Microbiological and physico-chemical changes during manufacture of an Italian goat cheese made from raw milk

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Abstract

The aim of this work was to study the microbiological and physico-chemical changes throughout three cheesemaking replicates of Italian *Formaggelle di capra* cheese made from raw goat milk. Therefore, during the process, three samples of milk, curd and cheese at 3, 7, 11, 14, 21 and 30 days of ripening old cheese were taken from three cheesemaking replicates. The average of total mesophilic bacteria and *Enterobacteriaceae* count in raw milk was 5.27±0.57 and 3.8±1.02 Log cfu/mL, respectively. Lactic acid bacteria was the predominant bacterial group during the process, and they developed in different ways in each of the media used (M17 and MRS agar). Variability of microbial concentrations was observed between three cheesemaking replicates. A correlation between the presence of higher levels of *Enterobacteriaceae* in milk and the presence of other contaminants bacteria such as *Escherichia coli* β-glucuronidase-positive and coagulase-positive staphylococci was observed. In cheesemaking replicate n. 2, *E. coli* level was 5.07±0.03 Log cfu/mL and increased by about 1 log until the last week of ripening, when the level decreased to 5.69±0.2 Log cfu/mL. The milk used for the cheesemaking replicate n. 2 was found to be contaminated also by coagulase-positive staphylococci (3.18±0.06 Log cfu/mL), but the behaviour of this group appeared to be very variable. In this study a first step of process control and microbial groups study was performed and the cheesemaking process was registered in the website www.ars-alimentaria.it, the Italian site supported by the Italian Board of Health.

Introduction

In Europe the breeding of sheep and goats is a marginal agricultural activity (3.6% of the total value of livestock production) but, in some countries such as UK, Ireland, Spain, Romania and Italy, the production of sheep milk and goat milk has been growing since 1995 by about 10% (AND International, 2011). Numerous varieties of goat cheeses are produced in Italy. Maturation or ripening of cheese from goat milk is governed by many many factors including the wide variety of microrganisms used in culturing, and the forming and pressing techniques. The characterization of an artisanal cheese includes the study of the technological process of manufacture, the chemical and biochemical study of ripening process and the microbiological changes which take place.

In Northen Italy (Insubria area), a traditional goat cheese namely *Formaggella di capra* is produced with whole raw milk added with mesophilic and termophilic lactic acid bacteria as starter culture (www.ars-alimentaria.it). The manufacturing of this cheese with raw milk linked with the short ripening (about 30 days), could be considered as risk factors for the health of consumers. The aims of this research were i) to study the dynamics of different bacteria species (as a function of technological parameters) in a traditional Italian goat cheese during three cheesemaking replicate made at three different time from September to October 2012 and ii) to study the behaviour of pathogenic bacteria in naturally contaminated milk during the cheesemaking.

Materials and Methods

Cheese manufacture and sampling

To evaluate the microbiological variability between milk collected in three different days, a total of three cheesemaking replicates were performed from September to October 2012. The cheese, namely *Formaggella di capra,* was produced according to producer's specifications (Cosciani-Cunico *et al*., 2014) in the same farm located in Insubria region with whole raw goat milk. During the process, three samples (n=3) of raw milk, curd and cheese at 3, 7, 11, 14, 21 and 30 days of ripening were taken from three cheesemaking replicates (n=3). The samples were collected into sterile containers and brought refrigerated to the Institute for Experimental Veterinary Medicine of Lombardy and Emilia Romagna regions (*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna*; IZSLER) laboratory.

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Microbiological and physico-chemical analysis

For microbiological analysis on milk, counts were estimated by direct plate count. For curd and cheese, 25 g of samples were homogenized 1:3 (w:v) in sterile peptone water (PW) (CONDA, Madrid, Spain) for 3 min using a Stomacher blender. Decimal dilutions in sterile PW were prepared. Total mesophilic bacteria (TMB) were determined according to ISO 4833 method (ISO, 2003); the number of *Enterobacteriaceae* (ENT) was determined according to ISO 21528-2 method (ISO, 2004). For the enumeration of mesophilic Lactobacilli and Lactococci (MLB and MLC), the appropriate dilution were pour plating (1 mL) in de Man, Rogosa and Sharpe agar (MRS) and M17 agar (Microbiol Diagnostici, Uta, Italy) respectively and incubating at 37°C for 48-72 h. *Escherichia coli* β-glucuronidase-positive counts (Ec) were determined according to ISO 16649-2 method (ISO, 2001), and coagulase-positive Staphylococci (CPS) were determined following the ISO 6888-2 method (ISO, 1999). The pH value was measured on approximately 10 g of samples using a HI 223 Calibration checkTM Microprocessor pH meter (Hanna Instrument, Woonsocket, RI, USA) equipped with a Gel-Glass electrode (Hamilton, Switzerland). During the cheese manufacture the temperatures of milk, curd and cheese were monitored using electronic data loggers (cox tracer; Cox Technologies, Belmont NC, USA).

