

Microbial characteristics of *Pecorino* processed cheese spreads

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Abstract — This study was designed to compare the microbiological quality of *Pecorino* processed cheese spreads manufactured in a mechanized factory and in small artisanal dairies. The 118 factory-made spreads analyzed were characterized by generally low microbial counts (lower than 10^3 cfu·g⁻¹), while the 98 artisanal products showed a high variability in microbiological quality, with total aerobic mesophilic counts often exceeding 10^5 cfu·g⁻¹. From a qualitative point of view, coliforms, *S. aureus* and Gram negative psychrotrophs were isolated only in artisanal samples. Spore-forming bacteria, yeasts and moulds were isolated from both types of products but they were observed in higher percentages and with higher values in artisanal ones. The results of our study seem to confirm that the application of a well-standardized and mechanized procedure, which minimizes the manual handling of the product, combined with proper hygiene practices during manufacturing helps to reduce the risk of developing food safety and/or spoilage problems in this kind of dairy products. © Inra/Elsevier, Paris.

***Pecorino* processed cheese spreads / microbial characteristic / organoleptic characteristic**

Résumé — **Caractéristiques microbiologiques de crème de *Pecorino*.** On a effectué une étude pour évaluer la qualité microbiologique de 216 échantillons de crème de *Pecorino*. Parmi ces échantillons, 118 ont été produits dans une structure unique mécanisée et 98 dans différentes fromageries artisanales. Les résultats de notre enquête ont confirmé que la standardisation et la mécanisation de toutes les opérations de transformation dans une structure unique mécanisée est une condition indispensable pour obtenir des produits de bonne qualité. En effet l'absence de contact manuel avec le produit pendant les différentes phases de transformation a permis d'obtenir des produits de bonne qualité microbiologique qui ont gardé leurs caractéristiques initiales et qui n'ont pas subi de modifications physico-structurales jusqu'à trois mois. © Inra/Elsevier, Paris.

crème de *Pecorino* / caractéristique organoleptique / caractéristique microbiologique

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1. INTRODUCTION

Sardinia is one of the major ewe's-milk producing regions in Italy. This milk is usually processed in small dairies that produce a wide variety of traditional ewe's-milk cheeses including fresh, semi-hard and hard *Pecorino* cheese and soft and salty ripened Ricotta.

In recent years there has been an increasing demand by the consumer for cheeses of soft and creamy texture, of good flavour and free of contaminating microorganisms [17].

As a consequence, the surplus of *Pecorino* cheeses of good quality is being converted into processed cheese spreads, appreciated for their creamy texture and good flavour/taste. The resulting products are creamy *Pecorino* cheeses which undergo no further ripening.

These novel products are usually processed in small artisanal dairies that do not have sufficient technical and scientific knowledge to obtain processed cheese spreads of constantly good quality. Their variability in organoleptic and microbiological profile contributes to reducing the possibility of large-scale production and international trade.

For this reason, some producers decided to convert the surplus of their ewe's-milk dairy products using standardized technological parameters and a mechanized procedure in a single factory in order to obtain hygienically safe products of constantly high quality.

This study was designed to compare the microbiological quality of *Pecorino* processed cheese spreads manufactured in a mechanized factory and in small artisanal dairies.

2. MATERIALS AND METHODS

2.1. Cheese making

Pecorino processed cheese spreads are prepared by comminuting and mixing, with the aid of heat, one or more variety of *Pecorino* cheeses,

with ewe's-milk Ricotta, water and polyphosphates as emulsifying agents. The pH values and moisture content of the finished product usually range between 5.2–5.6 and 50–60 %, respectively.

All dairy products used as raw materials are made from pasteurized ewe's milk and the mixture for each batch process is prepared using fresh and hard *Pecorino* cheeses and soft and salty ripened good quality Ricotta. The mixture is normally obtained from 50 % fresh and 50 % ripened products. On reception, the raw materials are graded, selected and thoroughly cleaned and trimmed, if necessary. Then they are passed through a grinder and transferred to the cooker, when water and emulsifier (sodium polyphosphates) are added. The amount of water used depends upon the texture desired, while the emulsifier is added in the amount of 1–1.5 % of the weight of cheese used.

After this point, different manufacturing procedures are performed in artisanal dairies and in the mechanized factory.

In artisanal dairies the mixture is heated in cauldron at ca. 80 °C, as evaluated empirically by the cheese maker, with slow manual mixing until a creamy texture is achieved; after cooling at room-temperature for 1 h, plastic boxes are manually filled and sealed, and then stored at a constant temperature of about 5–6 °C.

