Serbian Traditional Goat Cheese

Subjects: Agriculture, Dairy & Animal Science

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Serbian goat cheese, quality relies on the use of milk collected from animals reared in organic farming systems. This organic milk contains more dry matter and nutrients; hence, its usage leads to the obtention of dairy products with exceptional nutritional and functional properties. Goat's milk harbors a specific taste, and it is known to cause less allergic reactions than cow's milk. The absence of adverse reactions is due to its low or minimal level of the αs1-casein fraction. Goat's milk is generally more easily digested (the fat globules are smaller) and represents a good source of calcium, phosphorus and vitamins.

traditional goat cheese ripening physico-chemical characteristics sensory properties antibiotic resistance safety

1. Introduction

The standard chemical composition of organic and conventional goat milk has been the subject of numerous studies and thereby obtained highly opposing results[1]*. Malissiove et al.*[2] *found no significant differences. On the contrary, Tudisco et al.*[3] *recorded higher fat content in organic milk, while Pajor et al.*[4] *apart from the fat, found higher protein content and non-fat dry matter in organic goat milk. Artisan goat cheese with a geographical origin is often produced from raw milk in the traditional way and has its own specific flavor, often robust and very complex, that makes them different from cow milk cheeses. Also, artisanal cheeses process a unique microbiological community.*[1][2]

2. Physico-Chemical Analysis

The results observed for goat's milk, whey, and cheese during 28 days of ripening are displayed in **Table 1** and **Table 2**. The dry matter in milk was 14.21%, and 7.7% in whey, while in cheese the highest content of dry matter was detected on the 21st day (52.64%), and the lowest content was measured on day 0 (38.91%). Total protein content in milk was 3.58%, while in whey it was 1.36% (**Table 1**). In cheese (**Table 2**), the highest values for protein content were also observed on the 21st day (15.31%) and the lowest on day 0 (12.31%). The pH values for milk and whey were 6.52, whilst the cheese pH ranged between 4.75 and 6.55. Salt amount varied from 0.69% to 0.96%. Total ash content ranged between 2.03% and 1.30%, with the lower values corresponding to longer ripening periods. The quality of goat's milk fat and protein is an important factor because it defines the ability of milk to be processed and has a relevant role in the nutritional and sensory quality of the products obtained from it.[3]

Table 1. Physico-chemical parameters of goat's milk and whey.

		Goat Milk	Whey
Chemical characteristics	Dry matter content (%)	14.21 ± 0.13	7.7 ± 0.00
	Milk fat content according to Gerber (%)	4.60 ± 0.00	0.4 ± 0.01
	Ash content (%)	0.77 ± 0.01	0.53 ± 0.01
	Total protein content (%)	3.58 ± 0.02	1.36 ± 0.01
	pH value	6.52 ± 0.02	6.52 ± 0.02
	Titratable acidity (°SH)	6.53 ± 0.09	6.36 ± 0.04
	a_w value	0.945 ± 0.00	0.946 ± 0.00
Color parameters (D65)	L*	82.44 ± 0.09	39.22 ± 0.03
	a*	−3.16 ± 0.01	−2.92 ± 0.07
	b*	6.85 ± 0.00	4.53 ± 0.02
	Dominant wavelength (nm)	568.56 ± 0.02	565.17 ± 0.27

Data are presented as mean ± SD; nd—data not determined.

Table 2. Physico-chemical parameters of goat's cheese during ripening.

		Day 0	Day 7	Day 14	Day 21	Day 28
Chemical characteristics	Dry matter content (%)	38.91 ± 0.16 ^{a}	41.80 ± 0.11 ^{b}	50.87 ± 0.28 ^{c}	52.64 ± 0.13 ^{d}	46.79 ± 0.06 ^{e}
	Milk fat content according to Van Gulik (%)	23.00 ± 0.00 ^{a}	25.83 ± 0.23 ^{b}	32.25 ± 0.25 ^{c}	33.5 ± 0.00 ^{d}	32.75 ± 0.25 ^{c,e}
	Fat in dry matter (%)	59.11 ± 0.00 ^{a}	61.96 ± 0.00 ^{b}	63.40 ± 0.00 ^{c}	63.64 ± 0.00 ^{c,d}	69.99 ± 0.00 ^{e}
	Ash content (%)	2.03 ± 0.17 ^{a}	1.60 ± 0.01 ^{b}	1.66 ± 0.002 ^{b,c}	1.66 ± 0.02 ^{b,c}	1.30 ± 0.01 ^{d}
	Total protein content (%)	12.31 ± 0.35 ^{a}	12.54 ± 0.35 ^{a}	14.34 ± 0.14 ^{b}	15.31 ±0.49 ^{b,c}	14.22 ± 0.36 ^{b,c}
	pH value	6.55 ±	5.30 ±	5.15 ±	4.98 ±	4.75 ±