Data analysis

Mean of bacterial counts and pH values

were averaged from three replicates samples (n=3) for each sampling time for three cheesemaking replicate (N=3). Boxplot analysis was performed to evaluate the variability of each parameter during three cheesemaking replicates: the bottom of the box is at the first quartile, that corresponding to 25th percentile, and the top is at the third, that corresponding to 75th percentile. The whiskers are the lines that extend from the top and bottom of the box to the lowest and highest observations that are inside the region. The horizontal line within the box corresponds to the median value and the vertical lines are an index of data variability Outliers are points outside of the lower and upper limits and are plotted with open circles. The red circles inside the box represent the media values.

Results

Temperature distribution registered during three cheesemaking replicates was extremely narrow throughout the process: during the fermentation step the milk was heated up to 33-35°C for a time of 1-1.5 h and subsequent temperature ranged from 12 and 15°C during the cheese ripening (data not shown). The development of the different microbial groups throughout the cheesemaking and ripening are shown in Figure 1.

The average of total mesophilic bacteria and *Enterobacteriaceae* count in raw milk was 5.27±0.57 and 3.8±1.02 Log cfu/mL respectively and grew during the cheesemaking. Lactic acid bacteria were the predominant bacterial group during the manufacture of *Formaggella di capra* cheese. In the first days of the process a more rapid growth of mesophilic Lactococci respect to Lactobacilli group was observed: on M17 agar, Lactococci showed an increase of over 4 logarithms in three days of process. The lactic acid bacteria developed in different ways in each of the media used in this study also during the ripening process. Microbial concentration variability was observed between three cheesemaking replicates (Figure 1), however this variability did not affect the pH values that remains very similar in all the cheesemaking replicates (Table 1). The evolution of pH and the different microbial group counts throughout the process of *Formaggella di capra* cheese in each replicates are shown in Table 1.

In our study, *E. coli* and coagulase-positive Staphylococci were detected in raw milk, with a large variability of microbial concentrations (Figure 1). In Figure 2 the contamination levels of milk and the behaviour of *E. coli* and coagulase-positive Staphylococci during the cheese manufacture are indicated. Even if the milk collected and used for cheesemaking replicates n. 1 and n. 3, has not be found contaminated at countable levels, during the ripening a growth of *E. coli* and coagulase-positive Staphylococci was observed. The presence of these bacteria allows us to study their natural behaviour within the food matrix, at difference of the challenge test in which the pathogens were artificially inoculated in the food, and to know their behaviour as a function of the production process. In the milk used for the cheesemaking replicate n. 2, the *E. coli* level was 5.07±0.03 Log cfu/mL, and increased by about 1 log in the curd. During the ripening the *E. coli* concentration ranged from 6.14 to 6.89 Log cfu/mL until the last week of ripening, when the level decreased to 5.69±0.2 Log cfu/mL (Figure 2). The milk was found to be contaminated also by Coagulase-positive Staphylococci (3.18±0.06 Log cfu/mL), but the behaviour of this group appeared to be very changeable.

Discussion

Goat milk production is a dynamic and developing industry that is fundamental to the wellbeing of hundreds of millions of people worldwide and is an important part of the economy in many countries (Silanikove *et al*., 2010). The microbiological quality of goat milk was assessed and high levels of total bacteria and *Enterobacteriaceae* were enumerated (>5 Log cfu/mL). Similar counts have been observed in goat milk by Fontecha *et al.* (1990) and Buffa *et al.* (2001). Since the *Enterobacteriaceae* counts are widely used as a contamination index, an elevated number in milk indicates deficient handling during milking and collection. A high number of *Enterobacteriaceae* may not be considered as pathogenic, but their presence might indicate a potential contamination by more dangerous organisms.

