CHARACTERISTICS OF GOAT MILK PRODUCED IN THE ASPROMONTE MASSIF (CALABRIA, ITALY)

CARATTERISTICHE DEL LATTE CAPRINO PRODOTTO IN ASPROMONTE

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ABSTRACT

Physico-chemical parameters – pH (mean 6.55-6.81), fat (mean 3.95-5.68%), protein (mean 3.57-4.30%), lactose (mean 4.63-4.95%), and dry matter (mean 14.02-15.28%) – microbiological parameters – somatic cell (mean 5.1-5.7 Log mL^{-1}) and total microbial count (mean 4.0-5.0 Log CFU mL^{-1}) – and coagulation parameters – rennet coagulation time (r - mean 13'03"-17'22"), curd firming rate (k_{20} - mean 1'37"-2'32"), and curd firmness after 30 min (a_{30} - mean 33.13-53.67) – were determined in 300

RIASSUNTO

Sono stati determinati, in 300 latti individuali di capre autoctone, i parametri fisico-chimici, pH (media 6,55-6,81), grasso (media 3,95-5,68%), proteina (media 3,57-4,30%), lattosio (media 4,63-4,95%) e sostanza secca (media 14,02-15,28%), i parametri microbiologici, cellule somatiche (media 5,1-5,7 Log mL^{-1}) e conta microbica totale (media 4,0-5,0 Log CFU mL^{-1}) ed i parametri lattodinamografici, tempo di coagulazione (r - media 13'03"-17'22"), velocità di formazione del coagulo (k_{20} -

- Key words: goat milk, microbial groups, milk coagulation, raw milk -

individual native goat milk samples. Six bulk milk samples were also analysed. Most of the individual milk samples conformed to the European Community and Italian standards, but the bulk milk quality must be improved.

media 1'37"-2'32") e consistenza del coagulo a 30 min (a_{30} - media 33,13-53,67). Sono stati analizzati anche sei latti massali. La maggior parte dei latti individuali esaminati rientra nei parametri stabiliti dalla legislazione italiana ed europea, mentre la qualità dei latti massali necessita di interventi migliorativi.

INTRODUCTION

In the Mediterranean basin the raising of goat herds has increased in recent years. The ability of goats to use relatively poor food resources and, even thrive on marginal lands that would otherwise be unsuitable for animal production, is creating an ever-increasing interest in this type of livestock. This is particularly true in areas where the traditional rural system uses almost exclusively the native goat breeds. The increase in the knowledge about the composition and quality of goat milk is certainly important (CERUTTI, 1997) in helping to safeguard a distinctive Mediterranean product that is part of the local history and culture. Goat milk is the third most produced milk in the world (KLINGER and ROSENTHAL, 1997) and its production has been rising steadily, partially because of its good nutritional value (GOMES and MALCATA, 1998). However, unlike cow milk, which is subjected to stringent hygiene and quality regulations, the microbiological standards for the production and distribution of goat milk are less severe. The specific microflora (CASLA *et al.*, 1996; ZÁRATE *et al.*, 1997) and the microbiological problems connected with the poor hygienic standards for goat milk are well described (CLEMENTI *et al.*, 1998; THAM *et al.*, 1990). Isolation of bacteria responsible for human infections, such as brucellosis (WYATT, 1999) and listeriosis (ABOU-ELEININ *et al.*, 2000), from raw goat milk is often reported. In particular, *Listeria monocytogenes* was identified in 17 out of 35 Listeria-positive samples; *Listeria* isolation rates were markedly higher during winter (14.3%) and spring (10.4%), compared to autumn (5.3%) and summer (0.9%).

On the Calabrian Aspromonte Massif, in an area overlooking the Ionian coast in the province of Reggio Calabria, milk is almost exclusively produced from native populations of goats. These goats are characterised by high adaptability, morphological heterogeneity and an ability to exploit all the vegetative resources available in this desolate environment. The area, situated prevalently between 300 and 800 m asl, is largely inaccessible to humans and is therefore used almost exclusively for goat production. Each dairy farm has 180-200 goats and a few sheep, all half-wild. The pasture is primarily natural, with over 50 forage species. Lactation lasts about 200 days – from January to July – and the daily production of goat milk in the whole area is 10 tons. Each goat is milked by hand twice a day in the early-morning and the evening and gives from 0.5 to over 1 L per day.

