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Concerns about the microbiological quality of traditional raw milk cheeses: a worldwide issue

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ABSTRACT.

Six types of unripened raw milk fresh (Robiola, Crescenza, Primo sale and Formaggella) and "pasta filata" cheeses (Mozzarella and Burrata) were evaluated for microbiological parameters. No Listeria monocytogenes or Salmonella spp. were detected, but high microbial counts were revealed. Significantly higher Total Viable Counts (TVC) (P=0.002) and Enterobacteriaceae counts (P<0.001) were observed in "fresh cheese" than in "pasta filata" samples. Values > 6 Log CFU/g were found in 81.3% of fresh vs 50% in pasta filata for TVC and 65.6% vs 12.5% for Enterobacteriaceae, respectively. An evident contamination by Escherichia coli, Coagulase-positive Staphylococci and Pseudomonas spp. was detected in all the cheeses: the causes could be the improper hygiene of the artisanal production practices and the permanence of the cheeses on the refrigerated shelves. A careful attention to the respect of the good manufacturing practices is suggested to avoid the presence of initial high bacterial loads.

1 Introduction

Recently, in south Europe a number of small-scale dairies have been consolidating their market, especially those close to rearing facilities, and some of them use raw milk for cheese production. The production of raw milk dairy products has brought forward certain food safety concerns as unpasteurized milk is recognized to be responsible for foodborne diseases (De Buyser *et al.* 2001; Ryser 2001). With the dairy farms serving as possible reservoirs (Jayarao *et al.* 2006), artisanal dairy products obtained from raw milk in small processing facilities could be extremely risky for the presence of potential pathogenic bacteria (Schoder *et al.* 2003; Latorre *et al.* 2009). Environmental conditions such as temperature, pH, water activity, salt concentration and competing microflora are the main factors that influence the growth of pathogenic bacteria in raw milk and in dairy products. Raw milk is known to be characterized by a complex microbial community: its high water content and neutral pH allow the growth of several microorganisms, including those of technological relevance like lactic acid bacteria (LAB), but also several spoilage or potentially pathogenic species can affect the quality and the hygiene of dairy products with severe repercussions (Oliver *et al.* 2005; Mendonça Moraes *et al.* 2009). In addition, many studies where milk was voluntarily inoculated with pathogenic bacteria have shown that these microorganisms are able to survive during the manufacturing process and/or the ripening period (D'Amico *et al.* 2010).

Thus, the combination of high initial microbial loads (due to use of unpasteurized milk and to the application of improper traditional practices) and the lack of bacterial inactivation (due to the absence of ripening) can lead to an increase of microbial risk level of the products.

The aim of the study was the microbiological evaluation of different types of unripened, raw milk cheese, produced in an artisanal small dairy plant in Italy in order to measure the loads of spoilage microorganisms and detect the eventual presence of foodborne pathogens.

2 Materials and methods

2.1 Experimental design

Samples of unripened, raw milk cheeses were obtained from a small artisanal cheese dairy retail placed in Italy and analysed in order to evaluate the microbial contamination. A total of 6 different cheeses were considered, grouped as "fresh cheeses" (Crescenza, Robiola, Primo sale, Formaggella) and "pasta filata cheeses" (Mozzarella and Burrata). A total of 8 samples for each type of cheese were collected, during four sampling sessions within the period May-July 2012. The samples were taken from the refrigerated shelf after a mean exposition time of 2 days (except for Crescenza, that was sampled after 5 days of exposition), then transported to the laboratory at a standard refrigeration temperature (4°C) and immediately analysed.

2.2 Microbiological analyses

Total Viable Count (TVC) was determined according to the ISO 4833:2003 method. The number of *Enterobacteriaceae* was determined by the ISO 21528-2:2004 method. *Escherichia coli* were enumerated according to the ISO 16649-2:2001 method. Coagulase-positive

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Staphylococci were determined by the ISO 6888-1:1999 method. *Pseudomonas* spp. were enumerated on Pseudomonas selective agar with CFC supplement (Biogenetics, Ponte San Nicolò, I), incubated at 30°C for 48 hours. Yeasts and moulds were enumerated according with ISO 21527-1:2008 method. *Salmonella* spp. detection was performed by the methods ISO 6579:2002/Cor 1:2004. For microbial counts, 10 g of each sample were homogenized in 90 mL of a diluent solution (0.85% NaCl and 0.1% tryptone), and serial 10-fold dilutions were prepared. Detection of *L. monocytogenes* was performed according to AFNOR BRD 07/4–09/98 method.