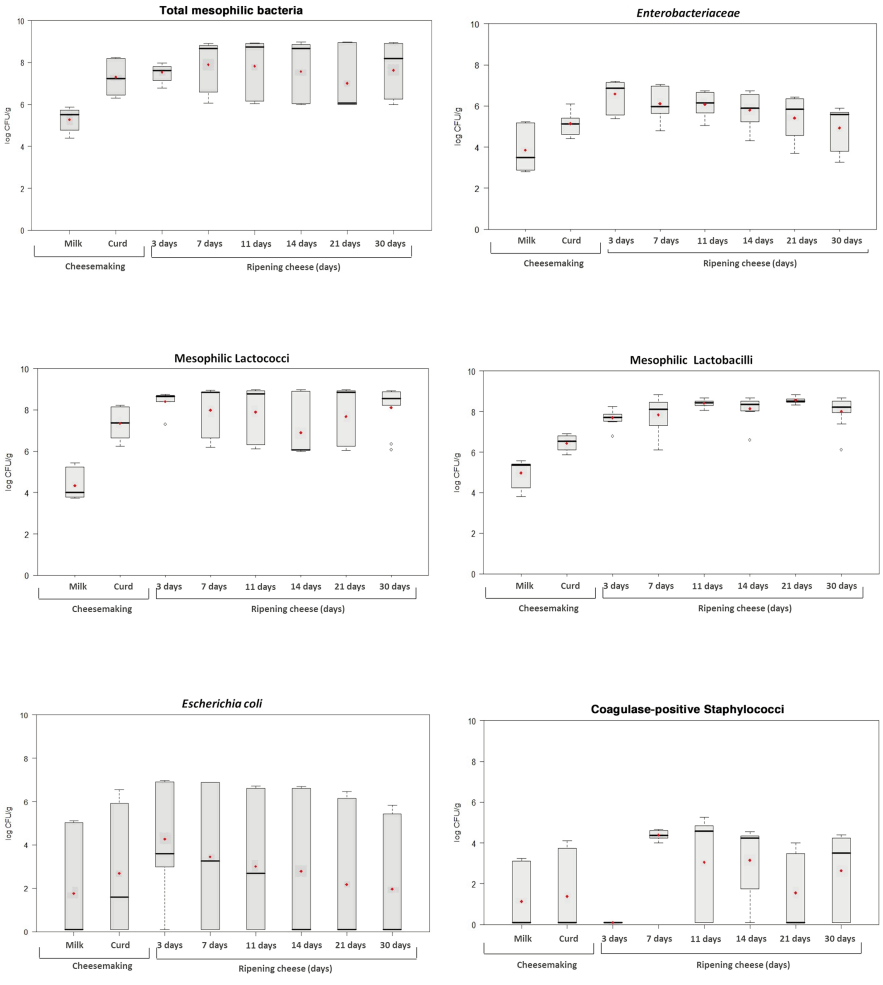

Figure 1. Changes in microbiological groups throughout the cheesemaking and ripening of *Formaggella di capra* cheese. Data represent the variability of nine samples [three replicates samples (n=3) for three cheesemaking replicate (n=3)]. Averages are reported as red circles inside the box.

Table 1. Average pH and microbiological counts in milk, curd and cheese during the cheese manufacture. Data represent the average values±standard deviation of nine samples [three replicates samples (n=3) for three cheesemaking replicates (n=3)].

Parameters		Cheesemaking		Ripening cheese (days)					
		Milk	Curd	3	7	11	14	21	30
Replicate 1	pH	6.56±0.04	6.51±0.01	5.33±0.01	5.24±0.04	5.20±0.03	4.99±0.04	5.07±0.10	5.02±0.04
	TMB	4.52±0.20	6.36±0.06	7.02±0.19	8.69±0.02	8.74±0.09	7.90±1.49	6.98±1.53	8.26±1.01
	ENT	2.86±0.05	5.13±0.25	5.48±0.09	5.10±0.29	5.20±0.23	4.49±0.15	4.01±0.43	3.50±0.25
	MLB	4.08±0.21	6.46±0.17	7.54±0.05	8.56±0.40	8.17±0.08	8.25±0.17	8.53±0.10	7.66±1.20
	MLC	3.84±0.12	6.48±0.18	8.58±0.15	8.86±0.03	8.80±0.07	7.98±1.50	7.99±1.51	6.88±1.05
Replicate 2	pH	6.6±0.02	5.59±0.03	5.34±0.01	5.28±0.02	5.13±0.01	5.05±0.02	5.07±0.02	5.07±0.05
	TMB	5.80±0.06	7.32±0.19	7.62±0.17	8.84±0.05	7.04±1.45	7.01±1.51	7.05±1.51	7.78±1.41
	ENT	5.20±0.03	5.76±0.46	7.16±0.03	6.99±0.05	6.69±0.03	6.61±0.17	6.4±0.04	5.76±0.14
	MLB	5.46±0.09	6.86±0.05	7.94±0.25	8.26±0.17	8.48±0.05	7.87±0.99	8.52±0.21	7.83±0.36
	MLC	5.31±0.09	7.39±0.13	8.15±0.67	8.89±0.05	7.17±1.40	6.03±0.04	7.07±1.45	8.79±0.19
Replicate 3	pH	6.62±0.01	6.45±0.01	5.12±0.01	5.20±0.02	5.14±0.01	5.10±0.01	5.04±0.01	5.08±0.05
	TMB	5.49±0.08	8.21±0.02	7.91±0.09	6.41±0.34	8.00±1.42	7.90±1.35	7.98±1.36	6.83±1.07
	ENT	3.50±0.04	4.54±0.10	6.82±0.04	5.92±0.09	6.05±0.15	5.87±0.05	5.84±0.13	5.54±0.22
	MLB	5.37±0.13	5.97±0.11	7.49±0.56	6.93±0.94	8.50±0.14	8.35±0.30	8.62±0.17	8.52±0.2
	MLC	3.84±0.12	8.17±0.04	8.6±0.17	6.49±0.31	8.02±1.41	7.06±1.44	7.99±1.37	8.69±0.27