On the Calabrian Aspromonte Massif goat milk is usually processed to make the distinctive Caprino d'Aspromonte cheese. It is manufactured in small dairies which do not have adequate techni-

cal and scientific knowledge for large-scale controlled production.

To date no research has ever been conducted on the goat milk produced on the Aspromonte Massif. Therefore, the purpose of the present work was to study the physico-chemical, microbiological, and coagulation characteristics of the raw milk. These characteristics were used to identify the hygienic problems connected with the raw milk, because the milk quality is fundamental and preliminary to the success of the cheese-making process and, therefore, to good cheese quality. The influence of the season, the farm, and the interaction season/farm were also studied.

MATERIALS AND METHODS

Three dairy farms, denominated I, II, and III, located at three altitudes in the province of Reggio Calabria, were chosen as representative of the production area. A total of 300 individual milk samples were collected by milking by hand directly into sterilised vessels in the early morning. Of these samples, 150 were simultaneously collected in the spring of 2000 (50 from each farm) and 150 were simultaneously collected in the winter of 2001 (50 from each farm). Each time the individual samples were collected, one bulk milk sample was also collected, giving a total of six bulk milk samples. The bulk milk samples were obtained by combining the bulk milk from the evening before, left at room temperature, with the early-morning bulk milk. The milk samples were transported to the laboratory in sterile, refrigerated containers (4°C) and maintained at this temperature for a maximum of 24 h.

The 300 individual milk samples were analysed using a pH-meter (model 355, Mettler Toledo, Greifensee, Switzerland), a Milkoscan (model 134, FossElectric, Hillerød, Denmark) and a Bactoscan (model 8000, FossElectric, Hillerød, Denmark), for the following parameters: pH, dry matter, fat, protein, lactose, somatic cell count and total microbial count. The rennet coagulation properties of individual milk samples were determined according to DURANTI (1995). Rennet coagulation time (r in min), curd firming rate (k_{20} in min) and curd firmness measured 30 min after the addition of rennet (a_{30} in mm) were determined using a lactodynamograph (Maspres, Firenze, Italy) in 10 mL of milk at 35°C with the addition of 200 μL of standard calf rennet (CHR Hansen, Corsico, Italy) with an original strength of 160±8 IMCU mL^{-1}, diluted to 1.6%.

The six bulk milk samples were also analysed for the above-listed physico-chemical parameters as well as for the following microbiological parameters: total microbial count, according to APHA (1978), on Plate Count Agar (Oxoid LTD, Hampshire, England) with the addition of 1 g L^{-1} of skim milk (Merck, Darmstadt, Germany) and incubated aerobically at 32°C for 72 h; coliforms, according to APHA (1978), on Violet Red Bile Agar (Fluka, Buchs, Switzerland) incubated aerobically at 32°C for 24 h; *Escherichia coli*, according to NMKL (1993), on 3M Petrifilm for *E. coli* and coliforms (3M Microbiology Products, St. Paul, USA) incubated aerobically at 37°C for 48 h; mesophilic and thermophilic lactobacilli, according to DE MAN *et al.* (1960), on MRS Agar (Biokar Diagnostics, Beauvais, France) acidified to pH 5.4 with acetic acid and incubated anaerobically, using a gas-pack catalyst (Oxoid LTD, Hampshire, England), at 22°C for 96 h or at 45°C for 48 h; mesophilic and thermophilic coccal-shaped lactic acid bacteria, according to TERZAGHI and SANDINE (1975), on M17 Agar (Fluka, Buchs, Switzerland) incubated anaerobically at 22°C for 96 h or at 45°C for 48 h; ente-

rococci, according to NMKL (1992), on Slanetz-Bartley Agar (Biokar Diagnostics, Beauvais, France) incubated aerobically at 45°C for 48 h; yeasts, according to IDF (1990), on Yeast Glucose Chloramphenicol Agar (Fluka, Buchs, Switzerland) incubated aerobically at 22°C for 96 h. The spread plate technique (0.1 mL) was used for all media, except Violet Red Bile Agar where the pour plate technique (1 mL) with overlay was used. Results are expressed as a logarithm (log) of colony forming units (CFU) mL^{-1} of milk.

All the analytical determinations were repeated twice and the reported values are the means of two analyses. The effect of season, farm, and the interaction between season and farm was statistically evaluated by ANOVA analysis (SAS system), with the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

where Y_{ijk} is the middle experimental value, μ is the general mean, α_i is the season effect, β_j is the farm effect, $(\alpha\beta)_{ij}$ is the effect of the interaction between season and farm, and eijk is the residual error. The significance of the paired comparison was determined by the Student *t* test.