In the mechanized factory the mixture is heated in closed stainless steel cheese blender by direct steam injection to 90–92 °C for 8 to 10 min, with constant agitation; after rapid cooling at 75 °C, the hot cheese spread is passed directly from the cooker to the filling machine where plastic boxes are automatically filled and sealed; the boxes are then stored at a constant temperature of 5–6 °C.

We analyzed a total of 216 batches of *Pecorino* cheese spreads: 118 manufactured in one mechanized factory and 98 made in different artisanal dairies.

For each batch two samples were taken, brought to the laboratory under refrigeration and kept at 5 °C until testing. On each sample organoleptic and microbiological characteristics were evaluated immediately and after three months of storage at 5 °C.

2.2. Sensory evaluation

Organoleptic analyses were performed following the recommendations of IDF standards [10], by a panel composed of 10 tasters. The

attributes judged were appearance (fresh-moist), texture (creamy and smooth), colour (white) and flavour/taste (delicate and sweetish), with scoring on the following scale: 1, very poor; 2, poor; 3, fair; 4, good; 5, very good.

2.3. Microbiological analyses

For microbiological analyses, 25 g of sample were homogenized with 225 mL of a sterile solution of 2 % sodium citrate in a Stomacher 400 Lab-Blender (P.B.I.) for 2 min, and decimal dilutions were made.

Total aerobic mesophilic counts were carried out with Plate Count Agar (PCA) incubated at 30 °C for 24–48 h. Coliforms and *E. coli* were estimated on Violet Red Bile Agar (VRBA) incubated for 24 and 36 h at 37 °C and 44 °C, respectively. *Staphylococcus aureus* was enumerated on Baird-Parker Agar (BPA) incubated at 37 °C for 24 to 48 h. Gram-negative psychrotrophs were determined by surface plating on PCA incubated at 7 °C for 10 d. Yeasts and moulds were evaluated using Potato Dextrose Agar (PDA) with chloramphenicol incubated at 25 °C for 5 to 20 d.

All samples were also examined for the presence of aerobic and anaerobic spore-forming bacteria. After heat-treatment at 80 °C for 15 min, numbers of *Bacillus* and *Clostridium* were determined in Tryptic Soy Agar (TSA) incubated at 37 °C for 24–48 h and in Reinforced *Clostridium* Medium (RCM) incubated in anaerobiosis at 37 °C for 48–72 h, respectively.

For each sample and from every medium, different colony types were picked according to morphological appearance and further purified for subsequent identification.

The bacteria were identified on the basis of morphological and biochemical characteristics and classified according to the Bergey's Manual of Systematic Bacteriology [9].

Yeasts were identified on the basis of the cellular morphology and biochemical characteristics and classified according to Barnett [3].

Moulds were identified by gross colonial morphology and/or by slide culture [1, 8].

2.4. Enterotoxin determination

The presence of *S. aureus* and *B. cereus* enterotoxins in the samples was determined using reversed passive latex agglutination test kits (Oxoid SET-RPLA and BCET-RPLA, respec-

tively) following the manufacturer's instructions. The sensitivity of these assays is 1 µg enterotoxin per g food and 4 ng·g⁻¹ food for *S. aureus* and *B. cereus*, respectively.

3. RESULTS

The data from sensory evaluation of the *Pecorino* processed cheese spreads analyzed, reported in *table I*, show that the majority of factory-made products, both at production and after three months, received very favourable scores for appearance, texture, colour and flavour/taste. A slight decrease in the sensory evaluation was observed, after three months of storage, in those few spreads characterized by a higher microbial presence and may presumably be attributed to enzymatic modifications caused by some bacteria or yeasts.

The artisanal *Pecorino* spreads received medium to low scores for the same characteristics. Major defects of these products were bitter/acidic flavours and sandy textures.

The distribution of total aerobic mesophilic counts in artisanal and factory-made spreads is presented in *figure 1*. From a quantitative point of view, of the 118 samples produced in the mechanized factory, only a small percentage showed values slightly higher than 10³ cfu·g⁻¹ (9 % and 11 % at production and three months later, respectively).

On the contrary, of the 98 spreads produced in artisanal dairies, more than 40 % exceeded the value of 10³ cfu·g⁻¹ both at production and three months later and among these the majority (32 % and 29 %, respectively) showed counts over 10⁵ cfu·g⁻¹.

Table II reports the frequency of isolation and the microbial species recovered from the samples examined.

E. coli were never detected in either type of spread, while coliforms, *S. aureus* and Gram negative psychrotrophs were isolated only in artisanal samples. In particular, col-

Table I. Mean values and standard deviation for the sensory characteristics of the different spreads at production and three months later.