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		Day 0	Day 7	Day 14	Day 21	Day 28
		0.01 [a]	0.01 [b]	0.01 [b,c]	0.00 [d]	0.01 [d,e]
	Titratable acidity (°SH)	8.53 ±0.37 [a]	37.86 ± 0.99 [b]	53.60 ± 1.60 [c]	61.60 ± 0.80 [d]	65.20 ±0.40 [e]
	a_w value	0.941 ± 0.00 [a]	0.937 ± 0.00 [a]	0.931 ± 0.00 [a]	0.929 ± 0.00 [a]	0.939 ± 0.00 [a]
	NaCl content (%)	0.72 ± 0.00 [a]	0.80 ± 0.04 [b]	0.69 ± 0.01 [c]	0.92 ± 0.01 [d]	0.96 ± 0.04 [d,e]
Color parameters (D65)	L*	88.49 ± 0.69 [a]	87.14 ± 0.00 [a]	87.95 ± 1.19 [a]	87.46 ± 0.77 [a]	nd
	a*	−1.88 ± 0.04 [a]	−1.32 ± 0.44 [b]	−1.82 ± 0.09 [a,c]	−1.98 ± 0.16 [d]	nd
	b*	10.30 ± 0.43 [a]	10.95 ± 3.21 [a]	12.51 ± 1.11 [b]	12.03 ± 0.37 [b]	nd
	Dominant wavelength (nm)	573.24 ± 0.09 [a]	574.27 ± 0.21 [a]	573.81 ± 0.11 [a]	573.51 ± 0.19 [a]	nd

Data are presented as mean ± SD; nd—data not determined; means within a row marked with different letters differ significantly ($p < 0.05$).

The goat cheese colors were measured using the CIE Lab color system. It was evident that goat cheese samples (**Table 2**) presented higher values (L*, a, b) in comparison to milk (**Table 1**). The whiteness of goat cheese did not change significantly during 21 days of ripening. However, the storage period of time had a significant effect on red/greenness (a) and yellow/blueness (b) coefficients. The Y value of goat cheese changed from 73.09.15% (day 0) to 70.95% (day 21), while dominant wavelengths during storage ranged from 573.24 nm (day 0) to 574.27 nm (7th day). Total color differences of goat cheese (ΔE) were estimated, and the highest change was observed after 14 days of ripening (2.40), with the lowest alterations occurring after 7 days (1.04).

3. Sensory Properties

The goat cheese sensory features were analyzed during 28 days of ripening, using the 5-point hedonic scale, based on the evaluation of appearance, color, texture, cross-section, odor, and taste. The results obtained are shown in **Figure 1**. On day 0, consumers described the cheese as compact, with a cross-section without cavities, having a typical odor and mild taste, as is usually associated with this food product. After 7 days of ripening, the cheese was defined as harboring an acceptable color, typical odor, and a slightly bitter taste. On day 14, they indicated an intense taste with increased bitterness, while after 21 days of ripening the cross-section presented a small number of cavities, characteristic smell, and pronounced bitterness. On the 28th day, it was not possible to carry out the predicted sensory analysis, due to the development of molds on the cheese surface. Overall, the