RESULTS AND CONCLUSIONS

The physico-chemical and microbiological parameters determined on the 300 individual milk samples are reported in Fig. 1-3. The significance of the comparisons, the significant effects, and the least square means are reported in Tables 1 and 2.

The pH of the milk (Table 2), which is indicative of the hygienic state of the goat and the milking procedure, was always lower in the spring (mean 6.55-6.60) than in the winter (mean 6.80-6.81). The spring values fell within the acceptable range for goat milk (ANIFANTAKIS and KANDARAKIS, 1980; KALANTZOPOULOS, 1993; SIMOS *et al.*, 1991; VEINOGLOU *et al.*, 1982; VOUTSINAS *et al.*, 1990). The second (6.40<pH<6.59) and third (6.60<pH<6.79) frequency classes were the most representative in spring, and the fourth (pH≥6.80) was most representative in winter (Fig. 1). A comparison among farms did not show significant differences, while a comparison between seasons showed significantly lower pH levels (P<0.01) in the spring.

Fat content (Table 2) varied significantly (P<0.01) with seasonal variations being lower in the winter (mean 3.95-4.98%) and higher in the spring (mean 4.20-5.68%). Similar levels have been reported for Greek goat milk (KALANTZOPOULOS, 1993; SIMOS *et al.*, 1991; VEINOGLOU *et al.*, 1982), while GRAPPIN *et al.* (1981), JOUBERT (1973) and PIERRE *et al.* (1998) reported lower values. The second frequency class (Fig. 1) (3.50%<fat<4.99%) was the most numerous. A comparison among farms, for each sampling period, was significant (P<0.05-0.01), except between farm I and farm III in the winter. The milk with the highest fat content was always produced on farm II.

The protein content (Table 2) was not statistically significant between spring (mean 3.57-4.11%) and winter (mean 3.74-4.30%). Analogous values have been reported for goat milk produced in Sardinia (COSSEDDU and PISANU, 1979), India (QURESHI *et al.*, 1981), and Libya (GNAN *et al.*, 1985); while, other authors have reported notably lower values (MBA *et al.*, 1975; MENA and ESCAMILLA, 1977; PRIMATESTA, 1979). The second frequency class (Fig. 1) was the most representative (3.50%<protein<4.99%). The comparison among farms was significant (P<0.01), with higher protein content in the winter milk from farm I and in the spring milk from farms I and II. This explains the significant season x farm interaction.

