



Autochthonous Starter Effect on the Microbiological, Physicochemical and Sensorial Characteristics of Moroccan Goat's Milk Cheeses

Ouiam El Galiou¹, Juan Antonio Centeno², Said Zantar³, Mohammed Bakkali¹, Javier Carballo², Amin Laglaoui¹

¹Equipe de Recherche en Biotechnologies et Génie des Biomolécules, Université Abdelmalek Essaadi, Faculté des Sciences et Techniques, BP 416, Tangier, Morocco.

laglaouiamin@yahoo.fr

²Área de Tecnología de los Alimentos, Facultad de Ciencias de Ourense, Universidad de Vigo, Campus Universitario s/n, 32004 Ourense, Spain.

carbatec@uvigo.es

³Station d'ionisation de Boukhalef, INRA de Tanger, Morocco.

zantar_said63@yahoo.fr

ABSTRACT

The effect of adding four *Lactococcus lactis* subsp. *Lactis*, *Lactococcus lactis* subsp. *lactis* Bv. *diacetylactis* and *Lactobacillus paracasei* autochthonous strains as single starters (A and B) on the microbiological, physicochemical and sensory characteristics of Moroccan goat's milk cheese was investigated. Batches with a commercial starter (C) and with raw goat's milk without starter (D) were also made as controls. The results indicated that the different starter cultures used significantly affected the compositional characteristics of Moroccan goat's cheese. At the first day of ripening, the pH values of cheeses manufactured with any type of starter were significantly higher than those made without starter. No significant differences ($P > 0.05$) in values of total solids (T.S.), proteins, ash, FAT and lactose content were found between any of the batches after 7 days of ripening. The autochthonous starters in Moroccan cheesemaking were successful at reducing the levels of coliforms earlier in ripening than in cheeses made without starter and even in those made with the commercial starter. These results suggest that the combined activity of *L. Lactis* subsp. *lactis*, *L. Lactis* subsp. *Lactis* bv. *diacetylactis* and *L. paracasei*, produces cheese with good sensory characteristics.

Indexing Terms/Keywords

Goat milk cheese, autochthonous starter, cheese manufactured process, ripening, sensorial quality.

Academic Discipline and Sub-Disciplines

Biotechnology.

Subject Classification

Microbiology.

Type (Method/Approach)

Experimental.

Council for Innovative Research

Peer Review Research Publishing System

Journal: JOURNAL OF ADVANCES IN BIOTECHNOLOGY

Vol .4, No. 1.

www.cirjbt.org , jbteditor@gmail.com

INTRODUCTION

Goat's milk is becoming increasingly important in Morocco, especially because of the popularity of its products, in particular cheese. Manufacture of most cheese varieties involves combining four ingredients: milk, rennet, microorganisms and salt, which are processed through a number of common steps such as gel formation, whey expulsion, acid production and salt addition, followed by a period of ripening. A variation in ingredient blends and subsequent processing has led to the evolution of all these cheese varieties. While variations in processing parameters such as cook temperature and curd handling techniques play a major role in determining the characteristics of each cheese type, the cheese microflora play a critical and pivotal role in the development of the unique characteristics of each cheese variety [1].

Starter's cultures are mainly species of lactic acid bacteria that are deliberately added to milk where their primary role is to start the production of lactic acid for the cheese manufacturing process [2]. It is well known that starter cultures fulfil other important functions: inhibition of spoilage and pathogenic microorganisms, improvement of cheese keeping quality, direct and indirect contributions to flavour and aroma. Starter cultures may be classified into two large groups: traditional (including artisanal, natural starters and multiple strain culture starters) and defined or commercial starters [3] are daily produced and propagated at the cheese plant. Overall, the composition of natural starter cultures is complex, variable and subjected to unpredictable performance [4,5].

Autochthonous cultures have also been used in Tetilla cheese [6], Proosdij-type cheese [7], Reggianito Argentino cheese [8], Turkish White Pickled Cheese [9], Cheddar cheese [10] and New Zealand Cheddar cheese [11] in order to study their influence on the ripening process and improve their sensory characteristics. The use of starter cultures containing lactic acid bacteria is an essential requirement in the manufacture of most cheeses, if cheese of consistent quality is to be produced [12]. The primary role of starter bacteria is to produce lactic acid at a controlled rate. Moreover, in some cases, the enzymes originating from starter (proteinases and peptidases) play a major role in the formation of small peptides and amino acids, which serve as precursors of flavour compounds in cheese [13, 14].

