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Microbiological and Chemical Characterization of a Typical Italian Cheese: Robiola di Roccaverano

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Robiola di Roccaverano is a traditional Italian goat's milk cheese carrying a Protected Designation of Origin (PDO). The present work studied both cheese microflora and cheese physicochemical characteristics to obtain a more accurate description of this PDO product. Multivariate statistical analysis (PCA) was performed to evaluate the influence of cheesemaking (artisanal and industrial), ripening time, and season of production on cheese characteristics. Multiplex PCR and fatty acid methyl esters (FAMEs) were used to identify the kind of milk employed by Robiola di Roccaverano producers. The results obtained highlight some product differences between the artisanal and industrial products. These differences were most evident in the microbiological data. The use of PCA allowed cheese samples to cluster on the basis of their age (fresh or ripened), the origin of production (artisanal and industrial), and even the season of production. Gross composition, microbiological parameters, and gas chromatographic analyses of FAMEs provided the most important parameters for Robiola di Roccaverano cheese characterization.

KEYWORDS: PDO cheese; characterization; chemical parameters; microbiological analysis; identification of milk; PCA

INTRODUCTION

The authenticity of typical dairy products has become a relevant topic for producers, researchers, and consumers. When a typical food acquires a reputation beyond national borders, it can be in competition with different products of the same name. For this reason the European Community created in 1992 three protection systems called PDO (Protected Designation of Origin), PGI (Protected Geographical Indication), and TSG (Traditional Specialty Guaranteed). During the past few years several studies of microbiological and chemical characteristics of PDO cheeses have appeared. For example, the work of Di Cagno (*1*) regarded the microbiological, compositional, biochemical, and sensory characteristics of some typical Italian cheeses (Pecorino Romano, Fiore Sardo, and Canestrato Pugliese). Other studies highlighted the influence of cheesemaking (e.g., milk origin, thermal milk treatment) on chemical and microbial composition of Manchego, Anevato, and Canestrato Pugliese cheeses (*2, 3*).

Goat's milk cheese represents today only a small fraction of total cheese production, but it has a relevant social and economic importance in the Mediterranean area. The quality of goat's milk cheese is closely related to the region of production and its traditions. The interaction of pedoclimatic conditions, autochthonous goat's genetic variations, and anthropic components creates such specific environments that they would be extremely difficult to reproduce elsewhere (*4*).

Many papers have attempted to differentiate cheeses according to their origin: good results have been obtained using classical multivariate techniques such as principal component analysis (PCA). Pham and Nakai (*5*) performed a chemometrical analysis of HPLC data to discriminate Cheddar according to ripening time. Muir et al. (*6*) used PCA to study the diversity in flavor and texture between farmhouse Cheddar and factory-produced Cheddar. Pillonel et al. (*7*) identified the origin of Emmental cheese samples produced in various European countries using the same approach.

Robiola di Roccaverano cheese is the only Italian goat's milk cheese that has been granted a PDO. It is produced only in Val Bormida (Piedmont region), and its name comes from the main village of production (Roccaverano). Robiola di Roccaverano is produced by 37 artisanal dairies and by the sole industrial dairy located in the PDO area. According to PDO regulation, Robiola di Roccaverano is made from raw goat's milk, but the

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PDO legislation allows the addition of cow's milk and ewe's milk (*8*). The industrial producer uses raw goat's milk with additions of pasteurized cow's milk and selected starters. The artisanal dairies use only raw goat's and cow's milk (no pasteurization) and traditional techniques (no starter addition). Each Robiola has its own unique flavor. This can be due to the different flowers, herbs, and pasture used to feed goats, which can confer specific characteristics to the milk used for Robiola production. The microflora of cheeses made from raw milk following traditional manufacturing procedures may show different characteristics in diversity and richness; the quality of these cheeses depends to a great extent on the composition of this nonstarter microflora (*9*).

Only a few studies have been performed on Robiola di Roccaverano cheese so far. Industrial Robiola chemical composition and free amino acids were studied by Coússon et al. (*10*), whereas Pattono et al. (*11*) studied the gross composition of Robiola produced from different artisanal dairies. Soncini et al. (*12*) focused on hygiene, performing microbiological analyses to evaluate total bacterial counts and pathogenic microorganisms (*Salmonella* and *Listeria monocytogenes*). In addition, some research on decarboxylating bacterial strains and biogenic amine content has also been performed on Robiola di Roccaverano cheese (*13*).

The present study analyzes together microflora and physicochemical characteristics of Robiola di Roccaverano PDO cheese to gain a more accurate description. Multivariate statistical analysis of both chemical and microbiological data was performed to evaluate the influence of cheesemaking, ripening time, and season of production on cheese characteristics.

MATERIALS AND METHODS

Cheesemaking and Samples. Robiola di Roccaverano samples were supplied by four traditional dairy producers (here named A, B, C, and D) and by the sole industrial producer in the PDO area (here named E). Robiola di Roccaverano is manufactured by the four different cheesemakers following the traditional method: goat's milk from two separate milking sessions is heated at 20−25 °C, and then cow's or goat's rennet is added. Robiola di Roccaverano is produced by the sole industrial producer using raw goat's milk and pasteurized cow's milk, and both rennet and starters are added (e.g., *Lactococcus lactis* subsp. *lactis, L. lactis* subsp. *cremoris*, *L. lactis* subsp. *diacetylactis*, *Leuconostoc citrovorum*, and *Leuconostoc dextranicum*). After rennet or starter addition, the milk is left quiescent for 24 h to coagulate. Then the curd so obtained is placed in plastic molds, salted, and matured for 3 days at 15−20 °C. Robiola cheese has a very thin and ivory-colored rind, but as it ripens it may take a straw yellow color. Robiola's rinds are quite small compared to other typical Italian cheeses: each rind weighs approximately 400 g, has a diameter of 10−14 cm, and is 4−5 cm thick. Robiola cheese is usually consumed fresh or within 20 days of ripening.

Two cheese samples were collected from each producer in three different seasons (winter, spring, and summer). One cheese sample from each batch was analyzed immediately (fresh product), whereas the other was analyzed after 20 days of ripening. The ripening of all samples was carried out in a fresh, damp chamber according to the PDO legislation.

Microbiological Analysis. *Salmonella* spp., *Listeria* spp., and *Staphylococcus aureus* were determined according to standard methods (*14–16*). Each cheese sample (10 g) was homogenized in a Stomacher Laboratory-Blender 400 (PBI International, Milan, Italy) with a sterilized sodium citrate solution (2% w/v, 90 mL) at 45 °C for 1 min. Serial dilutions were prepared in peptone water and depth-inoculated in duplicate on specific agar media for viable counts. Coliforms were counted on Violet red bile agar (VRBA) (Biokar Diagnostics, France) at 37 °C for 24 h and *Escherichia coli* on Tryptone bile X-glucoronide medium (TBX) (Biolife Italiana, Italy) at 44 °C for 24 h. Staphylococci were determined on Baird−Parker agar (Biokar Diagnostics) after incubation at 37 °C for 24−48 h. Mesophilics aerobic bacteria were counted on plate count agar (Fluka BioChemika, Switzerland) at 30 °C for 72 h. Lactic flora were counted on DeMan, Rogosa, and Sharpe agar (MRS) (Biokar Diagnostics) and lactococci on M17 agar (Biokar Diagnostics); both incubations were performed at 37 °C for 48 h. Lactobacilli were enumerated on Rogosa agar (Biolife Italiana) acidified with acetic acid and incubated at 30 °C for 5 days. Oxytetracycline glucose yeast extract agar (OGYE-agar) (Biokar Diagnostics) was used for mold evaluation by a surface-plate method with incubation at 22 °C for 5 days. Microbial counts were expressed as a logarithm (log) of colony-forming units (cfu) per gram of cheese sample.

