

Microbiological quality of Pecorino Siciliano “primosale” cheese on retail sale in the street markets of Palermo, Italy

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SUMMARY

Pecorino Siciliano (PS) “primosale” is a traditional Sicilian fresh soft cheese made from sheep's milk. Short-ripening time and production from unpasteurized or raw milk can facilitate bacterial contamination of PS “primosale”. The microbiological quality of “primosale” on retail sale in the street markets of Palermo, Italy was studied by detecting the common food pathogens *Listeria monocytogenes* and *Staphylococcus aureus* and indicator microorganisms, such as *Escherichia coli*, *Enterobacteriaceae* and *Staphylococcaceae*. In our study, 4% and 44% of the samples, respectively, did not comply with the acceptability levels fixed by European regulations for *S. aureus* and *E. coli*. A high contamination of bacteria belonging to *Enterobacteriaceae* and *Staphylococcaceae* was found in 42% and 50% of the cheeses analyzed, respectively. Such results indicate poor husbandry and poor hygiene practices during milk collection or preservation or during cheese production processes and handling. In addition, the retail sale conditions may have played a role in cheese contamination since a correlation was found between poor microbiological quality and some selling parameters. This study emphasizes the need to improve production hygiene throughout the PS food chain in line with the traditional cheese-making procedures. Labelling of PS with clear information on whether the cheese was prepared from raw milk also requires improvement.

KEY WORDS: Pecorino Siciliano, Microbiological quality, Retail sale, Street markets

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INTRODUCTION

Traditional dairy productions of the European countries are certified by the European authorities through the assignment of the ‘Protected Designation of Origin’ (PDO) status which fixes the requisites of authenticity for products from defined geographical regions (European Commission, 1996). Pecorino Siciliano (PS) is a typical PDO hard cheese produced in Sicily, Italy, according to ancient manufacturing techniques. This PDO cheese is made from raw ewe's milk without the addition of starter cultures. The ripening is ex-

clusively carried out by the indigenous bacteria population present in milk and in the dairy environment. Raw milk microflora, in particular native lactic acid bacteria, is considered to increase the diversity of flavour in PDO cheeses, and is involved in the production of the typical characteristics of cheese contributing to flavour development (Steele and Ünlu, 1992; Martley and Crow, 1993). In Italy, current regulations require unpasteurized cheeses to have at least 60 days of ripening for safety reasons (Official Gazette, March 1997). The 60-day holding period offers an alternative to pasteurization based on the assumption that any bacterial pathogens present in fresh cheese would die within this period. PS cheese complies with regulations since it is normally ripened for about 4-6 months or longer. However, according to the local tradition and to the PDO status regulation, PS can be consumed after shorter ripening periods provided it is produced with thermized milk. In par-

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particular, PS "primosale", meaning the first salting of the cheese, is a short-ripening time variant of PS which is matured for 7-15 days only and thus belongs to the fresh soft cheese category (European Commission, 1996). Nowadays, dairies producing PS "primosale" mainly use thermized milk that is treated at a sub-pasteurization temperature (57-68°C for at least 15 s) designed to eliminate spoilage bacteria before being inoculated with autochthonous starter cultures. However, production at farmhouse level is still present in some areas of the island where PS "primosale" is produced from raw ewes' milk without the addition of any natural or selected starter culture. Cheeses belonging to the fresh soft cheese categories are an appropriate substrate to allow the preservation of vitality and multiplication of several bacteria due to high moisture, high pH and low concentration of NaCl. Reports regarding food-borne disease outbreaks involving dairy products and food surveillance indicate fresh cheese as a potential vehicle of food pathogens such as *Salmonella* spp., *Listeria monocytogenes* and enterotoxigenic *Staphylococcus aureus* (De Buyser *et al.*, 2001; MacDonald *et al.*, 2005; Dominguez *et al.*, 2009; Di Pinto *et al.*, 2010). Though a few studies are available on the autochthonous microflora of traditional PS cheese (Randazzo *et al.*, 2006; Vernile *et al.*, 2008), information on its microbiological safety is absent in the literature.