Tableau I. Valeurs moyennes et écarts type pour les caractéristiques sensorielles des différents échantillons de crème de *Pecorino* aussitôt après la production et trois mois après.

	Factory-made spreads <i>n</i> = 118		Artisanal spreads <i>n</i> = 98	
	Production	Three months later	Production	Three months later
Appearance (fresh-moist)	4.4 ± 0.49	4.1 ± 0.54	3.0 ± 0.8	2.7 ± 0.96
Texture (creamy and smooth)	4.4 ± 0.66	4.0 ± 0.45	2.1 ± 1.1	2.0 ± 1.3
Color (white)	4.2 ± 0.6	3.9 ± 0.7	2.8 ± 0.99	2.6 ± 0.92
Flavour/taste (delicate, sweetish)	4.5 ± 0.67	4.0 ± 0.63	2.9 ± 1.82	2.4 ± 1.57

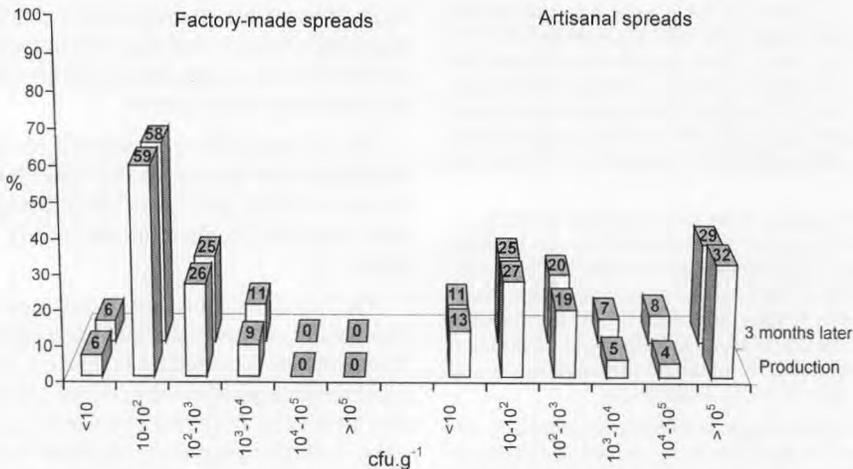


Figure 1. Total microbial counts of factory-made and artisanal spreads: % of samples presenting counts below and under 10^3 cfu.g⁻¹.

Figure 1. Dénombrements de la flore totale des échantillons de crème de *Pecorino* produits dans une structure unique mécanisée et dans différentes fromageries artisanales : % des échantillons avec des valeurs de flore totale soit inférieure à 10^3 cfu.g⁻¹ supérieure à 10^3 cfu.g⁻¹.

iforms and *S. aureus* presented similar mean values at production and three months later while the population of Gram negative psychroflora increased during the storage period. Coliforms consisted mainly of two *Enterobacter* species (*E. agglomerans* and *E. cloacae*) while *Citrobacter freundii* and *Klebsiella oxytoca* were isolated only sporadically. Gram negative psychroflora were

represented by the species *P. fluorescens* and *A. hydrophyla*.

Spore-forming bacteria, yeasts and moulds were isolated from both types of products but they were observed in higher percentages and with higher values in artisanal ones. In particular, *Bacillus* spores were present with mean values of about 100 cfu.g⁻¹ in factory-made spreads and

Table II. Frequency of isolation and microbial species recovered from the *Pecorino* processed cheese spreads at production and three months later. Mean values are expressed as log cfu-g⁻¹. Microbial species are reported in order of their frequency.

Tableau II. Fréquence d'isolement et espèces microbiennes retrouvées dans les différents échantillons de crème de *Pecorino* aussitôt après la production et trois mois après. Les valeurs moyennes sont exprimées en log cfu-g⁻¹. Les espèces microbiennes sont ordonnées selon leur fréquence.