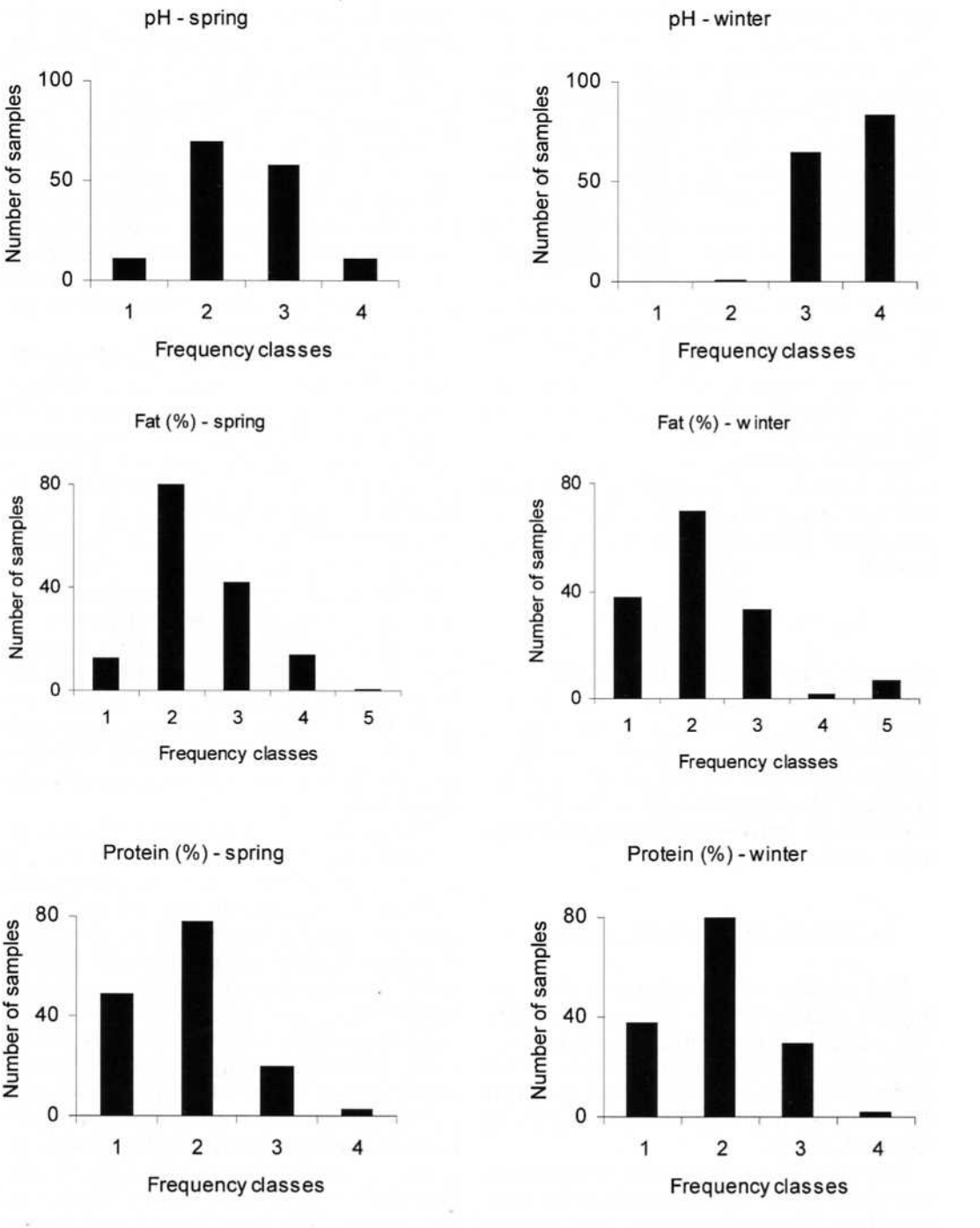

Fig. 1 - Trend of pH, fat, and protein in individual milk samples in the spring and winter. Frequency classes – pH: 1 (6.20-6.39), 2 (6.40-6.59), 3 (6.60-6.79), 4 (≥6.80); fat: 1 (2.00-3.49), 2 (3.50-4.99), 3 (5.00-6.49), 4 (6.50-7.99), 5 (≥8.00); protein: 1 (2.50-3.49), 2 (3.50-4.49), 3 (4.50-5.49), 4 (5.50-6.49).