According to the literature, the quality of the starting cheese-making milk, as well as the hygienic status prevailing throughout manufacture, packaging and handling of cheese, may be assessed specifically by determining viable numbers of *Enterobacteriaceae* in cheese samples (Kongo *et al.*, 2008). The presence of small numbers of *Enterobacteriaceae* is common in raw milk, but high numbers are indicative of poor husbandry, poor hygiene practices during milk collection or bad preservation (Tornadijo *et al.*, 2001; Lafarge *et al.*, 2004). Though not included in EU and national legislation, the search of bacteria belonging to the *Staphylococcaceae* family as indicators of microbiological quality of milk and cheese has also been used in the literature (Bautista *et al.*, 1986; Kongo *et al.*, 2008).

The main objective of this study was to assess the microbiological quality of PS "primosale" cheese at retail sale in the street shops of the historical old town food markets in Palermo. The presence

of *E. coli*, coagulase-positive staphylococci and *L. monocytogenes* was investigated, as suggested by the legislation on food security, but also the numbers of bacteria belonging to the families *Enterobacteriaceae* and *Staphylococcaceae* were considered indicators of milk quality and hygiene of the production process. In the meantime an investigation was conducted aimed at assessing the conditions of sale recording several parameters that could influence microbial contamination and bacterial growth.

MATERIALS AND METHODS

Sampling

Fifty PS "primosale" cheese samples were bought in the periods February-March and June-September 2009 in four historical markets of the city of Palermo from retail sale premises along the market streets. Any retail premises could be included in the study provided they sold cheese. One hundred gram cheese samples were purchased from every retail sale premise included in the study. Samples were cut and packed by the sellers and immediately (less than 2 hours) transported to the laboratory at refrigeration temperature of 4°C. At purchase, information on sample and premise was obtained by observation and enquiry and recorded on a handheld device. The parameters that were taken into account were related to:

1. cheese preservation: proximity with other foods, correct refrigeration, packaging, protection from sunlight;
2. manipulation: presence of a clean washable cutting board, dedicated knife for every type of food, use of plastic wrap for covering the cheese surface;
3. food sellers wearing of: coat or apron, hair cap and gloves;
4. the structural features of the retail sale: presence of a bathroom and a washbasin, permanent or temporary food premise; and, finally,
5. the labelling of cheese indicating: dairy of origin, denomination of cheese, thermal treatment of milk.

Microbiological analyses

Cheese samples were analyzed to evaluate the presence of food pathogens like *S. aureus* coagulase-positive and *L. monocytogenes*. Bacteria be-

longing to the indicator families *Staphylococcaceae* and *Enterobacteriaceae*, including *E. coli* glucuronidase-positive, were also sought and numbered. For each of the 50 samples, 10 g of cheese were added to 90 ml of peptone water (Oxoid, Basingstoke, UK) and homogenized for one minute in a Stomacher mixer (Seward, Thetford, Norfolk, UK). The homogenate was subjected to decimal serial dilutions in peptone water and 0.5 ml of each dilution were plated in duplicate on MacConkey agar medium (Oxoid), MacConkey MUG (4-methyl- β -umbelliferil-dglucuronide) (Oxoid) and Mannitol Agar Salt medium (Chapman medium, Oxoid), to search, respectively, for *Enterobacteriaceae*, *E. coli*, and *Staphylococcaceae*, including *S. aureus*. After incubation for 24-48 hours at 37°C, typical colonies were counted. Colonies grown on MacConkey MUG were tested for glucuronidase with exposure to an UV transilluminator at 366 nm. Fluorescent colonies were counted and confirmed as *E. coli* by biochemical tests with the API 20E system (BioMérieux, Marcy l'Étoile, France). Colonies grown on Chapman medium and showing yellow halo of lecithinase precipitation, typical of *S. aureus*, were tested for coagulase enzyme. According to EN ISO 11290-1 procedure (Anonymous, 2005), to evaluate the presence of *L. monocytogenes*, separate 25 g aliquots of each cheese sample were placed in sterile bags containing Listeria Enrichment Broth Base (Oxoid), homogenized in a Stomacher mixer for 1 minute, and incubated at 30°C for 24 hours. A second-step enrichment was performed diluting 0.1 ml of the first-step enrichment broth culture in 35 ml of Listeria Fraser Broth Base (Oxoid). Following incubation for 48 hours at 30°C, esculine-positive broths were plated on Oxford Listeria Selective Agar Base (Oxoid) and incubated at 37°C for 48 hours. Colonies exhibiting the typical central excavation and black halo were grown in pure culture and confirmed with the API-*Listeria* biochemical test (BioMérieux).

