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The effect of a commercial starter culture addition on the ripening of an artisanal goat's cheese (Cameros cheese)

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C. OLARTE, S. SANZ, E. GONZALEZ-FANDOS AND P. TORRE. 2000. The evolution of physicochemical parameters, and the most important microbial groups, were determined for the following three batches of 'Cameros' goat's milk cheese during ripening: Batch R elaborated with raw milk, Batch RS elaborated with raw milk and with the addition of a starter culture, and Batch PS elaborated with pasteurized milk and with the addition of the same culture. No differences in total solids (TS) or in the content of NaCl, fat and total nitrogen (expressed as percentages of TS) were found during the ripening. The pH, fat acidity and non-protein nitrogen (NPN, expressed as a percentage of TN) showed significant differences between the batches. The inoculated batches showed the fastest drop in pH at the beginning of the ripening period, but the cheeses of Batch R showed a higher degree of lipolysis and proteolysis. The addition of a starter influenced the microbiological quality of the cheeses. Differences in the counts of Enterobacteriaceae and faecal coliforms were found between Batches R and RS after 15 days. *Staphylococcus aureus* increased in number during the early period of ripening and attained a population above 6 log cfu g^{-1} in Batch R in the period from 5 to 10 days. However, enterotoxins were not detected in this Batch. Batch R showed lower values of lactic acid bacteria at the beginning of the ripening period, but no significant differences were found between batches in the period from 5 to 15 days of ripening. At the beginning of the ripening, *Lactococcus* was the main lactic acid bacteria, with *L. lactis lactis* being predominant. After 15 days, the lactic acid bacteria counts decreased in the three batches, especially in the cheeses of Batch PS (only 2·2 log cfu g^{-1} was found at 60 days), as lactococci (the only lactic acid bacteria present in Batch PS) are incapable of growing under the conditions found in cheeses at the end of their ripening period. At this time, *Lactobacillus* was the predominant genus in Batches R and RS, with *L. plantarum* predominant. No lactococci were found from day 30 in Batch R and from day 40 in Batch RS. The cheeses of Batch RS received the most favourable scores from the tasting panel for all attributes judged: cut appearance, colour, aroma, taste, texture and general acceptance.

INTRODUCTION

World production of goat's milk has increased in recent years, especially in the Mediterranean area. Like other countries of the region, Spain produces a large quantity of goat's milk (approximately 4×10^8 l $year^{-1}$). This milk is mainly used for on-farm cheese manufacturing, with or without thermal treatment. It is difficult to calculate the quantities of industrial cheese made only from goat's milk as it is generally blended with sheep's and/or cow's milk. Although 28 varieties of goat's cheese (Anon. 1990) are produced in Spain, its market

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share is very low. This is mainly due to a lack of data on production methods, biochemical and microbiological characteristics, which are an essential first step for manufacture under controlled conditions.

Given that the consumption of goat's milk cheese represents an alternative in the markets of developed countries, and that farming with small ruminants should be one of the objectives of aid programmes in developing countries (Le Jaoune 1990), an examination of the product quality is important. However, the information available on most goat's cheese is scarce and of little value for practical purposes. Among the Spanish cheeses, Majorero cheese (Martín-Hernández *et al.* 1984; Fontecha *et al.* 1990; Requena *et al.* 1992), Cendrat del Montsec cheese (Mor-Mur *et al.* 1992), Armada cheese (Tornadijo 1995; Fresno *et al.* 1996), Valdeteja cheese (Fresno *et al.* 1988; Carballo *et al.* 1994) and a few others have been studied relatively extensively. With regard to other countries, Greek goat's cheeses have been studied by Litopoulou-Tzanetaki and Tzanetaki (1992) and Tzanetakis *et al.* (1995). Park (1990) studied the characteristics of goat's cheese made in the USA and Sablé *et al.* (1997) studied a French goat's cheese.

Cameros is a fresh cheese traditionally made in the Cameros mountains (La Rioja, Spain) from goat's milk. Although its manufacture and consumption fell considerably in the 1960s, it is presently undergoing a period of recovery due to the support of programmes for the development of the rural environment promoted by regional and European governments. Since the end of the 1980s, therefore, small industries have been set up in the area, for the elaboration of this type of cheese in a semi-artisanal way, in an attempt to obtain a product with characteristics similar to the traditional cheese, but also incorporating the hygiene and health regulations legally established for this type of product. The production of Cameros cheese is very limited because of the special problems involved, i.e., the lack of goat's milk during some periods of the year and the situation of the producing area, which makes distribution especially difficult.