At the same sampling times, pH was measured by a pH meter (Amel instruments, Milano, I): three independent measurements were performed on each sample and means were calculated.

2.3 Statistical analysis

The values obtained from microbial counts were grouped in the two categories "fresh cheeses" and "pasta filata cheeses" and compared by Student t test. The threshold for statistically significant differences was settled at $P<0.05$.

3 Results and discussion

The results obtained from the enumeration of Total Viable Counts (TVC), *Enterobacteriaceae, Escherichia coli*, Coagulase-positive Staphylococci, *Pseudomonas* spp., yeasts and moulds are reported in table 1: the TVC in fresh cheeses (Crescenza, Primo sale, Formaggella and Robiola) ranged from 4.8 and 8.2 Log CFU/g while in pasta filata cheeses (Mozzarella and Burrata) ranged between 4.1 and 8.6 Log CFU/g. Considering *Enterobacteriaceae,* values in fresh cheeses ranged from 4.1 and 7.6 Log CFU/g while in pasta filata cheeses ranged between 2.8 and 8.0 Log CFU/g. *Pseudomonas* spp. in fresh cheeses ranged from 3.7 and 7.7 Log CFU/g while in pasta filata cheeses ranged between 4.1 and 6.2 Log CFU/g. Considering coagulase positive Staphylococci in fresh cheeses the loads were between the not detectable load (<2 Log CFU/g) and 5.5 Log CFU/g; Log CFU/g, while in pasta filata cheeses these microorganisms ranged between <2 and 3.3 Log CFU/g. Yeasts in fresh cheeses ranged from 3.6 and 7.6 Log CFU/g while in pasta filata cheeses ranged between <2 and 5.1 Log CFU/g. No evident contamination of the products with moulds was detected, except for Formaggella, that was the only product in which moulds were above the detection limit, with a mean value exceeding 5 Log CFU/g. All the pathogens researched were not detected in any type of cheese analysed.

Based on the physical-chemical characteristics, the typologies of cheeses considered in our study represented an optimal substrate for bacterial growth. The richness in nutrients and water content were associated with pH values that were not sufficiently low to result in a microbial inhibition, both in "fresh cheeses" (mean values ranging between 5.25 – 5.55) and especially in "pasta filata cheeses" (mean values ranging between 6.44 – 6.46).

Considering the potential presence of pathogenic bacteria in cheese samples, we must consider that, according to Reg. (EC) n. 1441/2007, in cheeses made from raw milk or milk that has undergone a lower heat treatment than pasteurisation, *Salmonella* spp. should be absent in 25 g. Moreover, in ready-to-eat foods able to support the growth of *L. monocytogenes*, like

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raw milk fresh cheeses, this pathogen must be absent in 25 g. In our study, none of these two microorganisms was detected in any sample.

Data from microbial counts are reported in table 1 (ranges) and in figure 1 (frequency distribution of raw data). Generally, a poor hygienical quality of all the types of cheese was observed: 81.3% of fresh cheese samples (Robiola, Crescenza, Primo sale and Formaggella) and 50% of "pasta filata cheese" samples (Mozzarella and Burrata) were found to be characterized by TVC loads higher than 6 Log CFU/g, and in particular 50% of fresh cheese samples resulted to be higher than 7 Log CFU/g. Crescenza samples were characterized by the highest loads: all the samples, showed values higher than 6 Log CFU/g; similar situations were detected for the other "fresh cheese" typologies except for Primo sale. In "pasta filata cheese" samples, the melting phase had probably determined a reduction of microbial loads, resulting in significantly lower (P=0.002) TVC values than those obtained from "fresh cheeses". A similar trend was observed for *Enterobacteriaceae* counts, with frequent presence of high values (fig. 1), especially in Crescenza samples (87.5% of the values > 6 Log CFU/g); also for this parameter, significantly lower counts (P<0.001) were detected in "pasta filata cheese" samples. High loads of *Pseudomonas* spp. were detected in all the products analysed, but without significant differences among the cheeses; our data were comparable with those obtained by Lanciotti *et al.* (2004) in Crescenza cheeses obtained from homogenized milk after 6 days of storage where the counts reached 6.38 Log CFU/g: these microorganisms, especially *P. fluorescens*, are considered the main responsible for the production of bitter peptides in traditional Crescenza (Ottaviani and Disegna 1987).