Statistical analysis

Statistical analysis of the data was undertaken using the Epi Info ver. 3.5.1 (Centers for Disease Control and Prevention, Atlanta, GA, USA) and Microsoft Excel software. Results were tested applying parametric tests for inequality of population means (ANOVA) in the case of distribution of

a numeric variable with respect to two groups and the chi-square test to assess the distribution of a non-numeric independent variable between two groups. A probability value of less than 5% was deemed to be significant.

RESULTS

Laboratory analysis

The results of the analysis of the 50 PS “primosale” cheese samples for compliance with the microbiological criteria prescribed in the European Regulation 2005/2073/EC are summarized in Table 1. Microbiological acceptability of samples was based on the detection of coagulase-positive staphylococci, *L. monocytogenes* and *E. coli* following the microbiological limits indicated for raw or thermized milk cheeses (European Commission, 2005). Overall, 27 of the 50 samples tested (54%) appeared not to be satisfactory according to the European Regulation 2005/2073/EC. This was mainly due to high levels of glucuronidase-positive *E. coli*. In 44% of the samples *E. coli* exceeded 10^3 CFU g⁻¹ and in 8% of the samples its presence was detected in quantities between 10^2 and 10^3 CFU g⁻¹. Coagulase-positive *S. aureus* exceeding EC regulation limits could be found only in three (6%) of the samples analyzed. In two of these samples *S. aureus* contamination levels were above 10^5 CFU g⁻¹ and unsatisfactory microbiological quality was also confirmed by high concentrations of *E. coli*. Finally, *L. monocytogenes* was not detected in any of the cheeses tested. Regardless of their microbiological acceptability based on the detection of common food pathogens and *E. coli*, the hygienic quality of the cheeses was also determined measuring the levels of contamination by *Enterobacteriaceae* and *Staphylococcaceae*. Based on the results obtained the samples were arbitrarily divided into 3 classes of hygienic quality (good, poor, bad) for each of the two families of indicators (Table 1). Bacteria belonging to the *Enterobacteriaceae* family were found in all 50 samples analysed in quantities ranging between $5.90 \cdot 10^2$ and $7.50 \cdot 10^9$ CFU g⁻¹. Forty-two percent of the samples displayed high contamination, exceeding 10^7 CFU g⁻¹. The presence of *Staphylococcaceae* was documented in all the cheeses analyzed except one. Quantities were al-

TABLE 1 - Classification of cheese samples according to the criteria of acceptability described by the European Recommendation 2005/2073/EC on microbiological quality criteria for raw and thermized milk cheeses and based on the presence of indicators of poor hygienic practice.