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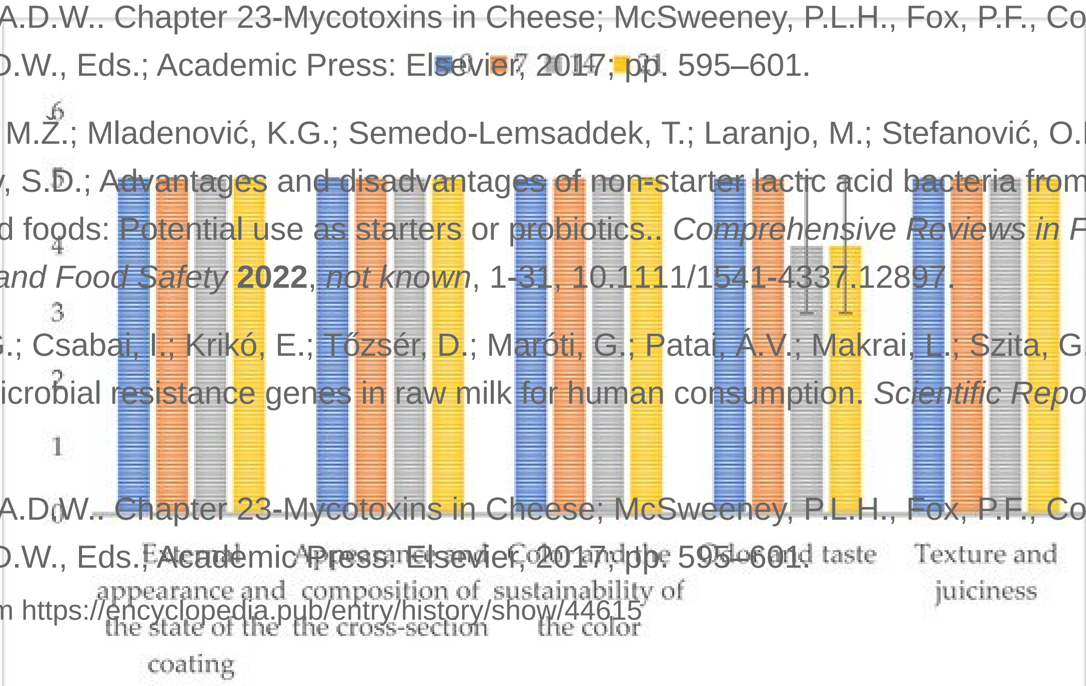

Figure 1. Sensory properties of traditional goat cheese during ripening.

The sensory characteristics and color of goat cheese are fundamental to consumer acceptance. Cheese color and flavor depend on the presence of fat globules and casein micelles.[4] Our results indicated that the highest changes in cheese color occurred after 14 days of ripening. This is probably due to the microbiological activity of non-starter lactic acid culture and complex biochemical processes (proteolysis, lipolysis, oxidation processes).[5][6]

4. Microbiological Analysis

4.1. Enumeration, Isolation, and Identification of Enterobacteriaceae

The counts of total enterobacteria present in goat's milk and cheese samples were assessed using the VRBG medium (**Table 3**). Briefly, microbial loads ranged between 9.09 × 10^4 CFU/g (day 0) and 1.24 × 10^8 CFU/g (14th day) of cheese, whilst in milk, the levels were similar to those observed at cheese day 0. As aforementioned, after 28 days of ripening molds developed on the surface of the cheese, which prevented the isolation of enterobacteria for further analysis.

Table 3. Total number of *Enterobacteriaceae* and aerobic mesophylic bacteria in goat's milk and cheese during ripening.

Sample	Day of Analysis	Total Number of *Enterobacteriaceae*	Total Number of Aerobic Mesophylic Bacteria
Milk [a]	0	1.44×10^4	1.63×10^3
Cheese [b]	0	9.09×10^4	3.6×10^6
Cheese	7	1.87×10^6	5.76×10^{10}
Cheese	14	1.24×10^8	5.24×10^{11}
Cheese	21	3.05×10^7	1.20×10^{11}
Cheese	28	1.07×10^5	3×10^7

[a] CFU/mL of milk, average values of three independent experiments; [b] CFU/g of cheese, average values of three independent experiments.

The total number of aerobic mesophilic bacteria present in goat's milk and cheese samples were addressed using a nutrient agar (**Table 3**). Microbial loads ranged between 3.6×10^6 CFU/g (day 0) and 5.24×10^{11} CFU/g (14th day) of cheese. It could be noticed that the initial number of aerobic mesophylic bacteria was lower compared to the total number of enterobacteria in milk. However, in cheese samples, the number of aerobic mesophylic bacteria was higher compared to the total number of enterobacteria.