Bacteria belonging to the genera *Lactococcus*, *Lactobacillus*, and *Streptococcus* are the most commonly used microorganisms in starter cultures. These lactic acid bacteria perform the primary acidification of the milk and participate in the maturation process via production of flavour compounds or their chemical precursors, either directly by cellular metabolism or indirectly by the release of enzymes [15].

There are not many technological studies on specifically improving the preparation and ripening conditions of goat's cheese in Morocco.

The objective of this work was to investigate the effects of adding various different starter cultures, composed of selected LAB strains isolated from Moroccan goat's cheese from raw milk, on the physico-chemical, microbiological and sensory characteristics of Moroccan goat's cheese made from pasteurized milk.

MATERIALS AND METHODS

Starter Cultures

The microorganisms used as cheese starters in this work had previously been isolated from Moroccan goat's raw milk cheese. After the technological and genetic characterization of 52 strains of LAB, 4 of them were selected to be used as single starters (A and B) based on their acidifying, proteolytic and diacetyl and acetoin production characteristics (Tab. 1).

Acidifying activity in skim milk (Oxoid) after 6 h at $30 \pm 0.1^\circ\text{C}$ was determined according to the IDF guideline [16]; proteolytic activity in skim milk (Oxoid), after 24 h incubation at $30 \pm 1^\circ\text{C}$, detected by a method employing the Folin-Ciocalteu reagent, diacetyl and acetoin production in skim milk (Oxoid) after 48 h incubation at $30 \pm 1^\circ\text{C}$ [17].

Tab. 1: Acidifying and proteolytic activity and diacetyl and acetoin of selected autochthonous strains.

Starter	Strains	Acidifying activity	Proteolytic activity	diacetyl and acetoin production
		% lactic acid after 6 h at 30 °C	mol L-glycine mL ⁻¹ milk	
Batch A	<i>L. lactis lactis</i>	4.13	26.30	22.54
	<i>L. lactis lactis</i> <i>bv. diacetyllactis</i>	4.20	38.22	24.73
	<i>Lactobacillus paracasei</i>	4.25	66.93	35.9
Batch B	<i>L. lactis lactis</i>	4.87	28.46	35.58
	<i>L. lactis lactis</i> <i>bv. diacetyllactis</i>	5.11	30.00	33.7
	<i>Lactobacillus paracasei</i>	6.11	17.95	67.63

Cheese Making

A total of 150 L of whole goat milk was pasteurized (74 °C, 15 s), and then divided into three vats, batch D was made with 50 L of non pasteurized milk, each batch comprising six cheeses. Therefore, 24 cheeses were made in total.

The cheeses were manufactured according to the experimental protocol shown in Figure 1.

The experimental design was four blocks of batches, as follows:

- Batch A: Experimental batch manufactured with pasteurized milk and an autochthonous starter composed by: one high acid producer isolate of *L. lactis subsp. lactis* (0.5% of active milk-culture), one low diacetyl producer isolate of *L. lactis subsp. lactis* bv. *diacetylactis* (0.5% of active milk-culture), and one highly-moderately proteolytic isolate of *L. paracasei* as adjunct culture (0.5% of active milk-culture)
- Batch B: Experimental batch manufactured with pasteurized milk and an autochthonous starter composed by: one low-moderate acid producer isolate of *L. lactis subsp. lactis* (0.5% of active milk-culture), one high-moderate diacetyl producer isolate of *L. lactis subsp. lactis* bv. *diacetylactis* (0.5% of active milk-culture), and one scarcely-moderately proteolytic isolate of *L. paracasei* as adjunct culture (0.5% of active milk-culture)
- Batch C: Experimental batch manufactured with pasteurized milk and a commercial starter composed by *L. lactis subsp. lactis*, *L. lactis subsp. cremoris* and *L. lactis subsp. lactis* bv. *diacetylactis* strains.
- Batch D: Raw-milk batch manufactured with the same milk unpasteurized and without added starter.