Microorganism	Microbiological quality		
	Satisfactory	Borderline	Unsatisfactory
<i>E. coli</i>	<10 ² CFU·g ⁻¹ 24 (48%)	10 ² ≤ x <10 ³ CFU·g ⁻¹ 4 (8%) ^a	≥10 ³ CFU·g ⁻¹ 22 (44%) ^b
<i>L. monocytogenes</i>	Not detected 50 (100%)	Detected-<10 ² CFU g ⁻¹ 0	≥10 ² CFU g ⁻¹ 0
Coagulase-positive staphylococci in cheeses made from raw milk	<10 ⁴ CFU·g ⁻¹ 48 (96%)	10 ⁴ ≤ x <10 ⁵ CFU·g ⁻¹ 0	>10 ⁵ CFU·g ⁻¹ 2 (4%) ^{a,b}
Heat treated milk	<10 ² CFU·g ⁻¹ 47 (94%)	10 ² ≤ x <10 ³ CFU·g ⁻¹ 1 (2%)	>10 ³ CFU·g ⁻¹ 2 (4%) ^{a,b}
	Hygienic quality		
Indicator microorganism	Good (≤10 ⁵ CFU·g ⁻¹)	Poor (10 ⁵ ≤ x <10 ⁷ CFU·g ⁻¹)	Bad (≥10 ⁷ CFU·g ⁻¹)
<i>Enterobacteriaceae</i>	9 (18%)	20 (40%)	21 (42%)
<i>Staphylococcaceae</i>	3 (6%)	22 (44%)	25 (50%)

^aOne sample had borderline levels of *E. coli* and unsatisfactory levels of coagulase-positive staphylococci. ^bOne sample had unsatisfactory levels of both *E. coli* and coagulase-positive staphylococci.

ways above 6 10³ CFU g⁻¹ and in 50% of the cases the bacterial load exceeded the value of 10⁷ bacteria per gram.

Selling conditions

Selling conditions of the cheese were analyzed based on the data collected at the retail sale (Table 2). Attention to cheese preservation was not optimal since 18% of the cheeses purchased were not stored or displayed at correct refrigeration temperature and 26% were not protected from sunlight. In 54% of the premises cheeses were stored in proximity with other foodstuffs and the different categories of products were not divided by compartments. According to our survey 88% of the sellers cut cheeses on clean washable boards and the use of plastic wrap to protect cheeses was quite generalized (74%) but only in one case were dedicated knives used for every type of dairy product (i.e. fresh, fresh-hard and hard cheese). The majority (84%) of the food

sellers wore clean coats, but they rarely had a cap to collect hair (12%) and gloves for food handling (2%). Shops had generally (84%) a permanent structure but in 75% of them a sink was not present and only 57.1% of the premises had a bathroom. Information on the cheese was hardly available, indeed a label appeared only on the products pre-packed by the dairy of origin (12%) and the exact denomination of the cheese as PS "primosale" was eventually indicated on a price sign (36%). The thermal treatment of the milk of origin was never indicated.

Premises and cheese details in relation to microbiological quality

Statistical analysis demonstrated significant correlation (P=0.0164) between plastic wrapping and poor microbiological quality of cheese related to *Staphylococcaceae* levels. On the contrary, a better quality of samples was observed where a hair cap was used (P=0.0109).

TABLE 2 - Sale conditions of Pecorino Siciliano "primosale" cheese recorded at the time of sampling.

Parameters related to	%
1) cheese preservation	
Protection from sunlight	74
Pre-packed	12
Refrigeration (4-8 °C)	82
Proximity with other foods	46
2) manipulation	
Use of a dedicated knife for cheeses and other foods for all cheeses	96
for cheeses of the same category	12
Clean washable cutting board	82
Plastic wrap	2
	88
	74
3) food sellers wearing	
Coat or apron	84
Gloves	2
Hair cap	12
4) structural features of the retail sale	
Washbasin	24
Bathroom	56
Permanent sale structure	84
5) labelling of cheese specifying	
Dairy of origin	12
Denomination	36
Thermal treatment of milk	0