Chemical Analytical Methods. pH, cheese moisture, and ash were determined according to the Italian Official Methods for cheese analysis (*17*). Total and water-soluble nitrogen were determined according to the Kjeldahl method using the Kjeltec system (Tecator, Sweden). Ripening index was calculated from the ratio of water-soluble nitrogen and total nitrogen. Protein content was obtained from the water-soluble nitrogen value using the conversion factor 6.38. SDS-PAGE was performed according to the method of Laemmli using a Mini Protean III dual slab cell apparatus (Bio-Rad Laboratories S.r.l., Segrate, Italy). The analysis of protein was also done on the urea-soluble fraction of cheese obtained using a 6 M urea solution at pH 8.5. Gels were stained with Coomassie Blue R-250 to reveal protein bands. Fluor-S Multimager and Quantity One software (Bio-Rad) were used for gel analysis. Gel bands were expressed as relative percentage for each sample and used as parameters for statistical analysis.

Four amino acids (tyrosine, histidine, tryptophan, and phenylalanine) and their corresponding biogenic amines (tyramine, histamine, tryptamine, and 2-phenylethylamine) were determined using an ion-pair high-performance liquid chromatography (HPLC) method (*13*). Analyses were carried out on a Shimadzu Class VP HPLC system equipped with a temperature controller (column oven CTO-10AS), UV−vis detector SPD-10A, using an ODS 2 column (4.6 i.d., 250 mm length, Waters), and two pumps.

Fatty acid methyl esters (FAMEs) were obtained by transesterification of triglycerides. They were extracted as total fat using dichloromethane in a Soxhlet apparatus for 14 h with a 1.5% sodium methylate in methanol solution. The reaction was carried out into a closed vial at 80 °C with periodic stirring. The content of each vial was then washed with water and diethyl ether until the organic phase was clear and transparent. After water elimination with Na_2SO_4, the solvent was removed and 1 mL of CH_2Cl_2 was added. FAMEs were analyzed on a Shimadzu gas chromatogaph 17-a ver.3, equipped with a DB23 column (J&W Scientific) 30 m, i.d. = 0.25 mm and film thickness = 0.25 μm. Hydrogen was used as carrier and a flame ionization detector (FID) as detector. The temperature program was as follow: 90 °C/10 °C min^{-1}/220 °C, with constant flow of 1.6 mL min^{-1}.

Separation and purification of free fatty acids (FFAs) was performed using solid phase extraction (SPE) columns. Two milliliters of diethyl ether/hexane solution (1:1) containing 2 g of fat were loaded into the column. FFAs were eluted with approximately 10 mL of 2% formic acid in diethyl ether solution. The solution was then concentrated with N_2 flow. FFAs were analyzed on a Shimadzu GC 17-a ver.3, on a Nukol column (Supelco) 30 m long, with an inner diameter of 0.25 mm and film thickness of 0.25 μm. Hydrogen was used as carrier, and FID was used as detector. The temperature program was as follow: 100 °C/30 °C min^{-1}/200 °C, with constant flow of 2.4 mL min^{-1}.

All chemical results are reported as the mean of three (or more) independent determinations ± standard deviation.

Identification of Milk Used for Robiola di Roccaverano Production. Multiplex Polymerase Chain Reaction (PCR) on DNA from the somatic cells of different animal species (*Ovis aries*, *Capra hircus*, *Bos taurus*) was employed to identify the kind of milk used by the different producers of Robiola di Roccaverano. DNA was extracted directly from cheese samples. Briefly, 2 mL of ammonium hydroxide, 2 mL of ethanol (100%), 4 mL of petroleum ether, and 200 μL of sodium dodecyl sulfate (SDS, 10% solution) were added to 10 mL of Robiola dilution. The mixture was then centrifuged, and the pellet was suspended in 2 mL of urea (6.0 M), 2 mL of ethanol (100%), 4 mL of

Table 1. Mean Counts[a] (log CFU/g) of the Principal Microbial Groups in Robiola di Roccaverano Cheese