TMB, total mesophilic bacteria; ENT, *Enterobacteriaceae*; MLB, mesophilic lactobacilli; MLC, mesophilic lactococci. Microbial values are expressed as Log cfu/g.

In fact, in our study we observed a correlation between the presences of higher levels of *Enterobacteriaceae* in milk collected during the cheesemaking replicate 2 and the presence of other contaminants bacteria such as *E. coli* and coagulase-positive Staphylococci.

The count of each bacterial group increased in curd and this was considered a normal process in the cheesemaking. This was due to the physical retention of bacteria in the coagulum and, in part, to the microbial growing during the coagulation and whey drainage (Fontán *et al*., 2001). The M17 agar is a selective medium for *Lactococcus,* a very active genus starting to ferment the lactose and to proliferate in the initial stages of the ripening process. The Lactococci decrease, observed in the later stages of ripening, can be related to their low ability to compete with other more acid-resistant microbial populations such as lactobacilli. In fact, Lactobacilli have a slower metabolism than Lactococci, and initially growth slower. However, since Lactobacilli are aciduric bacteria they are more tolerant to unfavorable conditions, concentration increase during the ripening process, till they are high to the end, when the pH values reach the optimum for growth (5.5). Lactobacilli include secondary flora which generally play a significant role during the ripening (Beresford *et al*., 2001).

In agreement with Cogan (2000) high densities of microorganisms were observed in cheese throughout ripening and they can play a significant role in the ripening process. In our study we observed the contamination of milk with *E. coli* and coagulase-positive Staphylococci at different level between the replicates. Leedom (2006) reported that occasionally a lactating animal's udder becomes infected with haemolytic streptococci of human origin, and this may result in milk-borne epidemics of scarlet fever or septic sore throat. The most prevalent trouble with a goat milk-borne bacterial contamination is the foodborne disease and the most prevalent cause is the presence of *Staphylococcus aureus* and its enterotoxin (Cremonesi *et al*., 2007). There are occasional reports or even outbreaks of alimentary toxicosis involving other pathogens, including *E. coli* (Espie *et al*., 2006).

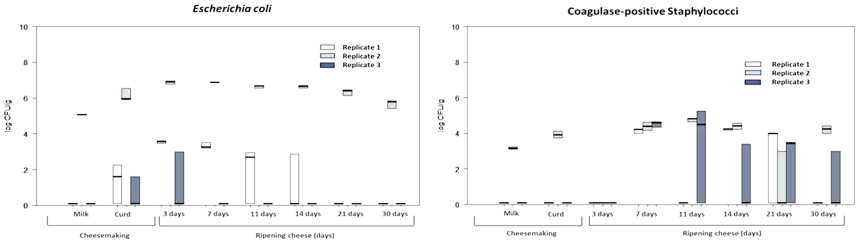

Figure 2. Changes in *E. coli* and coagulase-positive staphylococci groups in three cheesemakig replicates throughout the manufacture of *Formaggella di capra* cheese. Data represent the variability of three replicates samples.

Conclusions

The hygienic quality of milk herd should be of primary importance to any producer, especially where raw milk is used to make fresh cheese. Based on the potential pathogenic issues previously described, there is reason to implement more stringent food safety control systems in the dairy goat industry. An effective programme for prevention of zoonoses and pathogenic bacteria in food products can be assured if this includes regular monitoring of bacterial infections of herds by the appropriate national veterinarian authorities. Also, routinary testing of products by the dairies prior to release to the market is to be considered as necessary. In this work a first step of process control and microbial groups study was performed and the cheesemaking process was registered in website www.ars-alimentaria.it, the Italian site on quality and safety of Italian food supported by the Italian Board of Health.

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