DISCUSSION

This study demonstrated that more than half (54%) of PS "primosale" cheeses supposedly made from thermized milk on retail sale in the street markets of Palermo were of unsatisfactory or borderline microbiological quality according to criteria in EC Regulation 2005/2073/EC (European Commission, 2005). Though *L. monocytogenes* was not detected in any cheese sample, *S. aureus* was occasionally found above the limits for satisfactory samples. In our study, two samples had *S. aureus* levels exceeding the 10^5 CFU g⁻¹ limit which is considered to introduce a significant risk of production of enterotoxins that will remain in the cheese regardless of the recoverable level of this organism in the following ripening stages. Although Regulation 2005/2073/EC defines levels exceeding 10^5 CFU g⁻¹ as unsatisfactory in raw milk cheeses, in unripened soft cheeses made from milk that has undergone pas-

teurisation levels exceeding 10^2 CFU g⁻¹ also demand improvements in production hygiene. In cheeses made from thermized milk that has undergone a lower heat treatment than pasteurisation contamination levels above 10^3 CFU g⁻¹ should be viewed with suspicion, and the same limit should be applied to ripened cheeses made from pasteurised milk sampled on retail sale due to the likely reduction in staphylococcal levels during their shelf life (Little *et al.*, 2008). In a recent study on Monte Veronese cheese, an Italian PDO semi-hard cheese made with raw milk, *S. aureus* numbers in cheese were higher than the 10^3 CFU g⁻¹ limit in 78% of samples (Poli *et al.*, 2007). *S. aureus* was the most frequent pathogen associated with cheeses from raw or unspecified milk in food-borne disease outbreaks reported in France in 1992-1997 (De Buyser *et al.*, 2001). None of these studies could clearly demonstrate the origin of contamination which could derive from raw milk since *S. aureus* is the commonest cause of mastitis in dairy animals but also from post-processing contamination through unhygienic handling of products (Little and De Louvois, 1999).

However, almost all the samples that were not satisfactory did not comply with the EC regulations because of high levels of *E. coli* exceeding 10^3 CFU g⁻¹. Accordingly, 42% of the samples tested could be considered of bad hygienic quality referred to the levels of *Enterobacteriaceae*. *Enterobacteriaceae* are ubiquitous inhabitants of the gut of human beings and other warm-blooded animals. Members of this group include the generally harmless and commensal *E. coli* which owing to its occurrence in feces, ready culturability, and typically non-pathogenic character, has been adopted as a universal indicator of fecal contamination. The high viable counts of *Enterobacteriaceae* and *E. coli* exhibited by many of the cheeses we sampled may be ascribed to the use of raw milk and linked to poor husbandry of producing animals, poor hygiene practices during milk collection or bad preservation, possibly connected with lack of milk cooling. Otherwise, post-thermal treatment contamination must be hypothesized with organisms originally derived from raw milk or from manufacturing environments.

Since this study was not designed to collect any information on the conditions of milk collection,

milk preservation and cheese manufacture it is not possible to attribute with certainty the source of contamination to production or sale stage. However, the sale conditions may have influenced the multiplication of bacteria in contaminated cheese. In fact, in the traditional markets of Palermo it was quite common to find "primosale" cheese stored or displayed at room temperature and/or exposed to direct sunlight (Table 2). Analysis of the levels of *Enterobacteriaceae* showed that the samples not refrigerated had higher, though not statistically significant, contamination with respect to those correctly refrigerated. This mainly happened in the summer season (data not shown), which can be easily due to increased bacterial multiplication at higher temperature.