Some small dairies have recently begun producing ripened cheese from goat's milk. This kind of cheese has a longer shelf-life and its production and distribution are less problematic. Initially, the cheese-makers tried different options, such as the use of raw or pasteurized milk, with or without the addition of starter cultures. Characterization of this 'new' type of Cameros cheese is now necessary in terms of defining the microbiological, physicochemical and organoleptic characteristics of the final products obtained, as well as the changes which occur during ripening.

The aim of this work was to evaluate the quality and characteristics of ripened Cameros cheese, the influence of the thermal treatment of the milk, and the addition of starter cultures during ripening.

MATERIALS AND METHODS

Cheese-making and sampling

Three cheese batches were prepared in a pilot plant: Batch R was made from raw goat's milk without a starter, Batch RS used the same raw milk but with the addition of a starter, and Batch PS was prepared with pasteurized milk using the same starter. The batches were elaborated in duplicate.

The starter culture used was EZAL MA-400 ('artisanal') supplied by Rhône-Poulec Química (Texel-Meyhall, Madrid, Spain). According to the manufacturer, its composition is: *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* (15%) and *Streptococcus thermophilus* (5%).

The elaboration procedure in the pilot plant was as similar as possible to the method used by the cheese-makers themselves. The milk was coagulated at 32 °C, with the addition of about 0·15 ml commercial calf rennet (strength 1/10000; (Laboratorios Arroyo, Santander, Spain) l^{-1} milk; Ca_2Cl (0·2 $g1^{-1}$ maximum) was also added to improve curd firmness. After 1 h, the curd, compact at this stage, was cut by hand, placed in moulds and pressed for 12 h. The cheese was then dry-salted for 5 d at 18–20 °C and 70–80% humidity. The cheese-ripening was completed in a climate-controlled chamber at 14 °C and 90% humidity.

Batch PS was elaborated with pasteurized milk. The milk was heated to 74 °C and held at this temperature for 20 s. It was then cooled to the coagulation temperature. The phosphatase test (Lactognost Test; Heyl, Berlin, Germany) was carried out to determine whether pasteurization had taken place correctly.

After removing the cheeses from the moulds (day 0) and during ripening (2, 5, 10, 15, 30, 45 and 60 days), samples were taken and kept at refrigeration temperature (4 °C) until analysis. A maximum of 12 h elapsed between sampling and microbiological analysis. The sensory characteristics of the ripened cheeses were evaluated on day 60.

Physicochemical analyses

The pH was measured with a Crison model 2002 pH meter with a penetration electrode (Crison Instruments, Barcelona, Spain). Total solids (TS) were determined according to FIL-IDF 4 A: 1982 (Anon. 1982). The NaCl content was determined by the procedure described by Jonhson and Olson (1985). Fat content was analysed according to the Gerber procedure (Anon. 1969) and the acidity index of the fat was determined by a titration method (Casado 1991) after fat extraction (Soxtec System, Tecator Instruments, Höganäs, Sweden). Total nitrogen (TN) and non-protein nitrogen

(NPN) were determined according to FIL-IDF 25 : 1964 (Anon. 1964).

All analyses were performed in duplicate.

Microbiological analyses

Cheese samples (25 g) were homogenized for 1 min in 225 ml of a sterile solution (2% w/v) of sodium citrate using a Stomacher (IUL, Barcelona, Spain). Further decimal dilutions were prepared with the same diluent. Analyses were carried out using the following procedures.

Total aerobic mesophilic micro-organisms were enumerated on plate count agar (Difco) using the pour plate method with incubation at 31 °C ± 1 °C for 72 h (ICMSF 1978).

Enterobacteriaceae were determined on Violet Red Bile Glucose agar (Difco). The plates were overlaid prior to incubation at 37 °C ± 1 °C for 18–24 h (ICMSF 1978).

Faecal coliforms were determined by the MPN method for a three tubes series in Brilliant Green Lactose Broth (BGBL, Difco) incubated at 44 °C for 48 h (ICMSF 1978).

Micrococcaceae were determined on Mannitol Salt Agar (Difco); the plates were incubated at 37 °C ± 1 °C for 18–24 h (ICMSF 1978).