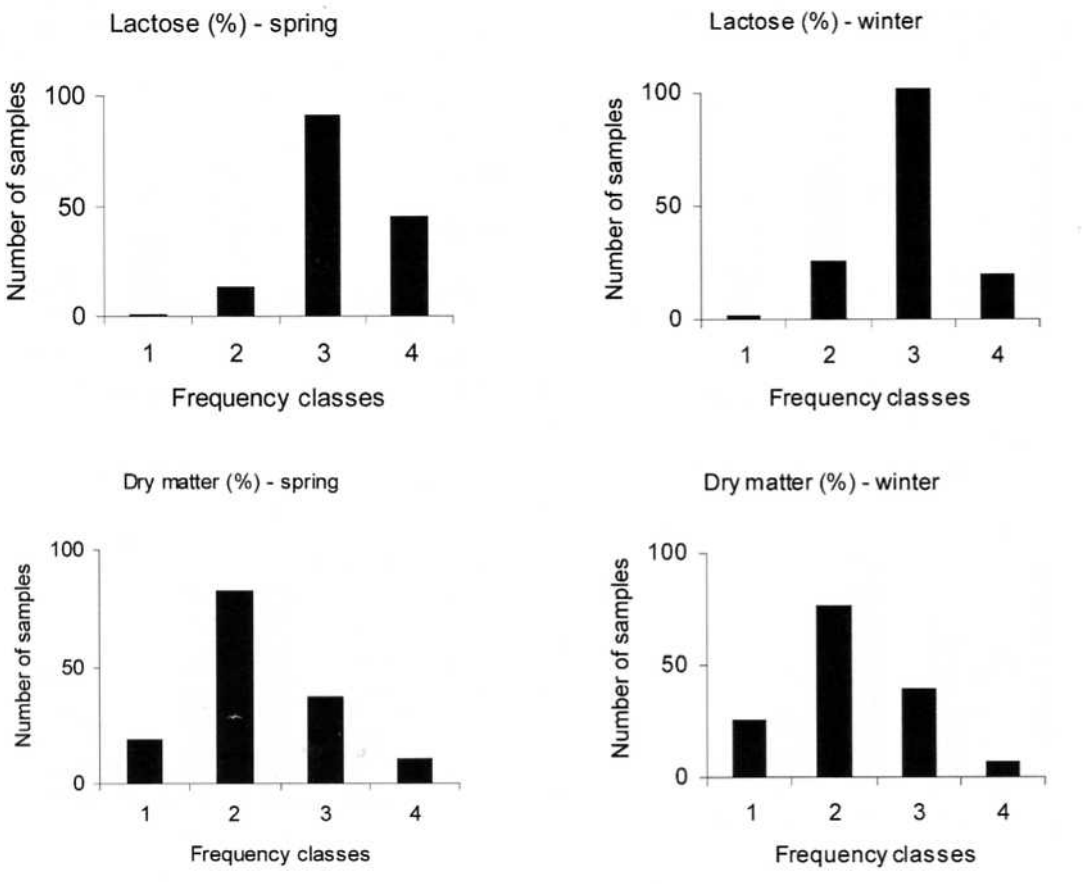

Fig. 2 - Trend of lactose and dry matter in individual milk samples in the spring and winter. Frequency classes – lactose: 1 (3.50-3.99), 2 (4.00-4.49), 3 (4.50-4.99), 4 (≥5.00); dry matter: 1 (11.00-12.99), 2 (13.00-14.99), 3 (15.00-16.99), 4 (17.00-18.99).

The average lactose values (Table 2) varied from 4.63 to 4.95% and were very similar to those reported in Greece for the Metsovo breed (SIMOS *et al.*, 1991) and in Italy for the Maltese breed (CLEMENTI *et al.*, 1998). The third frequency class (Fig. 2) was the most numerous (4.50%<lactose<4.99%). Significant differences ($P<0.01$) were noted between seasons and, for each season, among farms. This is quite important, considering that all the milk is processed into cheese. The season x farm interaction was also significant ($P<0.05$), with variability in the lactose content of the milk.

The very high dry matter values (mean 14.02-15.28%) (Table 2) are in agreement only with those reported for the Metsovo breed (SIMOS *et al.*, 1991). In contrast, the values reported for the British Alpine breed (DEVENDRA, 1972), the Saanen breed (CHANG and KIM, 1978; PILLA *et al.*, 1980) and for goat milk in general (MERIN *et al.*, 1988; MIDDLETON and FITZ-GERALD, 1981; SAWAYA *et al.*, 1984) are much lower. The second frequency class (Fig. 2) was the most representative (13.00%<dry matter<14.99%). Only the differences among farms for each season were significant. Compared to the others, farm

