milk, from the plates inoculated with nutrient agar, only 6 microorganisms were found; of the PDA and YMA boards, only one board contained the number of microorganisms needed to be considered. For Telemea cheese, only on the PDA and YMA medium, a number of colonies between 30–300 was identified.

Following the microscopic analysis of the microorganisms that developed during the thermostating conditions, most were identified as yeasts, respectively 7 colonies out of 9 identified and only two colonies of those examined were bacteria, this fact highlighting that both products are safe for human consumption, with a majority of yeasts found having no potential adverse effects on humans.

Comparing the microbiological results obtained from the analyzes performed on the samples of whole milk and Telemea cheese with the research found in the literature, the possibility of a certain degree of contamination was highlighted, favored by factors such as sanitary-veterinary hygiene, evolution of the degree of resistance of microorganisms, but also the formation of new species with unknown characters.

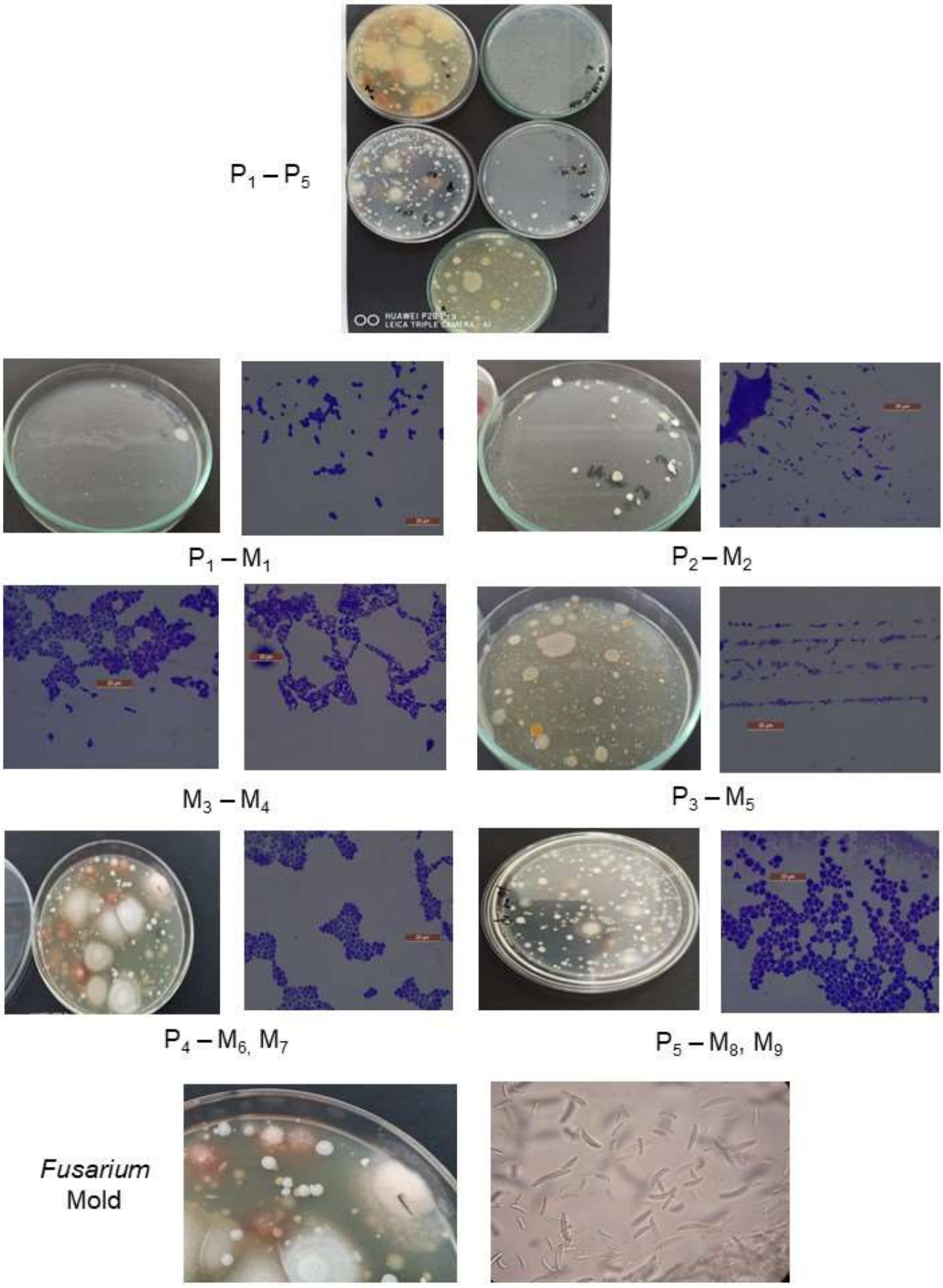

Figure 1 **Morphological characteristics of whole-milk and telemea cheese microbiota**

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