Figure 1: Flow diagram of manufacturing procedure for experimental Moroccan goat's cheese

Microbiological and physicochemical analyses

For the microbiological analysis, samples of milk and of cheeses at 1, 7 and 15 d were taken from each batch. Representative aliquots of cheese (10 g) were homogenized with 90 mL of a sterile salt tryptone for 2 min, then serial 10-fold dilutions were prepared and plated in duplicate [16]. Types of microbial counts determined were: lactic acid bacteria (LAB) on MRS agar (at pH 6.2; [18]) incubated anaerobically (Gas-Packan aerobic system, BBL, Cockeysville, Maryland, USA) at 30°C for 5 days and Gram-positive, catalase-negative cocci on M17 agar, after incubation at 30°C for 48 h; Enterococci on Slanetz and Bartley (Merck, Darmstadt, Allemagne), at 44°C for 48 h, total coliforms determined on violet red bile agar (VRBA) at 30°C for 3 days [19]; Fecal coliforms determined on violet red bile agar (VRBA) at 45°C for 3 days [19]; *Staphylococcus aureus* counts enumerated on Baird-Parker agar supplemented with egg yolk and potassium tellurite at 35-37 °C for 48 h [19]. All analyses were performed in duplicate.

For the physicochemical analysis, samples of the cheeses were taken at the same stages of ripening as for the microbiological sampling. The pH was measured by homogenizing 10 g of cheese with 20 ml of distilled water, using a Pocket pH meter (Amarell Electronic). Fat content was measured by the Gerber van Gulik method [20], Dry Extract was assessed by drying to constant weight at $102 \pm 1^\circ\text{C}$ [21]; Ash and lactose by the [22] method. Total nitrogen (TN) was measured using the Kjeldahl method; soluble nitrogen at pH 4.6 (SN) and soluble nitrogen in 12% trichloroacetic acid (TCASN) were determined according to [23] Ardö 1999 and then quantified using Kjeldahl. All analyses were performed in triplicate.

Sensorial Evaluation

On day 7 of ripening, one cheese from each batch was subjected to sensory evaluation by a mixed panel [24] of ten tasters who had been trained in tasting traditional cheeses, organoleptical analyses were performed following the



recommendations of IDF Standard 99C (1997) [17]. Tastes, aromas and textural characteristics were assessed on a scale from 1 to 7, the ideal values being 6 for all parameters.

Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) using Statistical Software. Post hoc testing was carried out using the Tukey test. A significant level of 0.05 was used for all statistical tests considering the cultures effect on cheeses.

RESULTS AND DISCUSSION

Effect of The Starters on the Physicochemical Characteristics During Ripening

Table 2 gives the results for the pH, Proteins, Fat content, dry extract, Ash and lactose contents.

Table 2: Changes during ripening in pH, proteins, fat content, dry extract, ash and lactose content of Moroccan goat's cheeses made with different starters.

Parameter	Starter	Days of ripening		
		1	7	15
pH				
	A	4.85 ± 0.01 ^a	4.75 ± 0.01 ^a	4.65 ± 0.02 ^a
	B	5.10 ± 0.06 ^b	5.01 ± 0.12 ^c	4.82 ± 0.09 ^b
	C	5.04 ± 0.03 ^b	4.98 ± 0.05 ^b	4.57 ± 0.03 ^c
	D	4.25 ± 0.04 ^c	4.06 ± 0.06 ^b	3.85 ± 0.01 ^{ac}
Protein g/100g				
	A	12.91 ± 0.25 ^a	13.48 ± 0.09 ^a	15.81 ± 0.39 ^a
	B	13.01 ± 1.11 ^a	13.75 ± 0.15 ^b	14.60 ± 0.71 ^b
	C	12.54 ± 1.02 ^a	13.76 ± 0.35 ^{ab}	14.57 ± 0.32 ^b
	D	11.61 ± 1.19 ^a	12.82 ± 0.27 ^c	14.66 ± 0.14 ^b
Fat content g/100g				
	A	28.12 ± 1.40 ^a	30.50 ± 0.24 ^a	33.45 ± 0.62 ^a
	B	27.55 ± 2.20 ^a	31.00 ± 0.37 ^a	34.60 ± 0.80 ^a
	C	28.13 ± 1.23 ^a	30.61 ± 0.35 ^a	33.39 ± 0.55 ^a
	D	25.75 ± 1.49 ^a	29.52 ± 0.27 ^c	33.82 ± 0.62 ^a
Dry Extract g/100g				
	A	47.56 ± 0.40 ^a	48.80 ± 0.28 ^a	49.81 ± 0.23 ^a
	B	48.32 ± 0.39 ^a	49.51 ± 0.51 ^a	50.21 ± 0.30 ^a
	C	47.61 ± 0.46 ^a	48.75 ± 0.79 ^a	49.62 ± 0.20 ^a
	D	46.52 ± 0.63 ^a	47.32 ± 0.62 ^a	48.49 ± 0.83 ^a
Ash g/100g				
	A	3.85 ± 0.33 ^a	3.33 ± 0.51 ^a	2.01 ± 0.12 ^a
	B	3.11 ± 0.62 ^a	2.27 ± 0.40 ^a	2.02 ± 0.23 ^a
	C	3.21 ± 0.53 ^a	2.27 ± 0.69 ^a	1.87 ± 0.31 ^a
	D	3.95 ± 0.41 ^a	3.40 ± 0.61 ^a	2.85 ± 0.42 ^a
Lactose g/100g				
	A	2.75 ± 0.10 ^a	2.49 ± 0.19 ^a	1.63 ± 0.04 ^a
	B	3.28 ± 0.15 ^a	2.75 ± 0.13 ^a	1.65 ± 0.07 ^a
	C	3.12 ± 0.01 ^a	2.43 ± 0.07 ^a	1.70 ± 0.09 ^a
	D	2.80 ± 0.26 ^a	2.33 ± 0.21 ^a	1.69 ± 0.03 ^a