According to Oikonomou et al.[7], the specific composition of the milk microbiota directly affects the subsequent development of dairy foods' characteristics. Microorganisms can bring about the fermentation of milk through the production of lactate and have a variety of different impacts on the sensory, texture, flavor, and organoleptic properties of the resultant dairies.[4][7]

After plate counting on VRBG agar, presumptive enterobacteria were isolated (10 bacteria per plate) and submitted to conventional microbiological characterization. All Gram-negative, catalase-positive, and oxidase-negative bacteria were further analyzed by biochemical tests and with Microgen GN-A and GN-B tests (**Table 4**). All isolates were incubated in the HiChrome coliform agar and citrate medium, where a characteristic growth (color of the colonies) and color of the medium were shown, respectively. For additional confirmation of species allocation, MALDI-TOF mass spectrophotometry was applied (**Figure 2**). The results obtained identified the following microorganisms: *Escherichia coli* (54 isolates), *Proteus mirabilis* (6 isolates), *Rahnella aquatilis* (1 isolate), *Pseudomonas* sp. (1 isolate), *Enterobacter cloacae* (2 isolates), and *Enterobacter asburiae* (1 isolate). It was observed that *E. coli* is the most dominant species in cheese, while *P. mirabilis* is the dominant in goat milk (**Figure 3**).

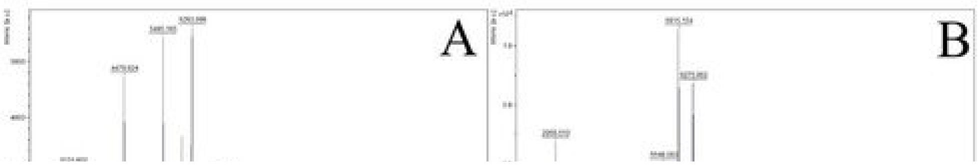

Figure 2. Mass spectra of the isolated bacteria (**A**)—*P. mirabilis*; (**B**)—*Pseudomonas* spp.; (**C**)—*E. coli;* (**D**)—*E. cloacae*; (**E**)—*E. asburiae*; (**F**)—*R. aquatilis*).

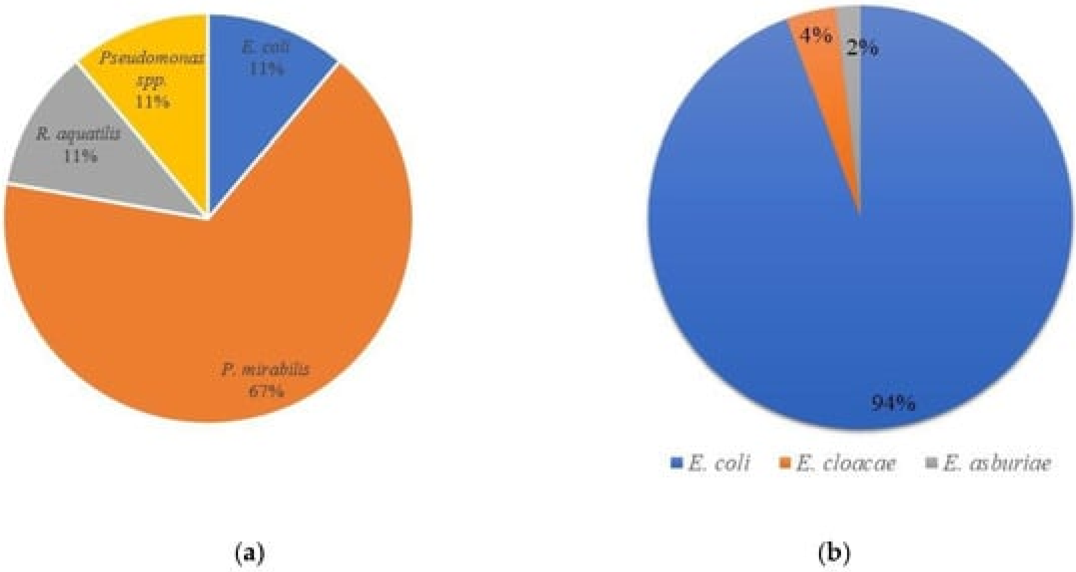

Figure 3. Distribution of Enterobacteriaceae in milk (**a**) and cheese (**b**).

Table 4. Microbiological analysis.