Staphylococcus aureus was enumerated by plating on Baird-Parker agar following the surface plate method. The incubation temperature used was 37 °C ± 1 °C (18–24 h). Suspected colonies were subjected to the DNAse test (Difco) (ICMSF 1978).

Yeasts and moulds were enumerated on Oxytetracycline Glucose Yeast Extract plates (Difco) following the surface plate method, with incubation at 22–25 °C for 5–7 d (ICMSF 1978).

Lactic acid bacteria were enumerated on MRS plates (Oxoid) using the pour plate method with incubation at 37 °C for 48 h in a 5% CO_2 atmosphere (de Man *et al.* 1960). MSE (Mayeux, Sandine and Elliker agar; Biokar Diagnostics, Beauvois, France) and Rogosa agar plates (Oxoid) were also inoculated and incubated under the same conditions. After incubation, five colonies were randomly taken from each plate of the MRS, MSE and Rogosa agar. The isolates were purified and afterwards kept in MRS broth until identification.

The isolated strains were identified according to the criteria of Wood and Holzapfel (1995): morphology, Gram, catalase, homo- or heterofermentation, growth capacity at 10 and 40 °C or at 6·5% of salt, hydrolysis of arginine, acetoin production, growth in bile-aesculine agar and utilization of substrates included in API 50 CH (BioMérieux).

All analyses were performed in duplicate.

Test for enterotoxins

Staphylococcal enterotoxins were extracted from 100 g samples by the procedure of Freed *et al.* (1982). Enterotoxins were detected using the ELISA sandwich technique of Fey *et al.* (1984). The reagents for the ELISA test were obtained from W. Bommeli, Bem, Switzerland.

Sensory analysis

Sensory analysis was performed at the end of the ripening period, following the IDF standards recommendation (FIL-IDF 99B 1995), by a panel composed of 15 tasters from this laboratory. The qualities judged were: cut appearance, colour, aroma, taste, texture and general acceptability, with scoring on a scale from 1 to 7 (1, very poor and 7, very good).

Statistical analysis

Analysis of variance was performed using the SYSTAT program for Windows: Statistics, version 5·0 edition (Evanston, IL, USA, 1992). Tukey's test was performed for comparison of means using the same program. Those means bearing different superscripts (^{a,b,c,d}) differ significantly ($P < 0{\cdot}05$).

The plate count data were transformed into logarithms prior to statistical treatment.

RESULTS AND DISCUSSION

Changes in the physicochemical characteristics during ripening

Table 1 shows the results obtained from the physicochemical analyses of the three batches at different moments of the ripening period.

As a result of the interchange with the environment, all the cheeses underwent a significant loss in humidity. This was detected as a continuous increase in total solids and NaCl content throughout ripening, causing a major fall in water activity. The drop in pH values and water activity was accompanied by a major decrease in microbial counts in these batches.

The total solids content increased considerably during the first 60 days of ripening, reaching very high values of around 80%.

When the physicochemical evolution of the three batches was compared, no significant differences were found for total solids content (TS), NaCl content, fat content (expressed as a percentage of TS) and total nitrogen content (TN, expressed as a percentage of TS) during the ripening period. It was therefore concluded that the thermal treatment of the

