SUMMARY

Study of hygienic-sanitary parameters and rheological characteristics of sheep, goat and buffalo milk, aimed at developing tools for improving the quality of traditional dairy products.

In the Lazio and Tuscany regions, sheep, goat and buffalo herds represent important economic sectors, in terms of number of heads, milk production and type of dairy products.
Consumer requests and European standards (EC Reg. 853/2004 - EC Reg. 1441/2007) increasingly urge dairies to produce cheeses with better quality and healthiness characteristics. In particular, it is necessary to monitor the production of raw milk cheeses, appreciated for their organoleptic characteristics and flavor.
The study therefore covered various aspects, concerning the quality of milk and cheeses:
1) Study on the determination of Milk Amyloid A (MAA) in milk for the diagnosis of subclinical mastitis in sheep and buffalo.
2) Development of a predictive model for determining the dairy attitude of milk using an automated method.
3) Microbial risk assessement of *E.coli* shiga-toxin producers (STEC) in raw sheep's milk cheese produced in farmhouse dairy.
4) Microbiological study on the presence of *E. coli* and coagulase positive staphylococci (SCP) in raw milk and in sheep, goat and buffalo cheeses, produced in farmhouse.
Milk Amyloid A: Milk Amyloid A protein for the early diagnosis of clinical and sub-clinical mastitis in sheep and buffaloes was studied. Milk Amyloid A (MAA) is a protein produced directly by the breast epithelium as a response to bacterial infection and is therefore an immediate and direct indicator of infection.
Udder halves samples were taken during lactation. Bacteriological examination was performed to identify the possible presence of mastidogenic bacteria and the number of somatic cells was determined using flow cell equipment. At the same time MAA was determined by ELISA test (Elisa "PHASE" TM Milk Amyloid A Mast (MAA) Assay kit). The statistical difference highlighted between the MAA values in healthy udder compared to udder with non-specific and specific subclinical mastitis allow us to consider the cutoff obtained of 27.36 µg / ml, as a first reliable value to differentiate the healthy udder from that affected by subclinical mastitis. The cut off obtained is higher than that present in the literature for bovine milk: from 1 µg / ml to 13.43µg / ml depending on the study carried out with variable specificity and sensitivity (Kovack et al., 2011; Sorensen et al., 2002). However, MAA would also be a good indicator in the sheep species to highlight subclinical mastitis.
Few studies have been carried out on MAA in buffaloes using the Elisa "PHASE" TM Milk Amyloid A Mast (MAA) Assay kit; in these studies the values shown for the MAA in buffalo milk were much lower than those of the bovine species. In the study carried out by us on quarter milk of 172 animals it would emerge instead that the ELISA test of the MAA would not be reliable in the buffalo species due to the too low value of MAA in the milk, compared to the bovine and ovine species, with frequent results below the threshold of detection of the kit, as confirmed by the high number of samples with values below 0 found during the study (48.05%).
Cheese-making ability: a study was performed to develop a predictive model to determine the milk coagulation properties using an automated method. The following lactodynamographic parameters were determined: rennet coagulation time (RCT), time to curd firmness (k20) and curd firmness at 30 minutes after enzyme addiction (a30). The model for determining the cheese-making ability for sheep, goat and buffalo milk was developed by comparing it with the direct method, performed by Formagraph. The bulk / individual milk samples were analyzed for the different quality parameters of the milk using automatic infrared equipment. For each sample the infrared spectra was stored and processed through specific statistical programs.

The results of the models obtained for the RCT and a30 parameter are shown below. For parameter k20 the values of R^2 do not allow practical use.

RCT	R^2	Bassa	Media	Alta
Sheep milk	0,495	<12,26	12,26 - 19,00	>19,00
Goat milk	0,582	< 6,00	6,00 - 10,30	>10,30
Buffalo milk	0,423	< 11,00	11,00 - 17,45	>17,45
a30	**R^2**	**Bassa**	**Media**	**Alta**
Sheep milk	0,159	<47,82	47,82 - 70,00	>70,00
Goat milk	0,460	<31,08	31,08 - 49,11	>49,11
Buffalo milk	0,234	<42,41	42,41 - 55,50	>55,50

It is necessary to continue the studies by implementing the data-set in order to improve the prediction in particular for the parameter a30.

Microbial risck assessment of STEC in raw sheep's milk cheeses: Shiga toxin-producing *E. coli* (STEC), widespread pathogens associated with severe foodborne disease, can contaminate milk during the milking process through faecal matter and survive, or grow, during cheese making if a pasteurization treatment has not been applied. Thus, a stochastic "farm-to-fork" model was developed to assess the risk of human infection by O157 STEC, one of the main pathogenic serotypes, associated with the consumption of a portion of raw sheep's milk cheese produced in a farmhouse dairy in Italy. The average risk of illness after the consumption of a portion of brief-, medium- and long-ripened cheese ranged between 1.61×10^{-4} and 4.03×10^{-4} for adults. Considering only a difference in serving size, the risk for children varied from 1.35×10^{-4} to 3.34×10^{-4}. Among the several intervention strategies simulated to mitigate the risk, administration of bacteriophages was, by far, the most effective measure with an average risk reduction of 34 times followed by use of probiotics and antimicrobials, which lowered the risk about 12 times. The sensitivity analysis showed that the probability that a shedder is present in the herd, the occurrence of the milk contamination with faeces and the within-herd prevalence of the pathogen were the parameters that most affected the risk. While further data is necessary to confirm the conclusion of this study, the model results might be able to assist producers and policymakers to manage the risk of STEC infection linked to such products.

E. coli and SCP in raw milk and cheeses: a monitoring was performed on the presence of coagulase positive staphylococcus (SCP) and *E.coli* in milk and raw milk cheeses in order to provide operational tools to suggest to dairies for the improvement of cheese microbiological quality. For this purpose, 108 samples of raw milk and 108 of related cheese on which *E.coli* and SCP were determined were taken from 15 dairies (6 sheep, 4 goats and 5 buffaloes).

The EC Reg. 1441/2007 does not foresee limits for *E.coli* in raw milk cheeses but only for cheeses obtained from heat-treated milk. 39%, 25.7% and 25% of the cheese samples respectively sheep, goats and buffaloes showed *E.coli* values higher than 1000 cfu/g.

As regards SCP, the minimum limit provided by Reg. CE 1441/2007 for raw milk cheeses is 10,000 cfu / g: 9.8% of sheep's cheese samples, 5.7% of goat's cheese samples and no samples for buffalo cheeses exceeded this limit. Only one sample of sheep's cheese exceeded the maximum limit for SCP of 100,000 cfu / g.

The values found in this study for *E.coli* and SCP do not show particular levels of contamination in the raw milk and in the cheeses.

Key words: STEC, cheese-making ability, Milk Amiloyd A.