The contamination by yeasts was widespread in "fresh cheeses", with 66.7% of the samples exceeding 5 Log CFU/g; also for this parameter, the highest mean value was observed in Crescenza samples, according to the studies of Fleet (1990) and Sarais *et al.* (1996), who detected in Crescenza and other soft cheeses a large number of these microorganisms (>6-7 Log CFU/g), responsible of cheese spoilage and unpleasant flavours. Limited, significantly lower counts (P<0.001) were detected in "pasta filata" cheeses (table 1). Our data were consistent with those obtained by Brooks *et al.* (2012) who found presence of yeasts in 58.3% of analysed raw milk cheeses even if they found a lower rate (17.1%) of those that exceeded the load of 5 Log CFU/g.

Table 1 Ranges of microbiological loads (expressed as Log CFU/g) and pH, obtained from analyses

		TVC	*Enterobacteriaceae*	*Pseudomonas* spp.	*E. coli*	CPS	Yeasts	Moulds	pH
Fresh cheeses	Crescenza	6.9-7.9	5.9-7.5	4.6-7.7	2.0-7.5	<2.0-5.4	4.6-7.6	<2.0	5.55
	Robiola	6.0-7.7	5.9-7.1	3.9-7.2	2.7-7.0	2.6-5.5	4.3-7.4	<2.0	5.25
	Primo sale	4.8-8.2	4.1-7.6	3.7-7.7	2.0-8.0	<2.0-3.0	3.6-6.3	<2.0	5.45
	Formaggella	5.8-7.5	5.6-6.9	6.4-7.0	<2.0-5.7	<2.0	4.0-6.8	4.8-5.7	5.50
Pasta filata cheeses	Mozzarella	5.0-7.1	3.2-6.0	4.5-5.6	2.0-6.0	<2.0-3.0	<2.0-4.7	<2.0	6.46
	Burrata	4.1-8.6	2.8-8.0	4.1-6.2	<2.0-7.9	2.0-3.3	<2.0-5.1	<2.0	6.44

The mean values are calculated using the countable values. TVC= total viable count; CPS= coagulase positive staphylococci

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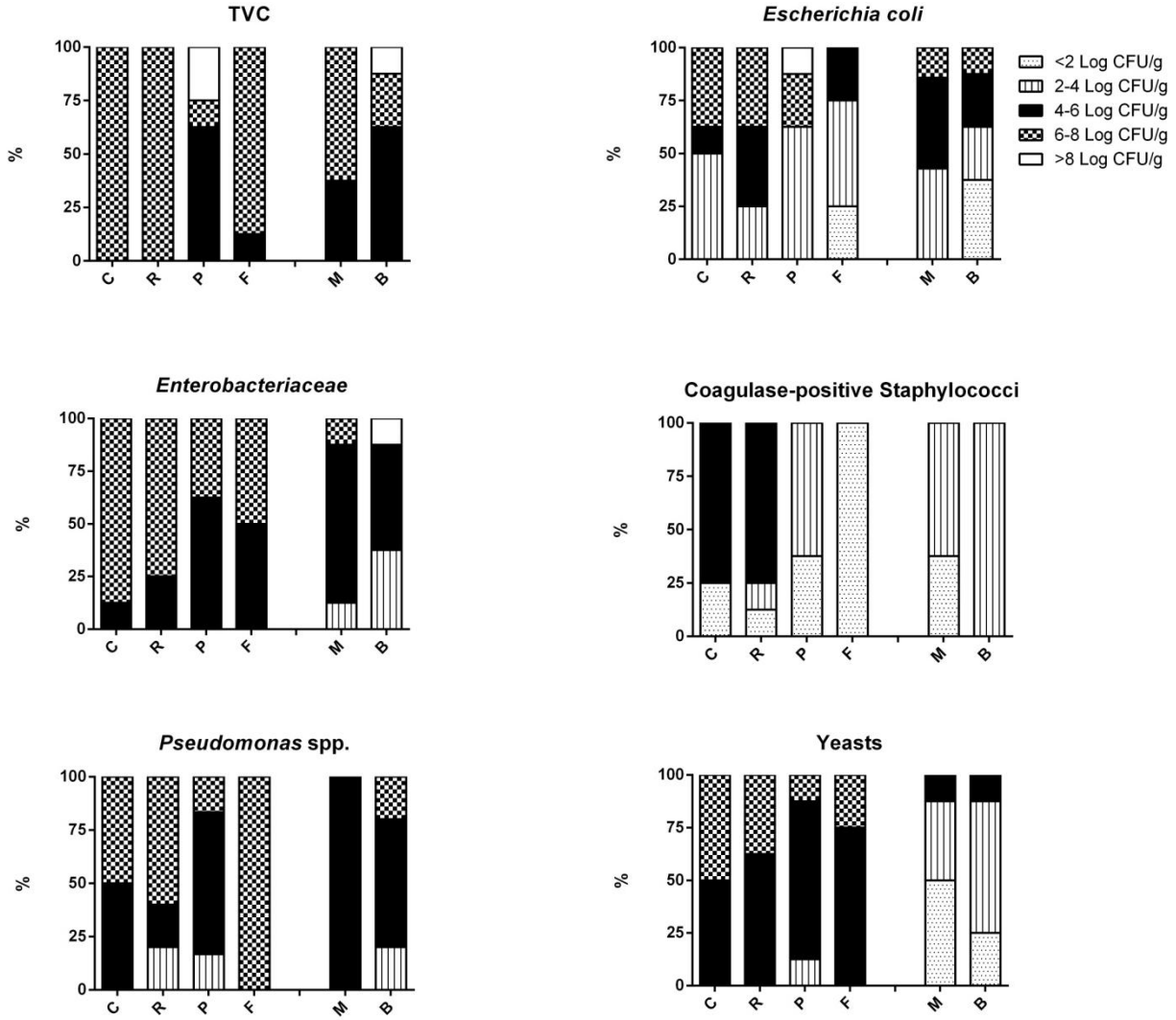
Figure 1: Frequency distribution of microbial counts in the samples.