Table 1 Evolution of physicochemical characteristics during ripening of cheese

		Batch Ripening time (days)							
		0	2	5	10	15	30	45	60
pH	R	6·78 ± 0·06	6·61 ± 0·09^{b}	4·89 ± 0·10^{b}	4·69 ± 0·07^{b}	4·53 ± 0·11	4·49 ± 0·07^{a}	4·58 ± 0·05^{a}	4·70 ± 0·07^{a}
	RS	6·81 ± 0·06	5·10 ± 0·09^{c}	4·87 ± 0·09^{b}	4·65 ± 0·10^{ab}	4·60 ± 0·13	4·65 ± 0·08^{b}	4·69 ± 0·11^{b}	4·95 ± 0·09^{b}
	PS	6·74 ± 0·10	4·85 ± 0·08^{a}	4·52 ± 0·07^{a}	4·56 ± 0·10^{a}	4·54 ± 0·09	4·56 ± 0·06^{b}	4·69 ± 0·11^{b}	4·98 ± 0·07^{b}
TS	R	49·11 ± 2·45	53·54 ± 2·41	56·05 ± 2·26	59·98 ± 2·25	64·40 ± 2·26	70·36 ± 2·28	74·38 ± 2·18	78·23 ± 2·08
	RS	52·44 ± 2·38	52·44 ± 2·03	54·12 ± 2·37	61·68 ± 2·34	68·00 ± 2·03	74·03 ± 2·21	78·04 ± 2·10	79·83 ± 2·31
	PS	44·95 ± 2·15	48·74 ± 2·54	51·12 ± 2·38	60·23 ± 2·23	66·37 ± 2·37	76·40 ± 2·29	80·37 ± 2·25	83·32 ± 2·47
NaCl	R	0·17 ± 0·08	0·49 ± 0·07	1·30 ± 0·10	1·91 ± 0·09	2·44 ± 0·11	2·95 ± 0·15	3·10 ± 0·06	3·45 ± 0·13
	RS	0·18 ± 0·11	0·45 ± 0·11	1·15 ± 0·14	2·04 ± 0·08	2·60 ± 0·12	2·87 ± 0·13	3·05 ± 0·14	3·20 ± 0·11
	PS	0·18 ± 0·13	0·51 ± 0·14	1·17 ± 0·10	1·89 ± 0·14	2·58 ± 0·09	2·89 ± 0·17	3·12 ± 0·16	3·25 ± 0·09
Fat (% TS)	R	50·19 ± 4·85	49·72 ± 4·86	51·08 ± 4·69	51·14 ± 5·05	50·65 ± 5·15	48·65 ± 4·93	50·01 ± 4·84	52·41 ± 4·79
	RS	48·65 ± 4·78	47·89 ± 5·07	51·40 ± 4·93	48·56 ± 4·94	47·42 ± 4·67	48·35 ± 4·70	47·19 ± 4·96	49·05 ± 4·73
	PS	48·12 ± 4·53	49·79 ± 4·80	51·73 ± 5·01	48·93 ± 4·76	50·07 ± 5·06	50·64 ± 4·77	49·65 ± 4·57	50·59 ± 4·66
Fat Acidity	R	0·17 ± 0·13	0·62 ± 0·08^{b}	0·98 ± 0·09^{b}	1·49 ± 0·10^{c}	1·67 ± 0·09^{c}	2·31 ± 0·07^{c}	2·75 ± 0·15^{c}	3·64 ± 0·13^{c}
	RS	0·16 ± 0·08	0·26 ± 0·10^{a}	0·45 ± 0·10^{a}	0·60 ± 0·12^{b}	0·81 ± 0·06^{b}	1·14 ± 0·13^{b}	1·63 ± 0·05^{b}	2·51 ± 0·08^{b}
	PS	0·17 ± 0·13	0·19 ± 0·08^{a}	0·20 ± 0·14^{a}	0·23 ± 0·09^{a}	0·31 ± 0·15^{a}	0·37 ± 0·10^{a}	0·51 ± 0·10^{a}	0·59 ± 0·10^{a}
TN (%TS)	R	5·31 ± 0·67	5·21 ± 0·74	5·24 ± 0·68	5·32 ± 0·73	5·22 ± 0·70	5·37 ± 0·72	5·20 ± 0·72	5·36 ± 0·71
	RS	5·26 ± 0·72	5·25 ± 0·73	5·43 ± 0·72	5·43 ± 0·74	5·48 ± 0·69	5·23 ± 0·73	5·38 ± 0·73	5·52 ± 0·74
	PS	5·42 ± 0·67	5·19 ± 0·70	5·31 ± 0·72	5·51 ± 0·69	5·67 ± 0·70	5·36 ± 0·71	5·34 ± 0·75	5·46 ± 0·71
NPN (%TN)	R	1·61 ± 0·56	2·26 ± 0·53	3·35 ± 0·55	3·93 ± 0·54	5·14 ± 0·56^{b}	6·42 ± 0·55^{b}	7·23 ± 0·57^{b}	7·72 ± 0·53^{b}
	RS	1·63 ± 0·53	2·16 ± 0·55	2·84 ± 0·50	3·57 ± 0·55	4·53 ± 0·52^{ab}	5·20 ± 0·53^{a}	5·35 ± 0·52^{a}	5·60 ± 0·55^{a}
	PS	1·48 ± 0·54	2·14 ± 0·55	2·53 ± 0·50	3·03 ± 0·53	3·72 ± 0·53^{a}	4·12 ± 0·53^{a}	4·54 ± 0·53^{a}	4·99 ± 0·54^{a}

The data are the average ± standard deviation values of two cheese productions with two samples from each. Means in the same column and parameter group bearing different superscripts differ significantly.

milk and the addition of starter culture did not influence the evolution of these parameters. These results agreed with the values found by other authors for cheeses with similar characteristics (Fontecha *et al*. 1990; Carballo *et al*. 1994; Fresno *et al*. 1996).