		staphylococci		coliforms		*E. coli*		mesophilic counts		lactobacilli		lactococci		lactic flora		molds	
cheese maker[b]	season	0 days[c]	20 days	0 days	20 days	0 days	20 days	0 days	20 days	0 days	20 days	0 days	20 days	0 days	20 days	0 days	20 days
A	spring	3.70[d] (0.00)	7.23 (0.12)	5.15 (0.12)	5.77 (0.04)	2.00 (0.01)	<1	11.48 (0.16)	13.08 (0.01)	7.92 (0.01)	7.98 (0.25)	8.96 (0.13)	9.18 (0.21)	9.23 (0.03)	8.81 (0.03)	6.45 (0.03)	5.84 (0.01)
	summer	5.84 (0.11)	7.34 (0.05)	5.98 (0.04)	5.30 (0.04)	<1	<1	9.81 (0.08)	9.18 (0.01)	8.30 (0.03)	8.52 (0.03)	9.52 (0.05)	8.08 (0.06)	8.97 (0.01)	8.26 (0.01)	6.38 (0.03)	4.93 (0.11)
	winter	5.79 (0.04)	7.26 (0.02)	3.68 (0.04)	5.26 (0.01)	2.30 (0.01)	<1	8.30 (0.06)	8.34 (0.04)	8.26 (0.06)	8.40 (0.01)	7.11 (0.04)	8.18 (0.04)	7.38 (0.02)	8.30 (0.02)	5.34 (0.03)	6.18 (0.04)
B	spring	4.54 (0.08)	8.11 (0.06)	5.18 (0.04)	5.82 (0.01)	2.30 (0.02)	<1	11.48 (0.00)	8.32 (0.08)	8.00 (0.01)	6.95 (0.47)	9.18 (0.04)	10.78 (0.33)	8.98 (0.02)	9.18 (0.08)	6.26 (0.02)	5.99 (0.03)
	summer	5.51 (0.11)	7.30 (0.01)	4.98 (0.01)	5.26 (0.04)	2.30 (0.01)	<1	9.08 (0.06)	8.38 (0.03)	8.36 (0.07)	7.83 (0.05)	8.30 (0.08)	8.00 (0.01)	8.45 (0.03)	8.38 (0.03)	5.72 (0.02)	4.81 (0.08)
	winter	5.48 (0.06)	7.26 (0.03)	3.90 (0.06)	5.26 (0.03)	2.00 (0.01)	<1	9.26 (0.01)	8.30 (0.01)	8.28 (0.01)	8.43 (0.01)	7.34 (0.01)	8.18 (0.04)	8.23 (0.02)	8.38 (0.00)	5.26 (0.11)	6.04 (0.05)
C[e]	spring	4.48 (0.00)		5.26 (0.02)		2.48 (0.02)		11.48 (0.18)		7.72 (0.01)		9.64 (0.49)		9.48 (0.03)		5.72 (0.06)	
	summer	5.11 (0.08)	6.08 (0.08)	6.11 (0.01)	4.23 (0.05)	2.00 (0.01)	<1	9.95 (0.07)	8.23 (0.06)	7.30 (0.01)	7.90 (0.52)	9.41 (0.06)	8.23 (0.03)	9.20 (0.06)	8.20 (0.02)	4.91 (0.08)	4.85 (0.21)
	winter	5.91 (0.01)	6.20 (0.04)	4.23 (0.05)	6.20 (0.05)	2.00 (0.02)	<1	8.15 (0.04)	7.43 (0.01)	7.28 (0.02)	8.32 (0.00)	7.34 (0.01)	8.23 (0.01)	7.30 (0.01)	8.28 (0.01)	5.20 (0.03)	6.23 (0.01)
D[e]	spring	4 (0.00)		5.18 (0.08)		2.30 (0.03)		9.49 (0.30)		8.15 (0.06)		9.18 (0.18)		9.00 (0.06)		6.36 (0.01)	
	summer	5.62 (0.14)	6.20 (0.03)	4.91 (0.02)	5.81 (0.03)	2.30 (0.03)	<1	8.34 (0.08)	8.15 (0.07)	8.99 (0.03)	8.32 (0.10)	7.93 (0.01)	7.99 (0.08)	8.38 (0.03)	8.15 (0.02)	5.48 (0.04)	6.04 (0.08)
	winter	5.71 (0.06)	6.23 (0.02)	4.08 (0.05)	5.89 (0.01)	<1	<1	8.38 (0.06)	8.15 (0.05)	9.00 (0.05)	9.41 (0.01)	8.20 (0.11)	8.04 (0.01)	7.30 (0.05)	8.26 (0.04)	4.72 (0.03)	5.91 (0.03)
E	spring	<2	8.11 (0.01)	1.56 (0.21)	5.72 (0.01)	<1	<1	7.96 (0.02)	9.00 (0.51)	6.68 (0.30)	6.59 (0.05)	6.99 (0.03)	10.18 (0.06)	7.08 (0.03)	7.64 (0.00)	6.26 (0.03)	5.85 (0.17)
	summer	<2	5.49 (0.11)	3.49 (0.13)	4.32 (0.01)	<1	<1	6.93 (0.02)	8.77 (0.01)	6.64 (0.08)	9.04 (0.06)	6.40 (0.02)	8.23 (0.04)	6.08 (0.21)	8.77 (0.10)	4.34 (0.16)	5.92 (0.04)
	winter	<2	5.63 (0.05)	3.30 (0.06)	3.88 (0.04)	<1	<1	6.77 (0.11)	7.08 (0.03)	6.42 (0.12)	8.18 (0.03)	7.00 (0.01)	8.04 (0.02)	6.48 (0.03)	8.26 (0.02)	5.32 (0.01)	6.00 (0.01)

[a] Mean values from two replicates. [b] A−D, artisanal products; E, industrial product. [c] Days of ripening. [d] Mean (SD). [e] Ripened samples were not analyzed. <2; <1 log CFU/g, detection limit for Staphylococci and *E. coli*.

petroleum ether, 800 μL of SDS (10% solution), and 130 μL sodium acetate (3.0 M). After centrifugation, the pellet was suspended in 400 μL of sodium hydroxide (0.1 M) and 16 μL of SDS (10% solution). DNA was extracted with phenol-chloroform (1:1), precipitated with ethanol (100%) and purified using a DNeasy Tissue Kit (Qiagen), following the manufacturer's protocol. The PCR reaction was performed with a final volume of 50 μL according to the protocol described by Bottero et al. (*18*). PCR products were analyzed by electrophoresis on agarose gel (2% agarose w/v).

Statistical Analysis. Analysis of variance (ANOVA, SYSTAT, version 8.0) was used to evaluate the influence of independent variables such as cheesemaking (artisanal or industrial process), ripening time, and season of production on the chemical and microbiological parameters measured on Robiola cheese.

Principal component analysis was applied to both chemical and microbiological data (a total of 97 parameters) using Mathematica software (Wolfram Research Inc., Champaign, IL). Of these 97 parameters, 9 were from compositional analyses, 30 were from FAME analysis, 11 were from FFA analysis, 21 were from SDS-PAGE analysis, 12 were from urea-PAGE analysis, 6 were from HPLC analysis (amino acids and biogenic amines), and 8 were from microbiological analysis.

PCA was performed on a correlation matrix. A normalization step was applied to each variable to avoid possible distortions arising from the different magnitudes of the numerical values associated with the different variables. This normalization involved a standardization of the variables to an equal variance of one by dividing them by their respective standard deviations (*19*).

RESULTS AND DISCUSSION

Microbiological Characteristics. The results of microbiological analyses for artisanal and industrial products are presented in **Table 1**.

These results show that of the 27 samples studied (artisanal and industrial, fresh and ripened), none contained pathogenic bacteria such as *S. aureus*, *Salmonella* spp., or *Listeria* spp. and all conformed to the Italian legislative standards (*20*).

All parameters from microbiological analyses were studied with ANOVA test to evaluate the possible differences between the artisanal and industrial products.

ANOVA showed that the average bacterial counts from the fresh industrial product (producer E) were significantly lower than the average counts from the fresh artisanal product (A−D) (staphylococci, coliforms, mesophilic counts, lactobacilli, and lactic flora $p < 0.001$; *E. coli* and lactococci $p < 0.05$). Conversely, mold counts showed no significant differences between fresh industrial and fresh artisanal products.

A decrease of coliforms and staphylococci counts was also observed in other studies comparing cheeses produced from raw and pasteurized milk (*4, 21*). A similar behavior was also observed for *E. coli*; in fact, it was present in almost all fresh artisanal cheeses, even though at low concentrations and always under the legislation threshold (*20*), whereas it was never found in fresh industrial products. Mesophilic counts, lactic flora, lactococci, and lactobacilli confirmed this finding with lower microbiological counts in the industrial products. This same trend has been also observed in other studies (*21, 22*). The behavior observed for all bacterial counts in the industrial product could be related to the cow's milk pasteurization. Grappin and Beuvier (*23*) reported that, in general, milk's bacteria population is reduced by at least 90% by pasteurization. Moreover, the low microbial counts observed in the industrial product may also denote a scarce activity of the starter bacteria used for industrial Robiola manufacturing.