C = Crescenza, R = Robiola, P = Primo Sale, F = Formaggella, M = Mozzarella, B = Burrata

Considering Coagulase-positive Staphylococci, 75% of the samples of Robiola and Crescenza exceeded the "m" limit indicated in Reg. (EC) n.1441/2007 for raw milk cheeses (4 Log CFU/g), while in all the other cheeses all the samples were below this limit, with constant low values (<2 Log CFU/g) in Formaggella samples. These results should be carefully considered, as *Staphylococcus aureus* counts between 3 and 5 Log CFU/g are recognized to be able to produce amounts of enterotoxins that could be of concern for consumers. More limited counts were detected in "pasta filata cheese" samples, according to the data obtained by Dambrosio *et al.* (2013) who found mean Staphylococci counts of 3 Log CFU/g in Burrata samples produced in Puglia (Italy). Raw milk cheeses are generally known as potentially contaminated by Staphylococci and this characteristic is also well recognized by the EU Regulation which settled a higher tolerance level if compared to fresh cheeses produced with

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milk that has undergone a lower heat treatment than pasteurization (m=2 Log CFU/g) and pasteurized milk (m=1 Log CFU/g).

The counts of *Escherichia coli* were very variable among the samples for all the cheese typologies, with the mean values ranging from 3.2 to 4.8 Log CFU/g. In previous studies, Coia *et al.* (2001) found that 98.6% of 735 raw-milk cheeses had *E. coli* counts below 4 Log CFU/g and Öksüz *et al.* (2004) detected that 82% of a total of 50 white pickled cheese manufactured from raw milk were characterized by loads below 3.8 Log CFU/g, while in the current work, the rate of samples with *E. coli* counts <4 Log CFU/g was evidently lower (42.3%). These parameters must be considered as potential hygienic criticisms, also if they cannot be linked to an actual risk for consumers. European legislation does not provide for limits for raw milk cheeses, even if a m limit of 2 Log CFU/g is settled by Reg. (EC) n. 1441/2007 for cheeses made from milk or whey that has undergone heat treatment.

The high loads detected could be due to an improper hygiene of the artisanal production practices and to the permanence of the cheeses on the refrigerated shelves (2 to 5 days depending on the typology), that increases the possibility of microbial replication. Improvements should be applied considering the usual transport and home storage procedures, that generally result in a further increase of microbial loads and in a limited residual shelf-life. A careful attention to the respect of the good manufacturing practices is suggested in order to avoid the presence of starting high bacterial loads. Moreover, a punctual control of the refrigerator temperature, coupled with a reduction of the shelf permanence, especially in the warm season, when temperature fluctuations are more likely, should be recommended.

4 Conclusion

The picture emerged from the microbiological monitoring of 6 different types of raw milk cheeses obtained from a small dairy plant, representative of a popular dairy retail typology in Italy, showed generally a very poor hygienical quality. An improvement of good manufacturing practices according with a more careful control of storage conditions is needed.

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