However, the pH, fat acidity and non-protein nitrogen (NPN, expressed as a percentage of TN) showed significant differences between batches. During the first 5 days of ripening, a sharp drop in pH values (about 2 units) was detected as a consequence of the production of acid by the micro-organisms. The inoculated batches showed the fastest drop in pH. This sharp drop in pH might be expected as lactococci (the main producers of lactic acid) were the dominant microbial group at the beginning of the ripening period. These differences decreased and no significant differences were found after 15 days of ripening. After 15 days, a slight increase in pH was observed; the increase was higher in Batches RS and PS. This could be explained by the low levels of lactic acid bacteria found and the high counts of yeasts and moulds (Poullet *et al*. 1991; Tornadijo *et al*. 1995).

Changes in fat acidity showed the evolution of lipolysis. The values obtained for this parameter are determined by the micro-organism groups present, taking into account the low lipolytic activity of lactic acid bacteria (Stadhouders and Veringa 1973). The lipolytic process is also conditioned by the pH values, as low pH values lead to a decrease in the activity of lipases present in the milk (Deeth and Fitz-Gerald 1983). Thus, Batch R showed a higher lipolytic activity than the batches to which starters had been added. After 10 days of ripening, Batch RS showed a higher lipolytic activity than Batch PS, although the levels reached were lower than Batch R.

The evolution of the NNP/NP could be interpreted as the degree of proteolysis. The values obtained in these cheeses could be explained by the low values of water activity and the pH reached; under these conditions, milk and microbial proteases show low activity (Fresno *et al*. 1988). Significant differences in proteolysis were found after 15 days between Batch R and Batches RS and PS. These differences could be explained by the longer permanence of proteolytic micro-organisms in the non-inoculated cheeses.

Changes in the microbial groups during ripening

Table 2 shows the results obtained from the microbiological analysis of the samples during the ripening process. The