On the contrary, the similar mold counts observed in artisanal and industrial products could be related to the analogous environmental contamination of the production area and manu-

Table 2. Mean Values[a] for the Gross Composition of Robiola di Roccaverano

		pH		moisture (%)		ash (%)		protein (%)		ripening index[c]		N sol (%)		ash (% dry matter)		protein (% dry matter)	
cheese maker[b]	season	0 days[d]	20 days	0 days	20 days	0 days	20 days	0 days	20 days	0 days	20 days	0 days	20 days	0 days	20 days	0 days	20 days
A	spring	3.80 (0.01)[e]	4.40 (0.02)	57.01 (0.52)	46.67 (0.99)	1.52 (0.04)	2.05 (0.31)	12.20 (0.50)	19.23 (1.51)	7.16	45.40	0.15 (0.00)	1.36 (0.03)	3.54 (0.09)	3.84 (0.58)	28.28 (1.16)	36.07 (2.83)
	summer	4.14 (0.01)	5.00 (0.01)	54.89 (1.19)	17.94 (0.22)	1.17 (0.04)	2.90 (0.08)	15.49 (1.46)	30.05 (0.24)	13.25	30.60	0.32 (0.00)	1.44 (0.01)	2.59 (0.08)	3.53 (0.10)	34.34 (3.24)	36.62 (0.29)
	winter	4.06 (0.00)	5.73 (0.03)	51.99 (1.71)	41.30 (1.00)	1.60 (0.01)	1.88 (0.10)	13.91 (0.08)	18.94 (0.05)	11.37	86.30	0.24 (0.01)	2.55 (0.02)	3.33 (0.01)	3.20 (0.17)	28.97 (0.17)	32.27 (0.09)
B[f]	spring	3.90 (0.01)		58.18 (0.34)		2.08 (0.06)		14.07 (1.35)		10.00		0.22 (0.01)		4.97 (0.14)		33.64 (3.23)	
	summer	4.12 (0.02)	4.92 (0.01)	45.18 (0.97)	13.69 (0.31)	1.29 (0.10)	3.08 (0.06)	19.70 (0.03)	31.56 (1.50)	9.44	28.63	0.29 (0.02)	1.42 (0.02)	2.35 (0.18)	3.57 (0.07)	35.94 (0.05)	36.57 (1.74)
	winter	4.70 (0.01)	5.54 (0.02)	52.66 (0.54)	45.63 (0.28)	1.43 (0.10)	2.41 (0.05)	13.75 (0.27)	17.49 (0.23)	9.70	47.57	0.21 (0.01)	1.31 (0.02)	3.02 (0.21)	4.43 (0.09)	29.05 (0.57)	32.17 (0.42)
C[f]	spring	4.21 (0.01)		59.13 (0.97)		1.37 (0.04)		14.13 (0.32)		6.86		0.15 (0.01)		3.35 (0.10)		34.57 (0.78)	
	summer	4.30 (0.01)	5.02 (0.01)	57.16 (0.98)	23.00 (0.22)	1.32 (0.05)	2.84 (0.06)	13.86 (0.49)	31.98 (0.36)	8.77	31.00	0.19 (0.01)	1.56 (0.01)	3.08 (0.12)	3.69 (0.08)	32.35 (1.14)	41.53 (0.47)
	winter	4.15 (0.00)	5.27 (0.01)	57.56 (0.16)	56.72 (0.17)	1.53 (0.21)	1.60 (0.03)	12.91 (0.46)	17.88 (0.10)	8.95	25.10	0.18 (0.00)	0.70 (0.02)	3.61 (0.49)	3.70 (0.07)	30.42 (1.08)	41.31 (0.03)
D[f]	spring	4.30 (0.02)		60.61 (0.58)		1.14 (0.18)		12.86 (0.38)		13.91		0.27 (0.01)		2.89 (0.46)		32.65 (0.96)	
	summer	4.12 (0.01)	5.19 (0.02)	55.06 (1.12)	21.99 (1.07)	0.95 (0.01)	2.36 (0.08)	15.67 (0.04)	31.39 (1.12)	15.50	35.00	0.37 (0.00)	1.72 (0.00)	2.11 (0.02)	3.03 (0.10)	34.87 (0.09)	40.23 (1.44)
	winter	4.21 (0.00)	5.68 (0.02)	45.93 (0.80)	40.18 (0.14)	1.04 (0.03)	1.67 (0.29)	15.13 (0.22)	21.88 (0.50)	9.09	95.30	0.21 (0.01)	3.27 (0.00)	1.92 (0.06)	2.79 (0.48)	28.01 (0.41)	36.58 (0.84)
E	spring	4.17 (0.01)	5.70 (0.01)	63.70 (0.71)	48.69 (0.57)	1.21 (0.04)	3.59 (0.08)	12.86 (0.38)	19.67 (0.31)	13.21	55.80	0.27 (0.01)	1.72 (0.10)	3.33 (0.11)	7.00 (0.16)	35.43 (1.05)	38.34 (0.60)
	summer	4.27 (0.02)	5.03 (0.02)	55.62 (1.98)	18.54 (0.24)	2.12 (0.06)	3.12 (0.72)	13.92 (0.39)	27.78 (0.00)	16.41	27.60	0.36 (0.00)	1.19 (0.01)	4.78 (0.14)	3.83 (0.88)	31.37 (0.88)	34.10 (0.01)
	winter	4.11 (0.00)	4.94 (0.01)	56.77 (0.33)	47.59 (0.13)	0.80 (0.21)	1.19 (0.11)	12.92 (0.09)	18.41 (0.28)	12.80	30.60	0.26 (0.01)	0.88 (0.00)	1.85 (0.49)	2.27 (0.21)	29.89 (0.21)	35.13 (0.53)

[a] Average data for three replicates. [b] A–D, artisanal products; E, industrial product. [c] Calculated as percentage ratio between water-soluble and total nitrogen. [d] Days of ripening. [e] Mean (SD). [f] Ripened samples were not analyzed.

facturing tools that are in contact with the milk and cheese during cheese production.

The significant differences in microbial counts observed in fresh samples between artisanal and industrial cheeses were not seen in ripened products.

A significant increase in staphylococci and coliforms counts was observed in artisanal cheese samples during ripening (staphylococci, $p < 0.001$; coliforms, $p < 0.05$). The same trend was observed also in industrial samples even though to a limited extent (statistically significant only for staphylococci, $p < 0.05$). The increase of these microbiological parameters during ripening has also been reported for other goat's milk cheeses such as Serra da Estrela Portuguese cheese (*24*). This phenomenon could partly be ascribed to the hygienic conditions used during either the milking or cheesemaking processes.

Conversely, *E. coli* presence was reduced in artisanal ripened cheeses ($p < 0.001$) as similarly observed in other studies on ripened goat's milk cheeses (*4*). This trend suggests that *E. coli* could be inhibited by some highly competitive microrganisms, which are able to prevail during ripening. *E. coli* was never found in ripened industrial cheeses, confirming the results obtained in fresh samples.

Ripening time did not significantly affect mesophilic and lactic acid bacteria (LAB) counts.

ANOVA test on fresh artisanal Robiola samples elicited a significant seasonal effect on mesophilic, LAB, and mold counts (winter < summer < spring, $p < 0.05$).

A significant effect of the season of production was also revealed for coliforms and staphylococchi ($p < 0.05$), but a different trend was observed (spring < summer < winter for staphylococchi and winter < spring < summer for coliforms). In any case the industrial product showed statistically significant seasonal differences.