	Factory-made spreads n. 118		Artisanal spreads n. 98	
	% positive (mean ± SD)		% positive (mean ± SD)	
	Production	3 months later	Production	3 months later
Coliforms	n.d.	n.d.	15.1 (3.3 ± 0,27)	14.9 (3.2 ± 0.19)
<i>S. aureus</i>	n.d.	n.d.	5.7 (1.9 ± 0,31)	6.1 (2.1 ± 0.10)
Gram- psychrotrophs	n.d.	n.d.	11.2 (2.4 ± 0,32)	12.1 (4.3 ± 0.12)
Yeasts	14.2 (1.1 ± 0,12)	14.3 (1.2 ± 0.13)	26.6 (2.3 ± 0,41)	27.2 (2.6 ± 0.38)
Moulds	16.6 (1.1 ± 0,12)	17.1 (1.1 ± 0.17)	31.4 (2.4 ± 0,21)	31.3 (2.1 ± 0.32)
<i>Bacillus</i>	27.8 (1.8 ± 0,08)	28.1 (1.6 ± 0.12)	64.7 (2.6 ± 0,24)	64.5 (3.4 ± 0.27)
<i>Clostridium</i>	19.2 (0.5 ± 0,17)	18.9 (0.6 ± 0.21)	24.1 (0.9 ± 0,27)	24.2 (0.8 ± 0.22)
Microbial species				
Coliforms		n.d.	<i>Enterobacter agglomerans</i> , <i>Ent. cloacae</i> , <i>Klebs. Oxytoca</i> , <i>Citr. Freundii</i>	
Gram- psychrotrophs		n.d.	<i>Pseudomonas fluorescens</i> , <i>Aeromonas hydrophila</i>	
Yeasts	<i>Debaryomyces hansenii</i>		<i>Debaryomyces hansenii</i> , <i>Kluyveromyces marxianus</i> ,	
	<i>Geotrichum candidum</i> , <i>Candida parapsilosis</i>			
Moulds	<i>Penicillium chrysogenum</i> , <i>P. frequentans</i>		<i>Penicillium chrysogenum</i> , <i>P. notatum</i> , <i>P. frequentans</i> , <i>P. sidowii</i>	
<i>Bacillus</i>	<i>B. subtilis</i> , <i>B. laterosporus</i> , <i>B. coagulans</i>		<i>B. cereus</i> , <i>B. subtilis</i> , <i>B. laterosporus</i> , <i>B. coagulans</i> , <i>B. pumilus</i>	
<i>Clostridium</i>	<i>C. glycolicum</i> ,		<i>C. sporogenes</i> , <i>C. glycolicum</i> , <i>C. sordellii</i>	

n.d.: not detected.

n.d.: non détecté.

higher than 10^3 cfu·g⁻¹ in artisanal ones. *Clostridia* spores were found in a low percentage in both types of samples and with values never exceeding 100 cfu·g⁻¹.

Finally, as can be seen from the table, there were also qualitative differences between the species isolated from factory-made and artisanal products.

Several yeast species were isolated from artisanal samples while only the species *Debaryomyces hansenii* was recovered from factory-made spreads.

Moulds were exclusively represented by some *Penicillium* species in both types of spreads.

Among *Bacillus* species, *B. laterosporus* and *B. subtilis* were isolated in both types of spreads, while *B. cereus*, *B. licheniformis* and *B. coagulans* were recovered only from artisanal samples.

As for *Clostridia*, none of the colonies isolated from the 216 samples was identified as *C. botulinum*, but no attempts were made to determine the presence of botulinum toxins. Three species were present in artisanal samples (*C. sporogenes*, *C. glycolicum* and *C. sordellii*) and only one, *C. glycolicum*, in the factory-made spreads.

Finally, it is important to note that neither *S. aureus* or *B. cereus* enterotoxins were detected in any of the spreads examined.

4. DISCUSSION

Among the most appreciated characteristics of *Pecorino* processed cheese spreads are their creamy texture and fresh appearance, as well as flavour/taste and aroma similar to those of the typical varieties of *Pecorino* Sardo cheese [13, 16]. In this study, the factory-made spreads obtained a very favourable evaluation for these characteristics while artisanal samples showed a major variability in organoleptic profile and therefore had lower scores.

The differences in scores may be in part attributed to the microbiological quantita-

tive and qualitative differences observed in the two types of spreads. In particular, among the species recovered exclusively from artisanal spreads it is worth noting the presence of the psychrotrophic *P. fluorescens*, *Aeromonas hydrophila* and *Bacillus cereus*, that have the potential to cause spoilage of dairy products, especially when they are kept refrigerated [6, 11, 17]. In fact, these species can grow at refrigeration temperatures [14, 15] and produce degradative enzymes which may cause bitterness and other off-flavours or textural defects in dairy products [4, 5]. In addition, some of these bacteria may represent a health risk: *A. hydrophila* is considered a possible food-borne pathogen [2, 18] and *B. cereus* is well established as a cause of food poisoning [7, 12].

Among the species recovered from artisanal samples, the presence of *C. sporogenes*, well known for its high proteolytic and lipolytic activity in milk [9] should also be stressed.