Table 2 Changes in log cfu g^{-1} of the main microbial groups during ripening

		Ripening time (days)							
	Batch	0	2	5	10	15	30	45	60
Mesophilic	R	6·75 ± 0·80	6·60 ± 1·22	8·45 ± 1·13	8·96 ± 0·49	8·20 ± 0·82	7·90 ± 1·14	6·95 ± 1·30	7·38 ± 0·79
	RS	6·71 ± 0·82	7·64 ± 0·62	8·00 ± 1·44	7·94 ± 1·24	6·30 ± 0·78	6·26 ± 1·29	6·20 ± 1·45	6·45 ± 0·99
	PS	6·26 ± 0·22	8·08 ± 0·85	8·11 ± 1·05	6·87 ± 0·95	5·00 ± 1·25	4·50 ± 0·74	4·38 ± 0·98	4·32 ± 0·81
Enterobacteraciae	R	2·30 ± 0·59	4·15 ± 0·44	5·31 ± 0·89	4·70 ± 0·56	3·45 ± 1·12	< 1	< 1	< 1
	RS	2·48 ± 1·21	3·41 ± 1·12	3·15 ± 0·75	1·90 ± 1·02	< 1	< 1	< 1	< 1
	PS	1·30 ± 0·10	2·38 ± 1·36	1·30 ± 1·13	< 1	< 1	< 1	< 1	< 1
Faecal coliforms	R	> 3·38	> 3·38	3·04	2·38	2·08	< 0·48	< 0·48	< 0·48
	RS	> 3·38	2·18	1·97	1·63	< 0·48	< 0·48	< 0·48	< 0·48
	PS	< 0·48	< 0·48	< 0·48	< 0·48	< 0·48	< 0·48	< 0·48	< 0·48
Micrococcaceae	R	3·26 ± 0·62	5·74 ± 1·40	5·86 ± 0·90	6·36 ± 1·23	6·04 ± 0·77	5·60 ± 0·61	5·08 ± 1·18	5·00 ± 1·07
	RS	3·08 ± 1·38	3·98 ± 1·20	4·23 ± 1·04	3·84 ± 1·29	3·30 ± 1·37	3·51 ± 1·33	3·26 ± 0·99	3·08 ± 1·67
	PS	2·90 ± 1·58	2·92 ± 0·94	2·70 ± 0·32	2·40 ± 1·16	2·41 ± 1·00	2·32 ± 0·68	2·28 ± 0·46	2·30 ± 1·09
Staph. aureus	R	4·20 ± 1·13	4·46 ± 1·13	6·41 ± 0·98	6·41 ± 1·63	5·70 ± 1·00	3·48 ± 0·21	< 2	< 2
	RS	4·32 ± 0·48	4·68 ± 1·38	4·38 ± 0·96	3·33 ± 0·63	2·90 ± 1·10	< 2	< 2	< 2
	PS	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2
Yeasts and molds	R	5·20 ± 0·79	5·51 ± 0·44	5·82 ± 0·96	6·84 ± 1·15	6·91 ± 1·15	6·62 ± 0·96	6·88 ± 1·20	6·92 ± 1·32
	RS	5·32 ± 0·84	5·88 ± 1·01	6·32 ± 1·36	6·67 ± 0·72	6·66 ± 1·31	6·49 ± 1·55	6·43 ± 0·38	6·53 ± 0·46
	PS	4·99 ± 1·24	5·60 ± 1·51	5·88 ± 1·64	6·50 ± 0·93	6·63 ± 1·58	7·00 ± 0·98	7·88 ± 1·06	7·94 ± 0·65
Lactic bacteria	R	4·60 ± 1·43	8·04 ± 0·75	10·07 ± 0·94	9·78 ± 0·76	9·40 ± 0·70	8·79 ± 1·02	8·51 ± 0·81	8·46 ± 1·47
	RS	8·40 ± 0·66	10·34 ± 0·74	11·00 ± 1·39	10·70 ± 0·84	9·18 ± 1·45	8·36 ± 0·83	8·08 ± 0·27	6·70 ± 1·17
	PS	7·79 ± 0·94	9·96 ± 0·87	9·75 ± 0·95	9·08 ± 0·52	8·20 ± 1·07	5·88 ± 0·64	3·70 ± 1·10	2·20 ± 1·00

The data are the average ± standard deviation values of two cheese productions with two samples from each.

highest counts of all microbial groups were generally reached on day 5.

The total aerobic mesophilic bacteria ranged from $> 10^7$ to $< 10^9$ cfu g^{-1}. These values were in agreement with those found in other goat's milk cheeses (Fontecha *et al*. 1990; Mor-Mur *et al*. 1992).

The differences found between Batches PS and RS, and Batch R, in the evolution of Enterobacteriaceae, faecal coliforrns and *Staph. aureus*, can be explained by the thermal treatment of the milk. The addition of starter influenced the microbiological quality of the cheeses, and differences in Enterobacteriaceae and faecal coliform counts were found between Batches R and RS after 15 d.ays The maximum level of Enterobacteriaceae was attained on day 5 in Batch R and on day 2 in Batches RS and PS. At that time, the pH had declined to 4·89 in Batch R and to 4·87 and 4·52 in Batches RS and PS, respectively. The faecal coliform counts decreased throughout the ripening period. The low counts of this group can be explained by the low pH of the cheese throughout ripening and the antagonistic action of the lactic acid bacteria (Babel 1977).

Micrococcaceae levels were relatively constant during the ripening period for each of the three batches, probably because the effect of low pH was counteracted by the salt tolerance of this group. Cheeses of the batch elaborated with pasteurized milk (PS) showed the lowest counts of Micrococcaceae (no more than 3 log cfu g^{-1}). Differences between batches elaborated with raw milk (about 2 log cfu g^{-1} higher in Batch R than Batch RS) could be related to the lower pH of the inoculated cheeses (Batch RS) at the beginning of the ripening period.