Species		***E. coli***		***P. mirabilis***	***R. aquatilis***	***E. cloacae***	***E. asburiae***	***Pseudomonas spp.***
Number of isolates		53	1	6	1	2	1	1
Origin (milk or cheese)		cheese	Milk	milk	milk	cheese	cheese	milk
Biochemical characteristics	Lysine	+	+	−	−	+	+	−
	Ornitine	−	−	−	−	+	+	+
	H_2S	−	−	−	−	−	−	+
	Glucose	+	+	+	+	+	+	+
	Mannitol	+	+	+	+	+	+	−
	Xylose	+	+	+	+	+	+	+
	ONPG	+	+	+	+	+	+	−
	Indole	+	+	−	−	+	−	+
	Urease	−	−	−	−	+	+	+
	VP	−	−	+	+	−	+	−

Species	*E. coli*		*P. mirabilis*	*R. aquatilis*	*E. cloacae*	*E. asburiae*	*Pseudomonas spp.*
Citrate	−	−	+	+	+	+	−
TDA	−	−	−	−	−	−	+
Gelatin	−	−	−	−	−	−	−
Malonate	−	−	−	−	+	−	−
Inositol	−	−	−	−	−	−	−
Sorbitol	+	+	+	+	+	−	+
Rhamnose	+	+	+	+	+	+	+
Sucrose	−	−	+	+	+	+	−
Lactose	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+
Adonitol	−	−	−	−	−	−	−
Raffinose	−	−	+	+	+	+	−
Salicin	−	−	+	+	+	+	−
Arginine	+	+	−	−	+	+	+
Growth on citrate medium	Green medium	Green medium	Blue medium	Blue medium	Blue medium	Blue medium	Blue medium
Growth on HiChrome coliform agar	Blue dark/violet	Blue dark/violet	Orange/yellow	Transparent white	Light pink	Light pink	Orange/yellow
Microgen GN-A and GN-B	*E.coli*	*E. coli*	*Enterobacter amnigenus biogroup 1*	*Pantoea agglomerans*	*Kluyvera ascorbata*	*Enterobacter gergoviae*	*Klebsiella oxytoca*
MALDI-TOF identification	*E. coli*	*E. coli*	*P. mirabilis*	*R. aquatilis*	*E. cloacae*	*E. asburiae*	*Pseudomonas spp.*
MALDI-TOF score	2.28 to 2.52		2.40 to 2.53	2.00	2.17 to 2.37	2.33	1.80
Proteolytic activity	−	−	−	+	−	−	−
Lypolytic activity	−	−	−	−	−	−	−
Antibiotic resistance profile	GEN	GEN	AMX, TET	S	AMX,	S	AMX, TET

Species	*E. coli*	*P. mirabilis*	*R. aquatilis*	*E. cloacae*	*E. asburiae*	*Pseudomonas spp.*
				TET		

“+”—Positive reaction; “−“—Negative reaction; the antibiotic short name whenever the isolate is resistant (GEN—gentamicin; TET—tetracycline; CL—chloramphenicol, AMX—amoxicillin; S—sensitive).

4.2. Proteolytic and Lipolytic Activities

The results of the investigation of the proteolytic and lipolytic activity of isolated bacteria from goat’s milk and cheese indicated that none of the isolates possess proteolytic or lipolytic activities (**Table 3**). The exceptions were *P. mirabilis* GM-6 and *R. aquatilis* GM-9, both isolated from milk, which showed proteolytic activity.

Enterobacteriaceae capable of synthesizing proteolytic and lipolytic enzymes are largely responsible for the deterioration of milk and dairy products, which may cause various issues in the dairy industry [4][8]. Masiello et al. [9] indicated that diverse bacteria found in pasteurized milk, exhibiting phenotypic characteristics such as the production of lipolytic and proteolytic enzymes, can result in milk spoilage. Except for *Pseudomonas* sp., the other genera of the Enterobacteriaceae family, such as *Enterobacter* and *Klebsiella*, usually do not display proteolytic and lipolytic activity [10], which was confirmed by the results.