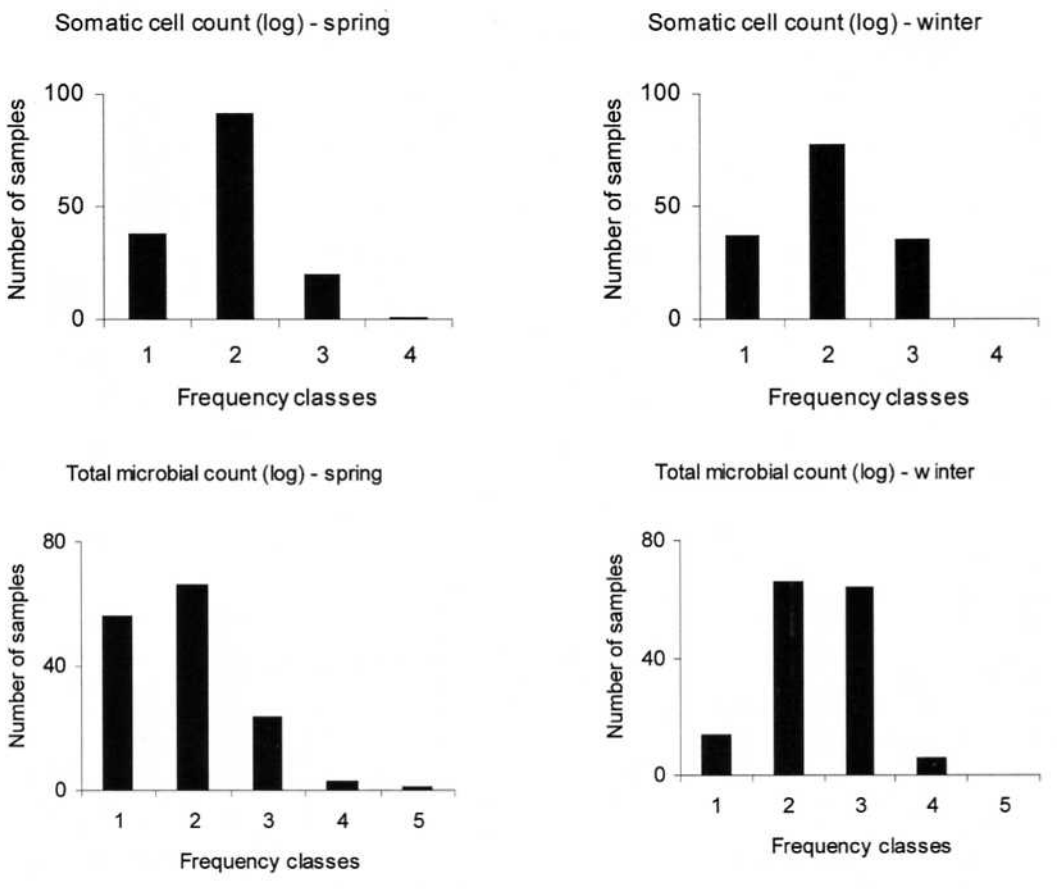

Fig. 3 - Trend of somatic cell count and total microbial count in individual milk samples in the spring and winter. Frequency classes – somatic cell count: 1 (4.00-4.99), 2 (5.00-5.99), 3 (6.00-6.99), 4 (≥7.00); total microbial count: 1 (3.00-3.99), 2 (4.00-4.99), 3 (5.00-5.99), 4 (6.00-6.99), 5 (≥7.00).

Table 1 - Significant effects regarding the physico-chemical and microbiological parameters determined on the 300 individual goat milk samples.

Significance effects	SED[a]	Season			Farm			Season x farm		
		MS[b]	F[c]	Pr[d]<F	MS	F	Pr<F	MS	F	Pr<F
pH	0.120	3.905	270.40	0.0001	0.023	1.61	0.2008	0.012	0.82	0.4412
Fat (%)	1.129	14.075	11.04	0.0010	40.558	31.81	<0.0001	1.479	1.16	0.3150
Protein (%)	0.597	0.231	0.65	0.4200	5.789	16.25	<0.0001	3.086	8.66	0.0002
Lactose (%)	0.249	1.393	22.43	<0.0001	1.330	21.42	<0.0001	0.238	3.83	0.0228
Dry matter (%)	1.390	0.900	0.47	0.4954	24.859	12.87	<0.0001	5.737	2.97	0.0529
Somatic cell count (log/mL)	0.498	0.523	2.11	0.1479	7.767	31.26	<0.0001	0.149	0.60	0.5503
Total microbial count (log/mL)	0.596	17.442	49.05	<0.0001	8.505	23.92	<0.0001	3.216	9.04	0.0002

[a] Standard Error Deviation; [b] Mean Square; [c] Fisher F; [d] Probability.

Table 2 - Least square means regarding the physico-chemical and microbiological parameters determined on the 300 individual goat milk samples. (a, b, c: P<0.05; A, B, C: P<0.01 indicate the significance of the comparison in the same row and by season).

Season	Spring						Winter					
Farm	I		II		III		I		II		III	
pH	6.573		6.604		6.555		6.813		6.807		6.796	
Fat (%)	4.199	Aa	5.685	B	4.689	Ab	3.952	A	4.977	B	4.345	A
Protein (%)	3.971	A	4.114	A	3.567	B	4.299	A	3.773	B	3.747	B
Lactose (%)	4.694	A	4.950	B	4.880	B	4.639	A	4.841	B	4.636	A
Dry matter (%)	14.024	A	15.282	B	14.294	A	14.066	a	14.636	b	14.294	ab
Somatic cell count (log/mL)	5.532	A	5.079	B	5.445	A	5.665	A	5.074	B	5.569	A
Total microbial count (log/mL)	4.948	A	4.028	aB	4.319	Bb	5.046		4.837		4.858	

II always produced milk with higher dry matter content.