a, b, c, d: means of the same column with different superscripts differ significantly ($p < 0.05$).



The drop in pH during the making and first stages of ripening of the Moroccan goat's cheese was lower in the batches produced from pasteurized milk inoculated with starter cultures than in the batch made from raw milk (batch D). The pH decreased in all cases up to day 15 of ripening.

Batch C underwent the highest drop in pH during coagulation and the first stages of ripening of the cheeses. Moreover, pH of batch D was lower for all sampling times than that of batches A, B and C. As the pH of cheese curd decreases there is a concomitant loss of colloidal calcium phosphate from the casein micelles and, below about pH 5.5, a progressive dissociation of the micelles into smaller casein aggregates [25].

Acidification at the appropriate rate and time is an essential and characteristic feature of cheese making, since it has a major impact on cheese quality [26, 27].

Ripening involves three primary biochemical events: glycolysis of residual lactose and its constituent monosaccharides (glucose and galactose), lipolysis, and proteolysis [28]. The pH decreased due to the production of organic acids (primarily lactic acid) with LAB responsible for most of the sugar fermentation.

When the physicochemical evolution of the four batches was compared, no significant differences were found for the protein content, fat content and dry extract content between batches. Similar observations were reported for Galotyri-type cheese made with different starter cultures by [29] Katsiari *et al*, 2009, who found no significant difference in contents of differences in gross composition (fat, protein and dry extract) made with *Streptococcus thermophilus*, *Lactobacillus delbrueckii subsp. Bulgaricus* or mesophilic lactic culture containing *Lactococcus lactis subsp. lactis* and *Lactococcus lactis subsp. Cremoris*.

The protein content values increased during ripening in all types of cheeses with no significant ($P > 0.05$) differences between batches from day 7 onwards. The Fat content values and the dry extract increased during ripening in all types of cheeses.

The lactose content decreased, corresponding with an increase in acidity, these results are consistent with those obtained by [30]. The differences observed in the lactose content may be related to the changes in pH; in fact, differences in pH values among different curds could produce differences in whey drainage. On the other hand, acidic pH values are not optimal for the stability and activity of bacterial β -galactosidase, as the enzyme considerably loses activity at pH 4.5 [31, 32].

Table 3: Changes During Ripening of Nitrogen Fractions of Moroccan Goat's Cheeses Made With Different Starters.

Parameter	Starter	Days of ripening		
		1	7	15
TN %				
	A	3.44 ± 0.03 ^a	3.58 ± 0.01 ^a	3.75 ± 0.01 ^a
	B	3.22 ± 0.21 ^a	3.41 ± 0.10 ^a	3.52 ± 0.04 ^b
	C	2.82 ± 0.01 ^b	3.02 ± 0.08 ^b	3.15 ± 0.05 ^c
	D	2.71 ± 0.01 ^c	2.85 ± 0.01 ^c	3.17 ± 0.03 ^c
SN/TN %				
	A	12.70 ± 0.11 ^a	15.44 ± 0.28 ^b	17.81 ± 0.23 ^a
	B	10.21 ± 0.05 ^b	11.20 ± 0.19 ^a	15.25 ± 0.44 ^a
	C	8.89 ± 0.67 ^a	13.56 ± 0.15 ^a	16.72 ± 0.07 ^a
	D	13.25 ± 0.26 ^c	18.61 ± 0.37 ^b	24.81 ± 0.08 ^b
TCASN/TN %				
	A	4.81 ± 0.04 ^a	5.87 ± 0.03 ^a	9.51 ± 0.04 ^a
	B	3.19 ± 0.03 ^b	5.11 ± 0.07 ^b	8.53 ± 0.01 ^b
	C	3.41 ± 0.11 ^b	4.55 ± 0.25 ^a	8.32 ± 0.34 ^b
	D	2.80 ± 0.03 ^c	6.50 ± 0.04 ^c	12.36 ± 0.01 ^c

a, b, c, d: means of the same column with different superscripts differ significantly ($P < 0.05$).