4.3. Antibiotic Resistance Profiles

Goat’s milk and cheese isolates showed different sensitivity to the tested antibiotics (**Table 3**). All isolates from the genus *Escherichia* showed resistance to gentamicin, but they were sensitive to amoxicillin, chloramphenicol and tetracycline. *E. asburiae* and *R. aquatilis* isolates were sensitive to all tested antibiotics, while *E. cloacae* isolates showed resistance to amoxicillin and tetracycline. Isolates from the genus *Proteus* showed sensitivity to gentamicin and chloramphenicol, but they were resistant to amoxicillin and tetracycline. *Pseudomonas* sp. showed resistance to tetracycline and amoxicillin. In general, there was no isolate that showed full resistance to antibiotics.

Antimicrobial-resistant determinants may be associated with non-pathogenic bacteria. Horizontal gene transfer (HGT) events can lead to their transference to pathogenic microorganisms, such as several members of the Enterobacteriaceae family, increasing the prevalence and dissemination of antimicrobial resistance along the food chain [11]. The likelihood of HGT is even greater if resistance genes are located in mobile genetic elements [12]. Nonetheless, in the research low levels of resistance were observed. All isolates showed susceptibility to chloramphenicol. *E. coli* isolates were resistant to gentamicin and susceptible to other tested antibiotics. *E. cloacae, P. mirabilis* and *Pseudomonas* spp. isolates showed resistance to amoxicillin and tetracycline, while *E. asburiae* and *R. aquatilis* isolates were susceptible to all antibiotics.

E. coli O157 Rapid Latex Agglutination Test

According to the *E. coli* O157 rapid latex agglutination test, none of the tested *E. coli* isolates belonged to the serotype *E. coli* O157.

Isolation and Identification of Molds

The enumeration of molds, as well as their isolation and identification, was determined in milk, whey, brine and cheese samples. In milk, 20 CFU/mL were detected, including *Alternaria alternata* and *Geotrichum candidum*. In whey, the total number of molds was 100 CFU/mL, while the identified species corresponded to *Acremonium strictum, Penicillium brevicompactum, Penicillium chrysogenum, Penicillium expansum, Penicillium glabrum* and *Talaromyces albobiverticillus*. The total number of molds in brine matched 80 CFU/mL, and the identified species were *Acremonium strictum, Penicillium aurantiogriseum, Penicillium glabrum* and *Penicillium thomii*. On the first day of cheese ripening (day 0), the total number of molds was 200 CFU/g, and the following species were identified: *Cladosporium macrocarpum* and *Penicillium aurantiogriseum*. On the 7th and 14th day of ripening, the total number of molds was less than 100 CFU/g, whereas in the 21st day, the total number corresponded to 5.4×10^5 CFU/g, and *Aspergillus flavus* was the dominant species (Supplementary Figures S1–S17).

As it was indicated, mold contamination in cheese can affect its organoleptic properties, influence the cheese quality and potentially produce secondary toxic metabolite mycotoxins, and therefore poses a potential health risk to consumers. Mycotoxin cheese contamination can occur indirectly via milk contamination or directly through mycotoxin-producing spoilage
molds. However, mold growth on the cheese surface does not necessarily indicate the presence of mycotoxins in cheese [13]. According to the obtained results, the molds found in cheese samples may indicate poor hygienic conditions during cheese production and/or storage, such as *Cladosporium macrocarpum*, or from the brine, such as *Penicillium aurantiogriseum*. Since the toxigenic mold *Aspergillus flavus* was found only in the cheese sample, after the 21st day of ripening, it is most likely that its occurrence resulted from cross-contamination.[4]

5. Conclusions

The present entry evaluated the physico-chemical, sensory, hygienic and safety characteristics of raw goat milk, whey, brine and traditional cheese during the ripening period of 28 days. The results show that the cheese under study belongs to the acid full-fat cheese group. A consumer panel attributed high scores to the goat cheese, until the 21st day of ripening. After this period, the overall features altered significantly, including bitterness, odor intensification and the development of molds on the surface. The presence of fungi, associated with Enterobacteriaceae, suggests that the hygiene of the production processes needs to be improved. The results indicate the need for better hygiene during milking and cheese production. Additionally, efforts are being made to control the production of traditionally made cheese, i.e., to place it under industrial conditions and to use pasteurized milk or starter cultures previously isolated from the same cheese in order to attain a standard of uniform quality and safety. Regarding microbial safety, the detection of putative pathogens and antibiotic resistances is recommended for active monitoring of traditional foods to avoid foodborne infections and/or the dissemination of resistant microorganisms along the food chain.[4]