Interesting information about microbiological characteristics of Robiola di Roccaverano was obtained by bacterial counts performed on different media. The number of lactococci (M17 agar) determined in fresh Robiola di Roccaverano samples was in general higher than that of lactobacilli (Rogosa agar). This fact highlighted that lactococci were the most represented organism of the lactic microflora of this PDO cheese, as confirmed by the similar counts obtained on M17 agar used for lactococci evaluation and on MRS agar used for lactic flora enumeration. The prevalence of lactococci has also been observed for Anevato cheese produced with raw goat's milk (*25*) and other goat's milk cheeses (*26*). This trend could be explained, in the fresh product, by the speedy metabolism of this genus, which quickly begins to ferment the lactose and to proliferate before other lactic acid bacteria.

Chemical Characteristics. The gross composition of all cheese samples (fresh and ripened) is presented in **Table 2**.

The compositional data of the fresh product are in accord with the more recent data available for Robiola di Roccaverano (*10, 11*) and confirm the data available in the literature for goat's or cow's milk fresh cheeses (*27*). The moisture content of the fresh product (ranging from 50 to 60%) was similar to that of Kopanisti and Pitktogalo cheeses from Greece or Azeitao cheese from Portugal. All of these cheeses are produced with a slow coagulation method and mild cooking temperatures (25 °C), which do not help whey clearance. Furthermore, all of the samples analyzed showed a composition similar to that of acid-coagulated cheeses such as cream cheese, Neufchatel, and other Quark cheeses produced with whole milk (*28*).

Table 3. Mean Values[a] (CV < 2%) of Relative Percentages for the FAMEs Composition of Robiola di Roccaverano Sample Analyzed

		C8		C10		C12		C14		C16		C18:0		C18:1		C18:2		C18:3		others	
cheese maker[b]	season	0 days[c]	20 days	0 days	20 days	0 days	20 days	0 days	20 days	0 days	20 days	0 days	20 days	0 days	20 days	0 days	20 days	0 days	20 days	0 days	20 days
A	spring	2.43	2.50	9.64	10.00	4.50	4.68	11.08	11.52	28.69	29.73	12.16	12.55	23.22	20.69	3.17	3.00	0.99	0.89	4.13	4.45
	summer	2.54	2.76	9.60	10.13	4.26	4.39	11.44	11.55	31.47	31.07	12.29	12.10	20.31	19.95	2.91	2.80	0.73	0.70	4.45	4.54
	winter	2.29	2.50	11.01	10.93	6.62	6.61	14.05	13.92	31.98	32.91	7.47	7.25	18.15	18.06	2.61	2.50	0.69	0.68	4.89	4.63
B[d]	spring	2.57		8.17		3.64		10.16		26.92		13.35		26.23		3.61		0.93		4.69	
	summer	2.63	2.39	9.67	8.59	4.39	3.82	11.18	10.84	29.76	28.92	12.23	13.29	20.28	21.95	3.82	3.85	1.06	1.34	5.05	4.99
	winter	2.27	2.93	11.14	12.31	6.19	6.63	13.33	13.74	30.62	30.75	8.21	8.01	19.26	17.53	2.87	2.73	0.87	0.84	4.88	4.53
C[d]	spring	1.84		7.59		4.09		11.26		30.04		11.11		22.82		3.21		1.08		6.54	
	summer	1.53	1.98	6.18	6.59	3.91	3.98	10.98	11.03	32.20	32.54	10.30	10.47	23.32	22.48	3.16	3.00	1.28	1.21	6.81	6.73
	winter	1.25	1.13	5.18	5.52	4.93	5.01	13.62	13.54	34.97	34.65	8.72	8.58	20.50	20.39	2.55	2.65	1.14	1.17	6.86	7.38
D[d]	spring	1.65		3.49		3.56		11.52		31.69		12.73		25.59		2.48		0.46		7.22	
	summer	1.51	1.63	5.15	5.17	3.30	3.32	10.89	14.04	29.97	29.72	13.55	13.24	24.42	22.40	2.88	2.67	1.16	1.07	7.03	6.74
	winter	2.40	1.26	4.46	3.71	3.68	3.21	12.04	9.63	30.81	28.96	12.14	11.84	24.93	30.21	2.73	3.01	0.99	1.00	6.71	7.17
E	spring	1.73	1.92	7.81	7.24	4.55	3.55	11.64	10.53	27.26	28.95	11.07	14.32	23.70	23.46	4.59	3.83	0.88	1.07	6.08	5.13
	summer	1.18	1.66	5.40	5.75	3.29	3.24	10.28	10.25	28.85	29.49	13.98	13.70	24.96	24.36	4.01	4.20	1.23	1.49	6.27	5.86
	winter	2.43	1.39	3.28	3.61	3.66	3.97	12.54	13.09	32.64	32.13	12.53	12.02	23.57	22.76	2.61	2.82	1.03	0.90	6.96	7.29

[a] Average data for three replicates. [b] A−D, artisanal products; E, industrial product. [c] Days of ripening. [d] Ripened samples were not analyzed.

The most recent PDO legislation (February 2005) requires a minimum value of 34% of protein (dry matter) and 3% of ash (dry matter), although the previous legislation (no longer valid in Italy but still used in Europe) requires a higher threshold with a minimum value of 38% of protein (dry matter) and 7% of ash (dry matter). The data obtained (**Table 2**) show that only a few cheeses conform to the new Italian legislation, whereas most have values that are well under the required threshold. This is most evident looking at the fresh products, where 6 of 15 samples presented values of <3% of dry matter and 10 of 15 samples had protein content lower than 34% of dry matter. Robiola di Roccaverano's dishomogeneous composition is not a new issue (*11*). In fact, this is why this new legislation was created; however, it seems to the authors (personal opinion) that the threshold value proposed is still too rigorous.

ANOVA test showed that the season affected significantly the pH ($p < 0.05$), moisture content, and protein content in the artisanal products, but did not have an effect on the industrial product.

All ripened samples showed some compositional differences due to the moisture contents, which were consistently lower than in the fresh samples ($p < 0.001$), especially in the samples ripened during the summer at a higher room temperature (13−23% of residual water).

As expected, ash and protein contents increased during ripening ($p < 0.05$) as a result of the moisture content decrease.

The industrial fresh product had a ripening index generally higher than the artisanal product: this fact is probably due to the use of starters, which improve the acidification phase prior to the rennet addition, and the various kinds and amounts of rennet added.

The ripening time had also a large influence on pH, which reached values higher than 5.5 ($p < 0.05$) and on the proteolysis rate, leading to an increase of the ripening index, reaching values until a maximum of 95% for an artisanal samples ($p < 0.05$).

The ripening index of ripened winter samples was in general higher than that of ripened summer samples, except for producer C. This fact can be explained by the lower water content observed in summer samples, whereas winter samples had a higher water content, which induced a higher proteolysis.

This information was also confirmed by the electrophoretic analysis of caseins, which revealed in the fresh product a high amount of α_{s1}- and β-caseins quite intact (data not shown), whereas ripened cheeses showed a lot of small fragments originating from the breakdown of α- and β-caseins.