In all cases the SN/TN and TCASN/TN levels increased significantly with the age of the cheeses. The SN/TN content was significantly higher ($P < 0.05$) in cheeses made without starter than in the other batches from day 7 onwards. The TCASN/TN content was significantly higher ($P < 0.05$) in cheeses made without starter from day 15.

The increased levels of proteolysis in the SN/TN content observed in cheeses made without starter can be attributed to less competition with *lactococci* so that a greater variety of bacteria with higher peptidase activity could have developed [33].



This primary proteolysis could enhance the formation of larger peptides which could lead, after day 7, in a secondary proteolysis, to a greater yield of small peptides and aminoacids reflected in the higher levels of TCASN/TN content, these values are in agreement with those reported by [33].

The SN/TN and TCASN/TN levels was significantly higher ($P < 0.05$) in cheeses made with highly-moderately proteolytic isolate of *L. paracasei* (Batch A) than the cheese made with scarcely-moderately proteolytic isolate of *L. paracasei* (Batch B).

Effect of the Starters on Microbiological Characteristics During Ripening

Table 4 lists the means of the microbiological counts in the milk and cheeses during ripening.

Table 4: Changes in log cfu-g⁻¹ of microbial groups during ripening of Moroccan goat's cheeses made with different starters.

Parameter	Starter	Milk	Days of ripening		
			1	7	15
Total lactic acid bacteria		6.32			
	A		9.97 ± 0.40	9.11 ± 0.18	8.63 ± 0.35
	B		8.12 ± 0.22	8.01 ± 0.21	7.92 ± 0.12
	C		9.4 ± 0.11	9.21 ± 0.65	9.15 ± 0.29
	D		8.62 ± 0.34	8.05 ± 0.16	8.88 ± 0.11
Enterococci		4.12			
	A		5.11 ± 0.03 ^a	4.81 ± 0.07 ^a	4.56 ± 0.14 ^a
	B		5.56 ± 0.01 ^b	5.33 ± 0.04 ^b	5.12 ± 0.16 ^b
	C		4.13 ± 0.14 ^c	4.05 ± 0.32 ^c	3.47 ± 0.20 ^c
	D		6.02 ± 0.09 ^d	5.92 ± 0.06 ^d	6.14 ± 0.08 ^d
Coliforms		1.45			
	A		1.03 ± 0.06 ^a	nd	nd
	B		nd	nd	nd
	C		1.15 ± 0.04 ^a	nd	nd
	D		5.55 ± 0.06 ^b	3.23 ± 0.03 ^b	2.55 ± 0.07 ^a

a, b, c, d: means of the same column with different superscripts differ significantly ($P < 0.05$). nd: not detected.

The counts of lactic acid bacteria on MRS were significantly ($P < 0.05$) lower in milk, however, from the first day of cheese ripening onwards, the counts evolved quite similarly in all types of cheeses, with the differences being small and not significant. The general trend for lactic acid bacteria (except in batch D) was a gradual decline as ripening progressed. The decrease in counts of LAB and *lactococci* during ripening is a phenomenon that has been observed and described by various authors [34, 35]. The decrease in a_w and the increase in salt/moisture ratio could have contributed to the decrease observed in the counts of LAB [36]. The growth rate and final population density of the lactic acid bacteria are affected by pH, salt, and moisture levels of the curd and by the temperature of ripening [37].

The mean *enterococci* counts in Batch A, B and C remained practically constant throughout ripening, although at the end of the ripening a very slight decrease could be observed. However, the counts in the commercial starter cheeses were in all cases significantly lower ($P < 0.05$) than for the other types at the same times of ripening.

Maximum counts of coliforms were observed on the first day of ripening for all batches excepted batch B, the highest being, as we expected, those of cheeses manufactured with raw milk (Batch D).