Staphylococcus aureus was only detected in batches elaborated with non-pasteurized milk (Batches R and RS); heat treatment of milk was effective against this micro-organism. The *Staph. aureus* counts decreased during the ripening period. This inhibition could be associated with low pH values, but other factors may also be involved, such as the type of starter used (Gilmour and Harvey 1990). According to Tuckey *et al*. (1964), cheese pH values above 5·3 were more favourable to *Staph. aureus* growth than pH values below this level. Higher levels of *Staph. aureus* in Batch R than in Batch RS could be explained by the fact that starter was not added and lower levels of lactic acid bacteria were reached (4 log cfu g^{-1} and 2 log cfu g^{-1} lower on days 0 and 2, respectively). pH values below 5·3 were recorded in Batch R after 5 days and in Batch RS after 2 days.

Staphylococcus aureus was not detected after 45 days in Batch R and 30 days in Batch PS, but counts above 6 log cfu g^{-1} were reached in Batch R. These levels could potentially produce enterotoxins, as enterotoxins have been detected in cheeses with *Staph. aureus* counts exceeding 7 log cfu g^{-1} (Tatini *et al*. 1971, 1973; Van Schouwenburg *et al*. 1979); in some cheeses, enterotoxin production may even occur at 6·80 log cfu g^{-1} (Tatini *et al*. 1973). Despite the high levels of staphylococci found in this study, no enterotoxins were detected in Batch R. This may have been due to the microflora present as this Batch was elaborated with raw milk (Noleto *et al*. 1987).

Batch R showed lower lactic acid bacteria counts than the inoculated batches during the first 2 days of the ripening period. However, no significant differences between the batches were found from day 5 to day 15 of ripening. After 15 days, the lactic acid bacteria counts decreased in the three batches. The decrease in lactic flora could be explained by the lower water activity in the cheeses (Tornadijo 1995). This was specially noticeable in the cheeses of Batch PS, in which the lactic acid bacteria present were exogenous, because it was elaborated with pasteurized milk with the addition of starter.

The lower bacterial counts of cheeses of Batch PS after 60 days (mesophilic, 2·23–3·06 log cfu g^{-1} lower than Batches RS and R, respectively; lactic acid bacteria, 4·50 and 6·20 log cfu g^{-1} lower than Batches RS and R, respectively) explain the high yeast and mould counts. These micro-organisms increased in number as the lactic acid bacteria counts decreased; this could explain the increase in pH values at the end of the ripening period.

Lactic acid bacteria isolated during ripening

Of 489 isolates, 378 were identified (77%). A large number of isolates was lost, particularly at the first sampling, probably because these strains were yeasts and other non-lactic acid flora.

The results are shown in Table 3. At the beginning of the ripening, *Lactococcus* was the principal lactic acid bacteria, *L. lactis lactis* being predominant. However, the percentage of lactococci decreased during ripening while *Lactobacillus* increased, with *Lact. plantarum* becoming predominant. No lactococci were found from day 30 for Batch R and from day 45 for Batch RS. This could be due to the ease with which lactobacilli grow at low pH values (McDonald *et al*. 1990) and their high resistance to salt (Sharpe 1979). The decrease in lactococci during the ripening period could be explained by the fact that this group is one of the most affected by the drop in water activity (Tornadijo *et al*. 1995).

In the cheeses of Batch PS elaborated with pasteurized milk, the lactic acid bacteria came from the starter culture and all the isolates were identified as *Lactococcus*. In these cheeses, the number of lactic acid bacteria at the end of ripening was very low.

At the end of ripening, the *Enterococcus* content was 33% in Batch R and 75% in Batch RS; *Ent. faecalis* was the main species isolated. This should be expected as the low water activity at the end of the ripening assists these lactic acid bacteria. *Enterococcus faecalis* has also been named as the most dominant enterococcal species in other artisanal goat cheeses such as Armada (Tornadijo *et al*. 1995) and Majorero (Fontecha *et al*. 1990). Only in white-brined raw goat's milk cheese (Litopoulou-Tzanetaki and Tzanetakis 1992) did *Ent. faecium* dominate over the other species of enterococci.

A low but constant percentage of isolates was identified as *Leuconostoc mesenteroides*. The role of this genus during the ripening of goat's cheese is not clear. Some authors have found high levels of *Leuconostoc* and suggest that they play an important role in ripening (Fontecha *et al*. 1990), whereas others have found only low levels of these micro-organisms (Tornadijo *et al*. 1995). However, results presented in the literature suggest that they have a role in the development of a balanced aroma and flavour, and *Leuconostoc* has therefore been included in the design of specific starter cultures (Mor-Mur *et al*. 1992; Requena *et al*. 1992).