This same finding has also been reported for other fresh cheeses (*29*). In fact, all ripened samples had a greater degree of proteolysis, with the production of abundant fragments from α_{s1}-casein (α_{s1}-I, α_{s1}-II, etc.) and in smaller measure the formation of γ-caseins from β-casein (data not shown).

The gas chromatographic analysis of fatty acids has been performed on both triglyceride fraction (**Table 3**) and free fatty acids (data not shown). The relative percentages of esterified fatty acids, even though reflecting the average composition of similar dairy products (*30*), showed a prevalence of saturated fatty acids as palmitic (28.94 ± 2.06%, average and standard deviation of all cheese samples), myristic (11.13 ± 1.14%), and stearic acid (10.85 ± 1.86%), if compared with the unsaturated fatty acids fraction (most represented: oleic acid, 21.40 ± 2.7%). The fat fraction showed smaller differences due to the ripening process among the FAMEs. Regarding the FFAs, the increases of short-chain fatty acid and of the relative percentage of oleic acid if compared to palmitic and stearic oils were observed (data not shown). This fact could be explained by a series of degradation processes that only the saturated acids have endured, with a consequent production of methylketones, alcohols, and other compounds.

Identification of Milk Used for Robiola di Roccaverano Production. The multiplex PCR experiments allowed us to differentiate with high specificity and sensitivity the milk used for Robiola di Roccaverano cheese production from three closely related species (goat, ewe, and cow). The results obtained confirmed that all of the producers used goat's milk. Moreover, the PCR pattern showed that two artisanal dairies (A and B) used pure goat's milk, whereas the other two (C and D) artisanal producers and the industrial producer used mixed milk (**Figure 1**). The PCR analysis allowed us to know which kind of milk had been added to the goat's milk by each producer: the artisanal dairies C and D added only cow's milk, whereas the industrial producer added a mixture of either goat's and ewe's milk or goat's and cow's milk depending on seasonal availability.

A confirmation of use of mixed milk can also be obtained by looking at the C10/C12 ratio, because a value smaller than 1 suggests the use of cow's milk, whereas a value bigger than 1.80 indicates the use of goat's milk (*30*). As observed with PCR the C10/C12 ratio confirmed that artisanal dairies A and B used only goat's milk (C10/C12 = 2.05 ± 0.26 SD), whereas the other producers used mixed milk (artisanal dairies C and D and the industrial producer E, C10/C12 = 1.39 ± 0.36 SD).

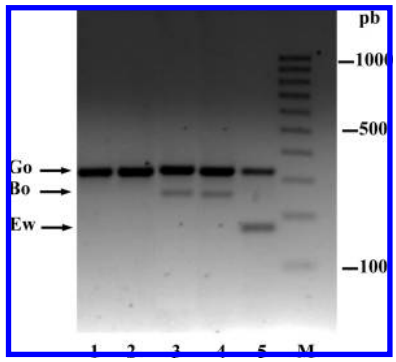

Figure 1. Agarose gel electrophoresis of PCR products amplified obtained from artisanal (A−D) and industrial (E) cheeses. Lanes: M, 100 bp ladder; 1, producer A, goat's milk (indicated as Go); 2, producer B, goat's milk; 3, producer C, mixture of cow's milk (indicated as Bo) and goat's milk; 4, producer D, mixture of goat's and cow's milk; 5, producer E, mixture of ewe's milk (indicated as Ew) and goat's milk.

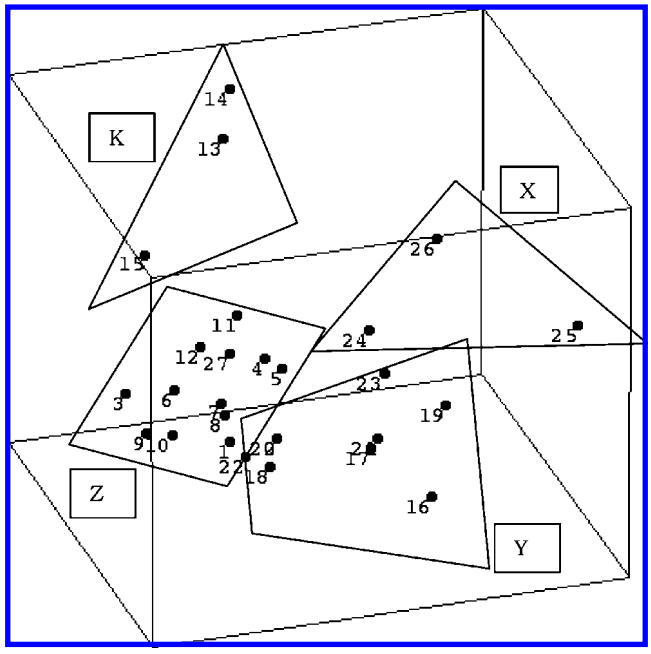

Figure 2. Three-dimensional PCA plot of all the chemical and microbiological data (fresh and ripened cheese samples): cluster X, industrial ripened cheeses; cluster Y, artisanal ripened cheeses; cluster Z, artisanal fresh cheeses; cluster K, industrial fresh cheeses. Total variance explained by the first three PCs = 34%.

Multivariate Statistical Analysis (PCA). All of the data resulting from the different analyses were further studied through PCA. The first aim was to combine these data in different ways to identify the productive origin (i.e., artisanal or industrial and possibly the single producers) and the season of production and to cluster the samples on the basis of ripening time. The second aim was to look for a reduction of the parameters needed to obtain a good discrimination of the samples. Several PCAs were performed to find the best combination of the parameters with the minor number of data possible. Only the PCAs that provided sufficient satisfactory clustering are shown.

The first PCA presented considers simultaneously all of the microbiological and chemical data for fresh and ripened samples (**Figure 2**). In this plot it is possible to observe a discrimination of cheeses as fresh and ripened, but no recognition of the season or of the producers can be seen.

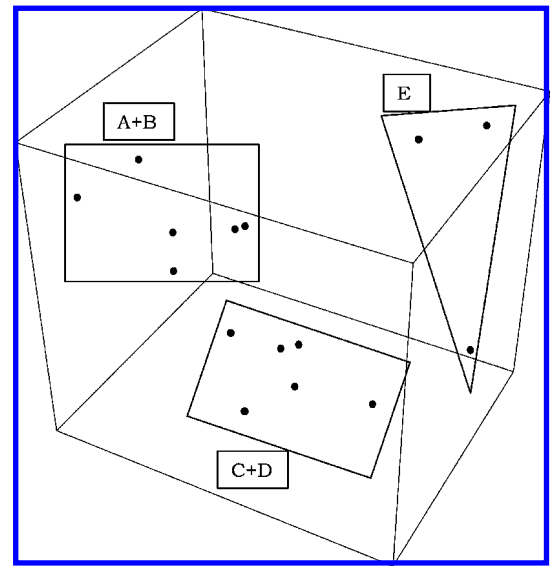

Figure 3. Three-dimensional PCA plot of all the microbiological and chemical data (fresh cheeses). Total variance explained by the first three PCs = 48%.