The somatic cell count of milk depends on the health of the udder, on milking hygiene and on many stress factors. WHITE and HINCKLEY (1999) reported that the detection of pathogen-free goat milk samples that contain more than 10^6 somatic cells/mL demonstrate that elevated somatic cell counts alone are not a valid indication of mammary infection in goats. Although reference values for goat milk have not yet been established, a comparative study was recently conducted on the effects of the testing laboratory, counting method, storage and shipment on somatic cell counts in goat milk (ZENG *et al.*, 1999). The somatic cell counts of these samples (Table 2) fell (mean 5.1-5.7 Log mL^{-1}) within the normal range for milk from healthy goats (HUNTER, 1984; POUTREL *et al.*, 1997; ROGUINSKI *et al.*, 1980; SCHULZ, 1994). The second frequency class (Fig. 3) was the most representative (5.00%<Log somatic cell count<5.99%). The differences among farms for this parameter were highly significant (P<0.01), showing lower somatic cell counts in both seasons in milk samples from farm II.

Regarding the total microbial count, the European Community Directive (CD, 1992) and Italian law (DPR, 1997) dictate that raw goat milk used to manufacture dairy products is permitted to have a maximum of $5.0x10^5$ microorganisms/mL at 30°C. These laws, however, do not take into account the important differences among the types of microorganisms present. The majority of the milk samples (Fig. 3) comply with this limit in both seasons. Total microbial count (Table 2) was significantly different between spring (mean 4.0-4.9 Log CFU mL^{-1}) and winter (mean 4.8-5.0 Log CFU mL^{-1}) and among farms, only in the spring. This explains the result of the season x farm interaction. In spring (Fig. 3) the vast majority of the milk samples were included in the first two frequency classes (3.00<Log total microbial count<4.99), while in winter most of the milk samples were included in the second and the third frequency classes (4.00<Log total microbial count<5.99).

According to CHIOFALO and MICARI (1987), the analysis of goat milk using a lacto-dynamograph shows five different types of tracings. Among these, type C corresponds to the tracing of a milk with average dairy quality, with r-values around 12 min, k_{20} about 6 min, and a_{30} around 32 mm. Type E corresponds to the tracing of a milk with a dairy quality

poorer than type C; effectively, its coagulation parameters are r-values around 19 min, k_{20} about 7 min, and a_{30} around 28 mm. The coagulation properties were determined on the individual milk samples in spring 2000 and in winter 2001 (Table 3); the three tested parameters showed normal behaviour in both seasons (CASTAGNETTI *et al.*, 1984; PIERRE *et al.*, 1998; STORRY *et al.*, 1983). The a_{30} parameter was lower in the spring milk samples than in the winter samples. This demonstrates that in spring there are more milk samples with lower coagulation ability, although most milk samples coagulated after the addition of rennet, both in winter and in spring (97% for farm I, 95% for farm II, and 92% for farm III, averaging the values of the two seasons). The coagulation profiles for these milk samples fell within types C and E, because they produced a soft clot, which was slow to compact, and was prone to spontaneous syneresis. The shorter coagulation time of the spring milk compared to the winter milk can be primarily attributed to the lower pH of the milk, as often described (REMEUF *et al.*, 1991).

The results of the physico-chemical and microbiological analyses, performed on the bulk milk samples in spring 2000 and in winter 2001, are reported in Table 4. The physico-chemical parameters and the somatic cell counts of the bulk milk were analogous to those of the 300 individual milk samples. It is interesting to note that three of the six bulk milk samples had low total microbial counts (5.2-5.7 Log CFU mL^{-1}), similar to the data reported by ESPIE and MULLAN (1987) and TIRARD-COLLET *et al.* (1991). On the contrary, the other three samples had very high total microbial counts (6.3-7.3 Log CFU mL^{-1}), similar to counts reported for goat milk used to manufacture different varieties of cheese (ABO-ELNAGA *et al.*, 1985; GUTIÉRREZ *et al.*, 1988; MAS MAYORAL *et al.*, 1991). The coliform content in four of the six samples (3.2-5.0 Log CFU mL^{-1}) was similar to other reports (LOPEZ-DIAZ *et al.*, 1995; MEDINA *et al.*, 1992), whereas the remaining two samples had notably higher values (6.6-7.0 Log CFU mL^{-1}). These alarming results are due to a few, easily identifiable causes. Firstly, in the production area, by tradition, the early-

Table 3 - Mean, minimum and maximum of the coagulation parameters determined on the 300 individual goat milk samples.