Similar to *Enterobacteriaceae*, high levels of *Staphylococcaceae* have also been attributed to microbial contamination linked to inappropriate manipulation and have been used as indicators of microbiological quality and hence of hygiene practices (Bautista *et al.*, 1986; Kongo *et al.*, 2008). In our study, the concentration of *Staphylococcaceae* was significantly higher in samples obtained from cheeses wrapped in plastic film compared to those from cheeses that were still in their original package or not wrapped at all. Apparently the use of plastic wrap to protect cheese surface from direct contact with the environment does not protect the cheese from bacterial contamination but, on the contrary, seems to increase its exposure. In fact, plastic foil films were not substituted at every cut but just reused. Repeated removing and repositioning could have promoted the transfer of bacteria from the cutting floor and the hands to the cheese. Samples collected from premises where food handlers did not wear head covering were significantly less protected from contamination by *Staphylococcaceae*, habitual residents of the human skin and scalp (Giraffa *et al.*, 1997). The use of a hair cap or net, besides direct protective effect, could also be an indicator of the attention of food handlers to hygiene practice. Also using the same knife for cutting different food categories was related (though not statistically) to poor microbiological quality calculated on the level of *Staphylococcaceae*. When a reduced number of cutting instruments are available the frequency of their use increases, exposing them to a higher risk of environmental con-

tamination. In UK, a wide survey on microbiological quality of retail cheeses demonstrated that a significantly higher number of samples that were cut to order was of unsatisfactory quality compared to those that were pre-packed (Little *et al.*, 2008).

It is now a legal requirement in the EU that all cheeses made with raw milk must be clearly labelled as such at all retail outlets since vulnerable groups, such as pregnant women and the immunocompromised, are advised not to consume soft mould ripened cheeses (European Commission, 2004). However, only a minority (12%) of the PS "primosale" cheeses sampled in Palermo had a label and it generally did not report any information enabling the purchaser to determine whether the cheese was heat-treated or prepared from raw milk.

This study demonstrated the overall poor microbial quality of PS "primosale" Italian soft cheese on retail sale in the street markets of Palermo. Though the frequency of isolation of *S. aureus* was low, it indicated that the risk related to this pathogen in PS "primosale" cheese must not be ignored and the high rate of unsatisfactory samples of this PDO cheese suggests the need for improving hygiene of production by preventing milk and cheese contamination in line with the traditional cheese-making procedures. The tendency to non-observance of good hygiene and food safety practices which is generalized in the premises of the traditional markets of Palermo may increase the risk of pathogens transmission raising the contamination levels of the products on sale. These results emphasize the need to apply and maintain good hygiene practices throughout the food chain when raw milk fresh or soft cheese with short ripening time is involved. More investigations on the risks related to the possible microbial hazards in fresh soft dairy products are required to check the healthiness of such products and improve controls by the local authorities. Labelling of PS cheeses with clear information on whether the cheese was prepared from raw milk also requires improvement.