Sensory characteristics

The sensory assessment of Batches R, RS and PS at the end of ripening is detailed in Table 4. The appearance of the cheeses on cutting received favourable scores, especially cheeses from Batch RS which displayed well distributed interior openings. The raw milk cheese openings were larger, probably due to an excess of coliforms at the beginning of the ripening process. Batch R received the lowest score for this parameter.

The colour of the cheese received very similar scores in all the batches but significant differences were detected in aroma, taste and texture. The cheeses of Batch PS had a very poor aroma and an acid flavour. Their sandy texture and acid flavour were probably due to the rapid initial acid production by the starter cultures. The low counts of lactic acid bacteria at the end of the ripening period, and the growth of other microbial groups, could explain these sensory defects.

The cheeses of Batch R showed a higher complexity of aroma and taste. However, the tasters detected a certain degree of saltiness and other abnormal flavour aspects, probably due to a longer presence of unfavourable micro-organisms (Enterobacteriaceae and faecal coliforms) during ripening.

The highest scores for general acceptance were for the cheeses of Batch RS. Batch RS also received very favourable scores for aroma and taste and showed an acceptable texture. These results suggest that the combined activity of micro-

Table 3 Changes in lactic acid bacteria species isolated during ripening

Batch		0 days		2 days		5 days		10 days		15 days		30 days		45 days		60 days	
		No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%
R	*Lactobacillus*																
	Lact. brevis	2	10	1	5	3	14	1	10	1	7	2	8				
	Lact. casei			2	10	1	5	2	20	3	21	4	17	4	29	2	11
	Lact. fermentum							1	10			2	8				
	Lact. paracasei					1	5			1	7	2	8			2	11
	Lact. plantarum	2	10	3	14	2	10	2	20	3	21	6	17	6	43	6	33
	Lact. rhamnosus	2	10	1	5	1	5										
	Lactobacillus sp.	2	10	1	5	4	19			2	14	2	8				
	Lactococcus																
	L. lactis lactis	3	15	5	24	5	24	2	20	2	14						
	L. lactis cremoris	2	10	4	19	3	14	1	10								
	L. diacetylactis	1	5														
	Enterococcus																
	Ent. faecalis	3	15	2	10			1	10	2	14	3	13	4	29	5	28
	Ent. faecium	1	5									1	4			1	5
	Leuconostoc																
	Leuc. mesenteroides	2	10	2	10	1	10					2	8			2	11
RS	*Lactobacillus*																
	Lact. brevis					1	5	1	5			1	6				
	Lact. casei			2	13	1	5	3	14			2	13				
	Lact. paracasei									1	10						
	Lact. plantarum					3	17	4	18	4	40	5	31	8	50	6	75
	Lactobacillus sp.									1	10	2	13	2	13		
	Lactococcus																
	L. lactis lactis	8	50	7	44	8	44	7	32	2	20	1	6				
	L. lactis cremoris	7	44	6	38	4	22	2	9	1	10	3	19	2	12		
	L. diacetylactis	1	6	1	6												
	Enterococcus																
	Ent. faecalis							3	14	1	10	2	13	2	12	2	25
	Leuconostoc																
	Leuc. mesenteroides					1	5	1	5					2	12		
PS	*Lactococcus*																
	L. lactis lactis	6	50	8	53	10	59	9	43	5	38	5	38	4	36	4	33
	L. lactis cremoris	5	42	6	40	6	35	10	48	7	54	7	54	7	64	8	67
	L. diacetylactis	1	8	1	7	1	6	2	10	1	8	1	8				

Table 4 Mean values and standard deviation for the sensory characteristics of cheeses at the end of ripening

Batch	Characteristics					
	Cut appearance	Colour	Aroma	Taste	Texture	General acceptance
R	3·85 ± 0·72	3·75 ± 0·98	4·12 ± 0·15	4·25 ± 0·18	3·91 ± 0·53	3·89 ± 0·65
RS	4·44 ± 0·23	3·35 ± 0·34	5·67 ± 0·54	4·98 ± 0·12	4·56 ± 0·21	4·72 ± 0·71
PS	5·85 ± 0·72	3·75 ± 0·65	3·45 ± 0·37	3·52 ± 0·65	3·41 ± 0·71	3·57 ± 0·19

organisms present in raw milk, together with the use of starter cultures, produces cheese with good sensory characteristics.

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