Afterwards they declined rapidly in all types of cheeses, disappearing completely after 7 d in cheeses made with autochthonous starters (batch A and B) and in cheeses made with commercial starters (Batch C), although they persisted at very low levels in batches made with raw milk with no added starter. 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 [38]. It is known that lactic acid bacteria inhibit the growth of pathogen, via the production of acidic compounds such as lactic acid and/or H₂O₂, diacetyl, bacteriocins, by consequence low pH was stored [39]. De Sousa (2002) [40] pointed out that a high total count of coliforms was the result of a poor hygienic handling causing post-pasteurization contamination.



Sensorial Evaluation

The sensory assessment of the various completed batches is summarized in Table 5.

Table 5: Organoleptic characteristics at 7 d of ripening of Moroccan goat's cheeses made with different starters.

Attribute	A	B	C	D
Colour	4.74 ± 0.02 ^a	5.01 ± 0.07 ^c	3.12 ± 0.16 ^b	4.61 ± 0.04 ^a
Cut appearance	3.65 ± 0.03 ^a	3.98 ± 0.05 ^c	3.71 ± 0.03 ^b	2.89 ± 0.08 ^b
Texture	2.91 ± 0.10 ^a	3.95 ± 0.03 ^b	2.55 ± 0.08 ^a	2.17 ± 0.17 ^c
Taste intensity	2.5 ± 0.09 ^a	2.33 ± 0.12 ^a	2.72 ± 0.07 ^b	3.48 ± 0.22 ^c
Taste quality	3.4 ± 0.08 ^a	3.75 ± 0.01 ^a	2.81 ± 0.16 ^b	2.22 ± 0.31 ^c
Aroma intensity	3.55 ± 0.03 ^a	3.82 ± 0.21 ^a	2.87 ± 0.11 ^b	2.31 ± 0.15 ^b
Aroma quality	3.61 ± 0.05 ^a	3.72 ± 0.14 ^a	2.65 ± 0.10 ^b	2.03 ± 0.07 ^c
General acceptance	3.65 ± 0.20 ^a	3.89 ± 0.28 ^a	2.63 ± 0.34 ^b	2.17 ± 0.12 ^b

a, b, c, d: means of the same column with different superscripts differ significantly (P < 0.05).

The most favourable scores for texture and cut appearance were obtained for cheese B.

The highest scores for taste intensity were for cheeses made with raw milk without starter (Batch D). Native flora of milk seems to be the main factor in producing the specific sensory properties of raw milk cheeses [41]. Therefore, the addition of native cultures to pasteurized milk could contribute to preserving the typical characteristics of traditional cheese.

For taste quality, batches manufactured with the B autochthonous starters received the best score. The highest scores for intensity and quality of aroma were for cheeses made with the A and B autochthonous starter.

The cheeses of Batch D 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. These results were in agreement with those found in other goat's raw milk cheeses [42].

CONCLUSION

The starters comprising native strains had an advantage over the commercial culture since, in general, they gave the cheeses made with pasteurized milk characteristics of flavour, aroma and texture similar to, or better than, those of cheeses produced from raw milk. The analysis of sensory and characteristics showed that strains *L. paracasei*, would be good candidates for inclusion as adjuncts in a starter culture for the manufacture of industrial Moroccan goat's cheese.

ACKNOWLEDGEMENTS

This study was financially supported by AECI project number A/017594/08. Our grateful thanks to J. Ignacio Garabal, Dairy Science and Technology Laboratory, Agricultural Research Center of Mabegondo (CIAM), Xunta de Galicia, for his precious help, skilful and technical assistance.

REFERENCES

- [1] Herreros, M. A., Fresno, J. M., Gonzalez Prieto, M. J. and Tornadijo, M. E. 2003. Technological characterization of lactic acid bacteria isolated from Armada cheese (a Spanish goats' milk cheese). *International Dairy Journal*. 13: 469–479.
- [2] Broome, M. C. 2007. Starter culture development for improved cheese flavour. In Weimer, B. C. (Eds.), *Improving the Flavour of Cheese*. CRC Press. Washington DC, pp. 157–176.
- [3] Limsowtin, G. K. Y., Broome, M. C. and Powell, I. B. 2003. Lactic acid bacteria, taxonomy. In: Roginski, H., Fuquay, J. W. and Fox, P. F. (Eds.). *Encyclopedia of dairysciences*. London: Academic Press. pp: 1470–8.
- [4] Coppola, S., Blaiotta, G., Ercolini, D., Moschetti, G. 2001. Molecular evaluation of microbial diversity occurring in different types of Mozzarella cheese. *Journal of Applied Microbiology*. 90: 414–420.
- [5] De Candia, S., De Angelis, M., Dunlea, E., Minervini, F., McSweeney, P. L. H., Faccia, M. and Gobbetti, M. 2007. Molecular identification and typing of natural whey starter cultures and microbiological and compositional properties of related traditional Mozzarella cheeses. *International Journal of Food Microbiology*. 119: 182–191.
- [6] Menéndez, S., Centeno, J.A., Godínez, R. and Rodríguez-Otero, J. L. 2000. Effects of various *Lactobacillus* strains on the ripening and organoleptic characteristics of Arzua-Ulloa cheese. *International Journal of Food Microbiology*. 59: 37–46.