It is interesting to note that the industrial cheese samples are separated from the artisanal samples, for both fresh and ripened samples, to form two separate clusters.

The second PCA performed results from the evaluation of all the data (chemical and microbiological) as in the previous one, but considering only the fresh cheese samples (**Figure 3**).

An interesting clustering of the samples according to their productive origin can be seen. The cheese samples of producers A and B, which used only goat's milk as demonstrated by the genetic analyses, are located close to each other and form one cluster, whereas the cheese samples of producers C and D, which used a mixture of goat's and cow's milk, are located in a different cluster. The cheese samples of the industrial producer (E) are located quite far from the artisanal samples, confirming what we previously learned by studying the entire cheese batch as a whole. This is probably due to the different operating conditions and the different milk added by the industrial producer (ewe's milk or goat's and cow's milk depending on the availability).

Looking for a parameter reduction, a PCA on microbiological data, FAMEs, and gross composition was performed, obtaining a plot (data not shown) similar to **Figure 3**, showing a very good clustering of the samples based on the productive origin (artisanal or industrial).

From only the microbiological data coupled with gross composition parameters the seasonal influence on cheese characteristics for both fresh (**Figure 4**) and ripened samples (**Figure 5**) was observed. In both cases only two principal components were required to obtain such discrimination.

In **Figure 4**, the artisanal cheese samples are located in three distinct clusters corresponding to the season of production (spring, summer, and winter), whereas industrial samples do not follow this trend (isolated cluster). This is probably due to the standardized operating conditions used by the industrial producer as well the cow's milk pasteurization.

In **Figure 5** for the first time the industrial product is located in the same cluster with artisanal samples. On the contrary, with respect to clusters shown in **Figure 4** three different clusters corresponding to the seasons of production were identified. It is important to note that with this analysis we also obtained the highest value of variance explained (70%). This fact allows us

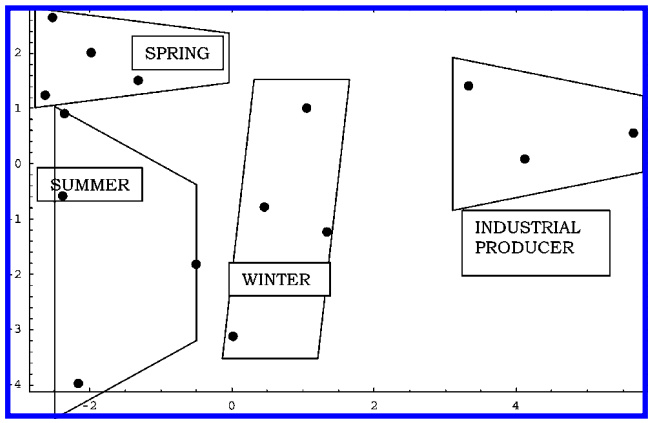

Figure 4. Two-dimensional PCA plot of microbiological data and gross composition (fresh samples). Total variance explained by the first three PCs = 67%.

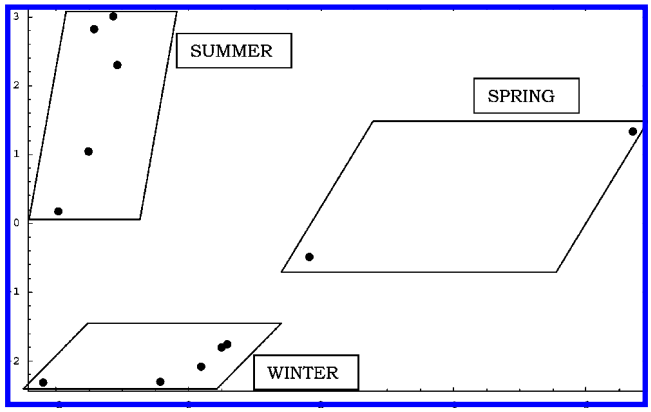

Figure 5. Two-dimensional PCA plot of microbiological data and gross composition (ripened samples). Total variance explained by the first three PCs: = 70%.

to prove that in the ripening the seasonal climate differences prevail over the other possible factors that induce variability among the artisanal and industrial product (as observed in **Figures 4** and **5**).

In summary, the analysis of variance (ANOVA) was able to reveal some differences in product characteristics of the four artisanal producers and the industrial one, particularly evident for microbiological parameters. With regard to the ripening time ANOVA showed that this factor had a large influence, mainly on gross composition, whereas the season affected the microbiological and chemical parameters in artisanal samples, but not in the industrial sample.

The use of multivariate statistical analysis (PCA) allowed us to obtain the clustering of cheese samples based on the age (fresh and ripened) or origin of production (artisanal and industrial) and even on the season of production. When a PCA was performed on all of the data considering all of the cheese samples together, a good discrimination based on cheese age (fresh and ripened clusters) was obtained. Performance of two distinct PCAs for ripened and fresh samples, respectively, using only selected parameters (microbiological data and gross composition) achieved the identification of the seasonal influence on cheesemaking, obtaining the clustering of the cheese samples based on the season of production.

This study demonstrated that by using the entire chemical and microbiological data set it is possible to discriminate between the industrial and artisanal products, but by performing a data reduction, information about the origin and the season of production was also obtained. Therefore, this statistical analysis showed that electrophoretic analyses (SDS- or urea-PAGE), gas chromatographic analyses of FFAs, and HPLC analyses (amino acids and biogenic amines) were not necessary because the resulting data sets were never able to produce a cluster based on producers or season of production. Gross composition, microbiological parameters, and gas chromatographic analyses of FAMEs were the most important parameters for Robiola characterization. In conclusion, the results of the PCA prove that it could be a useful method to recognize the typical characteristics of Robiola di Roccaverano PDO production.

ABBREVIATIONS USED

PDO, Protected Designation of Origin; PCA, principal component analysis; VRBA, Violet Red bile agar; TBX, Tryptone bile X-glucoronide agar; MRS, DeMan, Rogosa, and Sharpe agar; OGYE-agar, oxytetracycline glucose yeast extract agar; cfu, colony-forming units; HPLC, high-performance liquid chromatography; FAMEs, fatty acid methyl esters; FFAs, free fatty acids; SPE, solid phase extraction; PCR, Polymerase Chain Reaction; SDS, sodium dodecyl sulfate; FID, flame ionization detector; ANOVA, analysis of variance; LAB, lactic acid bacteria.

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LITERATURE CITED

(1) Di Cagno, R.; Banks, J.; Sheehan, L.; Fox, P. F.; Brechany, E. Y.; Corsetti, A.; Gobbetti, M. Comparison of the microbiological compositional, biochemical, volatile profile and sensory characteristics of three Italian PDO ewes'milk cheeses. *Int. Dairy J.* **2003**, *13*, 961–972.

(2) Xantopoulos, V.; Polychroniadou, A.; Litopoulou-Tzanetaki, E.; Tzanetakis, N. Characteristics of Anevato cheese made from raw or heat-treated goat milk inoculated with a lactic starter. *Lebensm-Wiss. -Technol.* **2000**, *33*, 483–488.