	I			II			III		
Farm	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Spring									
Rennet coagulation time (r in min)	13' 03"	7' 15"	24' 30"	15' 17"	10' 30"	23'	13' 19"	6'	23'
Curd firming rate (k_{20} in min)	1' 46"	1' 15"	9' 45"	2' 32"	1' 30"	8' 15"	2' 08"	1' 15"	8'
Curd firmness after 30 min (a_{30} in mm)	34.94	6.50	66.50	37.76	17.88	64.44	33.13	12.12	63.42
Winter									
Rennet coagulation time (r in min)	16' 42"	8' 45"	23'	15' 41"	10' 15"	22' 45"	17' 22"	12' 45"	21'
Curd firming rate (k_{20} in min)	1' 37"	45"	3' 45"	1' 41"	1' 30"	4'	2' 16"	1' 30"	4' 45"
Curd firmness after 30 min (a_{30} in mm)	53.67	34.86	62.20	47.26	18.24	67.42	47.05	29.72	63.82

Table 4 - Physico-chemical and microbiological parameters determined on the six bulk milk samples.

Season	Spring			Winter		
Farm	I	II	III	I	II	III
pH	6.38	6.45	6.39	6.67	6.64	6.70
Fat (%)	5.39	5.14	5.46	4.85	4.99	5.02
Protein (%)	4.39	4.12	3.66	4.28	3.63	4.73
Lactose (%)	4.75	5.00	4.99	4.66	4.84	4.88
Dry matter (%)	15.38	15.11	14.96	14.98	15.26	15.07
Somatic cell count (log/mL)	5.777	5.415	5.401	6.289	5.394	6.239
Total microbial count (log/mL)	7.322	5.643	5.204	6.322	6.978	5.716
Coliforms (log/mL)	7.000	4.964	3.176	3.934	6.568	4.398
Escherichia coli (log/mL)	4.934	3.380	2.301	2.778	6.568	1.845
Mesophilic lactobacilli (log/mL)	5.982	3.477	3.919	5.114	5.301	3.505
Thermophilic lactobacilli (log/mL)	4.041	2.462	2.580	3.255	4.041	4.000
Mesophilic coccal-shaped lactic acid bacteria (log/mL)	6.556	4.886	4.949	5.892	6.806	5.255
Thermophilic coccal-shaped lactic acid bacteria (log/mL)	6.633	5.602	3.845	4.857	6.633	4.114
Enterococci (log/mL)	3.968	3.973	3.778	3.255	4.602	4.079
Yeasts (log/mL)	3.643	2.580	2.380	3.699	2.114	1.954

morning bulk milk is added to the bulk milk of the evening before, which has been left unrefrigerated overnight in non-sterilised containers, in an environment that is far from aseptic. Secondly, the hand milking is performed in unhygienic conditions. By simply addressing these two factors, however, and improving the hygienic-sanitary conditions of the milking and management environment, the total microbial count and the coliform numbers could be lowered to meet acceptable levels. Among the lactic acid bacteria, the mesophilic coccal-shaped lactic acid bacteria predominated in winter (5.3-6.8 Log CFU mL^{-1}) and the thermophilic coccal-shaped lactic acid bacteria predominated in spring (3.8-6.6 Log CFU mL^{-1}). The number of coccal-shaped lactic acid bacteria was 1-2 Log units higher than the lactobacilli, as reported for other goat milk samples (FATICHENTI *et al.*, 1979; MAS MAYORAL *et al.*, 1991; TORNADIJO *et al.*, 1995). The number of enterococci (3.3-4.6 Log CFU mL^{-1}) was within the range observed for other goat milk samples (CLEMENTI *et al.*, 1998; FONTECHA *et al.*, 1990; ZARATE *et al.*, 1997). The number of yeasts was rather low (1.9-3.7 Log CFU mL^{-1}), around 30% of them fermented lactose with production of gas.

The results of this study show that the qualitative characteristics of most of the individual milk samples from the three farms conformed to the standards laid down by the Italian and European directives. Implementation of improved hygienic-sanitary practices in response to the needs manifested in the present study, together with a selective breeding program, may improve the quality of goat milk produced in the Calabrian Aspromonte Massif.

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