The presence of coliforms, *Staphylococcus aureus* and psychrotrophic species as well as the higher levels of yeasts and moulds observed in the artisanal spreads, is presumably an index of post heat-treatment contamination and suggests poor hygiene during production. Operations involving risk may be considered to be the slow manual mixing of the mixture in an open container and the manual handling and packing of the spreads.

On the whole, the factory-made spreads were characterized by generally low microbial counts and a lower presence of microbial species, indicating a better hygienic level of the processing conditions. In addition, these products maintained fairly good organoleptic and microbiological characteristics even three months after production.

Finally, in relation to the presence of health risks for the consumer, it should be stressed that none of the samples analyzed showed presence of *C. botulinum* or *S. aureus* and *B. cereus* enterotoxins.

The results of our study seem to confirm that the application of a well-standardized and mechanized procedure, which minimizes the manual handling of the product, combined with proper hygiene practices during manufacturing will help to reduce the risk of developing food safety and/or spoilage problems in these kinds of dairy products.

The achievement of these objectives could guarantee adequate quality of these new Sardinian dairy products and might also lead to access to the international market.

REFERENCES

- [1] Ainsworth G.C., Sparrow F.K., Sussman A.S., *The fungi*, Academic Press, New York and London, 1973.
- [2] Altwegg M., Ljungh A., *Aeromonas* as a human pathogen, *Crit. Rev. Microbiol.* 16 (1989) 253–286.
- [3] Barnett J.A., Payne R.W., Yarrow D., *Yeasts: Characteristics and identification* (2nd ed.), Cambridge University Press, Cambridge, 1990.
- [4] Bishop J.R., White C.H., Assessment of dairy products quality and potential shelf-life, A review, *J. Food Protect.* 49 (1986) 739–753.
- [5] Cousin M.A., Presence and activity of psychrotrophic microorganisms in milk and dairy products: a review, *J. Food Protect.* 45 (1982) 172–207.
- [6] Fricker C.R., Tompsett S., *Aeromonas* spp. in foods: A significant cause of food poisoning?, *Int. J. Food Microbiol.* 9 (1989) 17–23.
- [7] Gilbert R.J., Taylor A.J., *Bacillus cereus* food poisoning, in: Skinner F.A., Carr J.G. (Eds) *Microbiology in Agriculture, Fisheries and Food*, Soc. Appl. Bacteriol. Symposium Series n° 4 Academic Press, London, 1976, pp. 197–213.
- [8] Gilman J.C., *A manual of soil fungi*. The Iowa State University Press, Ames, Iowa, USA, 1957.
- [9] Holt J.G., Krieg N.R., Sneath P.H.A., Staley J.T. (Eds), *Bergey's Manual of Systematic Bacteriology* 9th ed. Williams & Wilkins, Baltimore, 1994.
- [10] International Dairy Federation Sensory Evaluation of Dairy Products, FIL-IDF standard 99, Brussels, Belgium, 1981.
- [11] Johnson K.M., *Bacillus cereus* in food-borne illness - An update, *J. Food Protect.* 47 (1984) 145–153.
- [12] Kramer J.M., Gilbert R.J., *Bacillus cereus* and other *Bacillus* spp., in: Doyle M.P. (Ed.), *Food-borne bacterial pathogens*, Marcel Dekker, New York, 1989, pp. 21–70.
- [13] Ledda A., Murgia A., Arrizza S., Indagine sulla tecnologia di fabbricazione del Fiore sardo. Giornata di Studio su "Nuovi aspetti della produzione del formaggio Fiore sardo", Sassari, 1978, pp. 8–22.
- [14] Meer R.R., Baker J., Bodyfelt F.W., Griffiths M.W., Psychrotrophic *Bacillus* spp. in fluid milk products: a review, *J. Food Protect.* 54 (1991) 969–979.
- [15] Palumbo S.A., Buchanan R.L., Factors affecting growth or survival of *Aeromonas hydrophila* in foods, *J. Food Safety* 9 (1988) 37–51.
- [16] Ricci C., Di Bucchianico R., D'Angelo M.G., Tecnologia di produzione del Pecorino d'Abruzzo: caratteristiche del prodotto finito e aspetti del processo di maturazione, *Latte* 3 (1995) 268–274.
- [17] Torelli F., Il consumatore e la domanda di prodotti alimentari, *Largo Consumo* 9 (1989) 140–149.
- [18] Wadström T., Ljungh A., *Aeromonas* and *Plesiomonas* as food and waterborne pathogens, *Int. J. Food Microbiol.* 12 (1991) 303–312.