- [7] Ayad, E. H.E., Verheul, A., Bruinenberg, P., Wouters, J. T. M. and Smit, G. 2003. Starter culture development for improving the flavour of Proosdij-type cheese. *International Dairy Journal*. 13: 159–168.
- [8] Candioti, M. C., Hynes, E., Quiberoni, A., Palma, S. B., Sabbag, N. and Zalazar, C. A. 2002. Reggianito Argentino cheese: influence of *Lactobacillus helveticus* strains isolated from natural whey cultures on cheese making and ripening processes. *International Dairy Journal*. 12: 923–931.
- [9] Karakus, M. and Alperden, I. 1995. Effect of starter composed of various species of lactic acid bacteria on quality and ripening of Turkish white pickled cheese. *Lebensmittel-Wissenschaft und-Technologie*. 28: 404–409.
- [10] Lynch, C. M., McSweeney, P. L. H, Fox, P. F., Cogan, T. M. and Drinan, F. D. 1996. Manufacture of Cheddar cheese with and without adjunct *lactobacilli* under controlled microbiological conditions. *International Dairy Journal*. 6: 851–867.
- [11] Crow, V., Curry, B. and Hayes, M. 2001. The ecology of non-starter lactic acid bacteria (NSLAB) and their use as adjuncts in New Zealand Cheddar. *International Dairy Journal*. 11: 275–283.
- [12] Cogan, T. M. and Hill, C. 1993. Cheese starter cultures. In Fox, P. F. (Ed.), *Cheese: Chemistry, physics and microbiology*, Vol. 1: General aspects 2nd ed. London, UK: Chapman & Hall, pp. 193–255.
- [13] Broome, M.C. and Limsowtin, G.K.Y. 1998. Starter peptidase activity in maturing cheese. *Australian journal of dairy technology*. 53: 79–82.
- [14] Urbach, G. 1993. Relations between cheese flavour and chemical composition. *International Dairy Journal*. 3: 389–422.
- [15] Broome, M. C., Powell, I. B. and Limsowtin, G. K. Y. 2003. Starter Cultures: Specific Properties. In: Roginski, H., Fuquay, J. W., Fox, P. F. Eds. *Encyclopedia of Dairy Sciences*. Vol 1. Academic Press, London, pp: 269-275.
- [16] IDF. 1995. milk and dairy products. Guide sampling (in french), standard 50C. International Dairy Federation. Brussels, Belgium.
- [17] IDF. 1997. Sensory evaluation of dairy products by scoring. Standard 99C. International Dairy Federation. Brussels.
- [18] Reuter, G. 1985. Elective and selective media for lactic acid bacteria. *International Journal of Food Microbiology*. 2: 55–68.
- [19] APHA. 2001. In: *Compendium of Methods for the Microbiological Examination of Foods*. Frances, P. D., Keith, I. Eds. APHA, Washington DC.
- [20] ISO. 1975. Cheese, determination of fat content Van Gulik method. Standard ISO 3433-1975 5(E): International Organization for Standardization.
- [21] IDF. 1982. Cheese and processed cheese-Determination of total solids content. IDF Standard 4A. Brussels, Belgium: International Dairy Federation.
- [22] AOAC. 1984. *Official Methods of Analysis*. 15e Edn. Association of Official Analytical Chemistry, Washington, D.C.
- [23] Ardö, Y. 1999. Evaluating proteolysis by analysing the N content of cheese fractions. *Bulletin of International Dairy Federation*. 337:4–9.
- [24] ISO. 1993. Norma 8586-1. Análisis sensorial. Guía general para la selección, entrenamiento y control de catadores.
- [25] Lawrence, R. C., Creamer, L. K. and Gilles, J. 1987. Symposium: Cheese ripening technology. Texture development during cheese ripening. *Journal of Dairy Science*. 70: 1748–1760.
- [26] Fox, P. F. and Cogan, T. M. 2004. Factors that affect the quality of cheese. In: *Cheese: Chemistry, physics and microbiology*. Fox, P. F., McSweeney, P. L. H., Cogan, T. M., Guinee, T. P. (Eds.). London, UK: Elsevier Academic Press 3rd ed. Vol. 1 pp: 583–608.
- [27] McSweeney, P. L. H. and Fox, P. F. 2004. Metabolism of residual lactose and of lactate and citrate. In: *Cheese: Chemistry, physics and microbiology*. Fox, P. F., McSweeney, P. L. H., Cogan, T. M. and Guinee, T. P. (Eds.). London, UK: Elsevier Academic Press 3rd ed. Vol. 1 pp: 361–371.
- [28] Hayaloglu, A. A., Guven, M., Fox, P. F., Hannon, J. A. and McSweeney, P. L. H. 2004. Proteolysis in Turkish white-brined cheese made with defined strains of *Lactococcus*. *International Dairy Journal*. 14: 599–610.
- [29] Katsiari, M. C., Kondyli, E. and Voutsinas, L. P. 2009. The quality of Galotyri-type cheese made with different starter cultures. *Food Control*. 20 : 113–118
- [30] Fresno, J. M., Tornadijo, M. E., Carballo, J., Gonzalez-Prieto, J. and Bernardo, A. 1996. Characterization and biochemical changes during the ripening of a Spanish craft goat's milk cheese (Armada variety). *Food Chemistry*. 55: 225–230.
- [31] Montanari, G., Zambonelli, C., Grazia, L., Benevelli, M. and Chiavari, C. 2000. Release of β galactosidase from *lactobacilli*. *Food Technology and Biotechnology*. 38: 129–133.