(3) Cabezas, L.; Sánchez, I.; Poveda, J. M.; Seseña, S.; Palop, M. L. L. Comparison of microflora, chemical and sensory characteristics of artisanal Manchego cheeses from two dairies. *Food Control* **2007**, *18*, 11–17.

(4) Caridi, A.; Micari, P.; Foti, F.; Ramondino, D.; Sarullo, V. Ripening and seasonal changes in microbiological and chemical parameters of the artisanal cheese Caprino d'Aspromonte produced from raw or thermised goat's milk. *Food Microbiol.* **2003**, *20*, 201–209.

(5) Pham, A. M.; Nakai, S. Application of stepwise discriminant analysis to high pressure liquid chromatography profiles of water extract for judging ripening of Cheddar cheese. *J. Dairy Sci.* **1984**, *67*, 1390–1396.

(6) Muir, D. D.; Banks, J. M.; Hunter, E. A. A comparison of the flavour and texture of Cheddar cheese of factory or farmhouse origin. *Int. Dairy J.* **1997**, *7*, 479–485.

(7) Pillonel, L.; Badertscher, R.; Bütikofer, U.; Casey, M.; Dalla Torre, M.; Lavanchy, P.; Meyer, J.; Tabacchi, R.; Bosset, J. O. Analytical methods for the determination of the geographic origin of Emmentaler cheese. Main framework of the project; chemical, biochemical, microbiological, colour and sensory analyses. *Eur. Food Res. Technol.* **2002**, *215*, 260–267.

(8) DLgs. Decreto Ministeriale 13/01/2006. Protezione transitoria accordata a livello nazionale alla modifica del disciplinare di produzione della denominazione di origine protetta "Robiola di Roccaverano" registrata con regolamento (CE) n. 1263/96 della

Commissione del 1° luglio 1996., no. 27 del 02/02/2006. *Gazz. Uff. Repub. Ital.* **2006**.
(9) Marino, M.; Maifreni, M.; Rondinini, G. Microbiological characterisation of artisanal Montasio cheese: analysis of its indigenous lactic acid bacteria. *FEMS Microbiol. Lett.* **2003**, *229*, 133–140.
(10) Coïsson, J. D.; Arlorio, M.; Martelli, A. Chemical characterisation of Robiola di Roccaverano DOP cheese. *Sci. Tecn. Latt-Cas.* **2000**, *51*, 38–49.
(11) Pattono, D.; Grassi, M. A.; Civera, T.; Turi, R. M. Characterisation of hand-crafted "Robiola di Roccaverano" goat milk cheese. *Ind. Aliment.* **2001**, *40*, 1351–1355.
(12) Soncini, G.; Valnegri, L.; Pisoni, G.; Quasso, A.; Moroni, P. Indagine valutativa su latte e formaggi di aziende produttrici di Robiola di Roccaverano. *Latte* **2002**, *27*, 39–42.
(13) Bonetta, Si.; Bonetta, Sa.; Carraro, E.; Coïsson, J. D.; Travaglia, F.; Arlorio, M. Detection of biogenic amine producer bacteria in a typical Italian goat's cheese. *J. Food Prot.* **2008**, *71*, 205–209.
(14) Association Française de Normalisation. Microbiologie des aliments, Recherche des *Salmonella*; Méthode de routine; AFNOR: La Plaine Saint-Denis Cedex, France, 1997; NF V 08-052.
(15) International Organization for Standardization. Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of *Listeria monocytogenes*. Part 2: Enumeration method; ISO: Geneva, Switzerland, 1998.
(16) International Organization for Standardization. Enumeration of *Staphylococcus aureus*, ISO 6888; ISO: Geneva, Switzerland, 1993.
(17) Metodi Ufficiali di analisi dei formaggi. n. 229, D.M. n.88, 21.4. *Off. Ital. J.* **1986**.
(18) Bottero, M. T.; Civera, T.; Nucera, D.; Rosati, S.; Sacchi, P.; Turi, R. M. A multiplex polymerase chain reaction for the identification of cows', goats' and sheeps' milk in dairy products. *Int. Dairy J.* **2003**, *13*, 277–282.
(19) Massart, D. L.; Vandegiste, B. G. M.; Deming, S. N.; Michotte, Y.; Kaufmann, L. *Chemometrics: a Textbook*; Elsevier Science Publishers: Amsterdam, The Netherlands, 1988.
(20) DPR. Decreto del Presidente della Repubblica 14/01/1997 no. 54 - Regolamento recante attuazione delle direttive 92/46 e 92/47 CEE in materia di produzione ed immissione sul mercato di latte e di prodotti a base di latte, no. 54 del 12/03/1997, serie generale; *Gazz. Uff. Repub. Ital.* **1997** (Suppl. Ordinario).
(21) Albenzio, M.; Corbo, M. R.; Rehman, S. U.; Fox, P. F.; De Angelis, M.; Corsetti, A.; Sevi, A.; Gobbetti, M. Microbiological and biochemical characteristics of Canestrato Pugliese cheese made from raw milk, pasteurized milk or by heating the curd in hot whey. *Int. J. Food Microbiol.* **2001**, *67*, 35–48.
(22) Psoni, L.; Tzanetakis, N.; Litopoulou-Tzanetaki, E. Characteristics of Batzos cheese made from raw, pasteurized and/or pasteurized standardized goat milk and a native culture. *Food Control* **2006**, *17*, 533–539.
(23) Grappin, R.; Beuvier, E. Possible implication of milk pasteurization on the manufacture and sensory quality of ripened cheese. *Int. Dairy J.* **1997**, *7*, 751–761.
(24) Macedo, A. C.; Malcata, F. X.; Hogg, T. A. Microbiological profile in Serra ewes'cheese during ripening. *J. Appl. Bacteriol.* **1995**, *79*, 1–11.
(25) Hatzikamari, M.; Litopoulou-Tzanetaki, E.; Tzanetakis, N. Microbiological characteristics of Anevato: a traditional Greek cheese. *J. Appl. Microbiol.* **1999**, *87*, 595–601.
(26) Litopoulou-Tzanetaki, E.; Tzanetaki, N. Microbiological study of white-brined cheese made from raw goat milk. *Food Microbiol.* **1992**, *9*, 13–19.
(27) Kalantzopoulos, G. C. Cheeses from ewes' and goats' milk. In *Cheese: Chemistry, Physics and Microbiology*; Fox, P. F., Ed.; Chapman and Hall: London, U.K., 1996; Vol. 2, pp 507−543.
(28) Guinee, T. P.; Pudja, P. D.; Farkye, N. Y. Fresh acid-curd cheese varieties. In *Cheese: Chemistry, Physics and Microbiology*; Fox, P. F., Ed.; Chapman and Hall: London, U.K., 1996; Vol. 2, pp 363−419.
(29) Arlorio, M.; Coïsson, J. D.; Travaglia, F.; Capasso, M.; Rinaldi, M.; Martelli, A. Proteolysis and production of biogenic amines in Toma Piemontese P.D.O. cheese during ripening. *Ital. J. Food Sci.* **2003**, *15*, 395–404.
(30) Belitz, H. D.; Grosch, W.; Schieberle, P. *Food Chemistry*, 3rd ed.; Springer-Verlag: Berlin, Germany, 2000.

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