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REFERENCES

- ANONIMOUS (2005). Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes*, Part 1: Detection method Amendment 1: Modification of the isolation media and the haemolysis test, and inclusion of precision data, Vol. EN ISO 11290-1: 1996/A1: 2004. European Committee for Standardization - Brussels.
- BAUTISTA L., BERMEJO M.P., NUNEZ M. (1986). Seasonal variation and characterization of *Micrococcaceae* present in ewes' raw milk. *J Dairy Res.* **53**, 1-5.
- DE BUYSER M.L., DUFOUR B., MAIRE M., LAFARGE V. (2001). Implication of milk and milk products in food-borne diseases in France and in different industrialised countries. *Int J Food Microbiol.* **67**, 1-17.
- DI PINTO A., NOVELLO L., MONTEMURRO F., BONERBA E., TANTILLO G. (2010). Occurrence of *Listeria monocytogenes* in ready-to-eat foods from supermarkets in Southern Italy. *New Microbiol.* **33**, 249-252.
- DOMINGUEZ M., JOURDAN-DA SILVA N., VAILLANT V., PIIHER N., KERMIN C., WEILL F.X., DELMAS G., KEROUANTON A., BRISABOIS A., DE VALK H. (2009). Outbreak of *Salmonella enterica* serotype Montevideo infections in France linked to consumption of cheese made from raw milk. *Foodborne Pathog Dis.* **6**, 121-128.
- EUROPEAN COMMISSION (1996). Regulation (EC) No 1107/1996. Registration of geographical indications and designations of origin under the procedure laid down in Article 17 of Council Regulation (EEC) No 2081/1992, Vol. L 148, 1-10. Off J Eur Comm.
- EUROPEAN COMMISSION (2004). Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin, Vol. L 139, 55-205. Off J Eur Union.
- EUROPEAN COMMISSION (2005). Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. In "Off J Eur Union", Vol. L 338, 1-29.
- GIRAFFA G., CARMINATI D., NEVIANI E. (1997). Enterococci isolated from dairy products: a review of risks and potential technological use. *J Food Prot.* **60**, 732-737.
- KONGO J.M., GOMES A.P., MALCATA F.X. (2008). Monitoring and identification of bacteria associated with safety concerns in the manufacture of Sao Jorge, a Portuguese traditional cheese from raw cow's milk. *J Food Prot.* **71**, 986-992.
- LAFARGE V., OGIER J.C., GIRARD V., MALADEN V., LEVEAU J.Y., GRUSS A., DELACROIX-BUCHET A. (2004). Raw cow milk bacterial population shifts attributable to refrigeration. *Appl Environ Microbiol.* **70**, 5644-5650.
- LITTLE C.L., DE LOUVOIS J. (1999). Health risks associated with unpasteurized goats' and ewes' milk on retail sale in England and Wales. A PHLS Dairy Products Working Group Study. *Epidemiol Infect.* **122**, 403-408.
- LITTLE C.L., RHOADES J.R., SAGOO S.K., HARRIS J., GREENWOOD M., MITHANI V., GRANT K., MCLAUCHLIN J. (2008). Microbiological quality of retail cheeses made from raw, thermized or pasteurized milk in the UK. *Food Microbiology.* **25**, 304-312.
- MACDONALD P.D., WHITWAM R.E., BOGGS J.D., MACCORMACK J.N., ANDERSON K.L., REARDON J.W., SAAH J.R., GRAVES L.M., HUNTER S.B., SOBEL J. (2005). Outbreak of listeriosis among Mexican immigrants as a result of consumption of illicitly produced Mexican-style cheese. *Clin Infect Dis.* **40**, 677-682.
- MARTLEY F.G., CROW V.L. (1993). Interaction between non-starter microorganisms during cheese manufacture and ripening. *Int Dairy J.* **3**, 461-483.
- OFFICIAL GAZETTE (March 1997). Regolamento recante attuazione delle Direttive 92/46, 92/47 CEE in materia di produzione ed immissione sul mercato di latte e prodotti a base di latte, Vol. Gazzetta Ufficiale della Repubblica Italiana del 12/03/1997. D.P.R. 14 gennaio 1997 n. 54.
- POLI A., GUGLIELMINI E., SEMBENI S., SPIAZZI M., DELLAGLIO F., ROSSI F., TORRIANI S. (2007). Detection of *Staphylococcus aureus* and enterotoxin genotype diversity in Monte Veronese, a Protected Designation of Origin Italian cheese. *Lett Appl Microbiol.* **45**, 529-534.
- RANDAZZO C.L., VAUGHAN E.E., CAGGIA C. (2006). Artisanal and experimental Pecorino Siciliano cheese: microbial dynamics during manufacture assessed by culturing and PCR-DGGE analyses. *Int J Food Microbiol.* **109**, 1-8.
- STEELE J.L., ÜNLÜ G. (1992). Impact of lactic acid bacteria on cheese flavour development. *Food Technol.* **1992**, 128-135.
- TORNADIJO M.E., GARCIA M.C., FRESNO J.M., CARBALLO J. (2001). Study of *Enterobacteriaceae* during the manufacture and ripening of San Simón cheese. *Food Microbiology.* **18**, 499-509.
- VERNILE A., GIAMMANCO G., SPANO G., BERESFORD T.P., FOX P.F., MASSA S. (2008). Genotypic characterization of lactic acid bacteria isolated from traditional Pecorino Siciliano cheese. *Dairy Sci. Technol.* **88**, 619-629.