- [32] Ladero, M., Ferrero, R., Vian, A., Santos, A. and Garcia-Ochoa, F. 2005. Kinetic modelling of the thermal and pH inactivation of a thermostable *b*-galactosidase from *Thermus sp.* Strain T2. *Enzyme and Microbial Technology*. 37: 505–513.
- [33] Gonzalez, J., Mas, M., Tabla, R., Moriche, J., Roa, I., Rebollo, J. E. and Cáceres, P. 2003. Autochthonous starter effect on the microbiological, physicochemical and sensorial characteristics of Ibores goat's milk cheeses. *Lait*. 83: 193–202.
- [34] Martley, F. G. and Crow, U. L. 1993. Interactions between non-starter microorganisms during cheese manufacture and ripening. *International Dairy Journal*. 3: 461–483.
- [35] Menéndez, S., Godínez, R., Hermida, M., Centeno, J.A. and Rodríguez-Otero, J. L. 2004. Characteristics of “Tetilla” pasteurized milk cheese manufactured with the addition of autochthonous cultures. *Food Microbiology*. 21: 97–104.
- [36] Herreros, M. A., Arenas, R., Sandoval, M. H., Castro, J. M., Fresno, J. M. and Tornadijo, M. E. 2007. Effect of addition of native cultures on characteristics of Armada cheese manufactured with pasteurized milk: A preliminary study. *International Dairy Journal*. 17 : 328–335.
- [37] Limsowtin, G. K. Y., Powel, I. B. and Parente, E. 1995. Types of starters. In: Cogan, T. and Accolas, J. P. (Eds.) *Dairy Starter Cultures*. VCH Publishers, New York, NY, pp: 101–129.
- [38] Babel, F.J. 1977. Antibiosis by lactic acid bacteria. *Journal of Dairy Science*. 60: 815–821.
- [39] Pitt, W. M., Harden, T. J. and Hull, R. R. 1999. *Listeria monocytogenes* in milk and dairy products. *Australian Journal of Technology*. 54: 49–65.
- [40] De Sousa, G.B., Tamagnini, L. M., Olmos, P. and González, R. D. 2002. Microbial enumeration in ready-to-eat foods and their relationship to good manufacturing practice. *Journal Food Safety*. 22: 27–38.
- [41] Grappin, R. and Beuvier, E. (1997) Possible implications of milk pasteurization on the manufacture and sensory quality of ripened cheese: A review. *Bulletin of the International Dairy Federation*. 327: 16–19.
- [42] Olarte, C., Sanz, S., Gonzalez-Fandos, E. and Torre, P. 2000. The effect of a commercial starter culture addition on the ripening of an artisanal goat's cheese (Cameros cheese). *Journal of Applied Microbiology*. 88: 421–429