Original article

Microbiological changes throughout the manufacturing and ripening of a Spanish goat's raw milk cheese (Armada variety)

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Summary — The microbial groups of technological interest (aerobic mesophilic flora, presumptive lactococci, presumptive lactobacilli, presumptive leuconostocs, presumptive enterococci, presumptive Micrococcaceae and moulds and yeasts) were enumerated throughout the ripening process in 4 batches of Armada goat's milk cheese (2 ripened in summer and 2 ripened in autumn). High counts of all microbial groups were observed in milk ($\log_{10}$ cfu/g: aerobic mesophilic flora 6.72–7.55, presumptive lactococci 6.53–7.40, presumptive lactobacilli 4.84–5.05, presumptive leuconostocs 5.47–5.68, presumptive enterococci 2.94–4.29, presumptive Micrococcaceae 4.47–4.66, moulds and yeasts 4.59–4.66); the highest counts being generally reached in all the microbial groups in 1-week-old cheese. Beyond this, they progressively dropped until the end of ripening. In the first 8 weeks of ripening, no significant differences were observed in the counts related to the season when the cheeses were ripened. Only in 16-week-old cheeses were the counts in ripened cheeses during the summer lower than those determined in autumn ripened cheeses. The lactic acid bacteria were the most abundant flora during manufacturing and ripening of this cheese. Lactococci (*Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*) dominated in milk (50% of the isolates carried out in M17 agar), curd (70% of the isolates carried out in M17 agar) and 1-week-old cheese (70% of the isolates carried out in M17 agar) and lactobacilli (mainly *Lactobacillus casei* subsp. *casei* and *Lactobacillus plantarum*) were the most abundant group during the last stages of ripening (92.5, 95 and 77.5% of the isolates carried out in Rogosa agar in 4-, 8- and 16-week-old cheese, respectively). M17 agar showed a moderate selectivity for the isolation of lactococci (45.71% of the isolates carried out in M17 agar were classified as lactococci). The selectivity of MSE agar for the isolation of leuconostoc was very low (only 16.42% of the isolates carried out in MSE agar were classified as leuconostocs). On the other hand, Rogosa agar and KAA agar showed a very high selectivity for the isolation of lactobacilli and enterococci, respectively

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(86.42% of the isolates carried out in Rogosa agar were classified as lactobacilli and 91.85% of the isolates carried out in KAA agar were classified as enterococci). Values of some physicochemical parameters (pH, a_w and moisture and NaCl contents) as well as the correlation coefficients between the values of these parameters and the microbial group counts throughout ripening were also determined.

Armada cheese / goat cheese / microbiological change / lactic acid bacteria

Résumé — Modifications de la flore bactérienne pendant la fabrication et l'affinage du fromage de chèvre Armada, une variété espagnole fabriquée à partir de lait cru. La présente étude a porté sur la numération de différents groupes microbiens d'intérêt technologique (germes aérobies mésophiles totaux, lactocoques, lactobacilles, leuconostocs, entérocoques et micrococcaceae présumés et moisissures et levures) lors de la fabrication et l'affinage de 4 fabrications de fromages de chèvre de la variété Armada (2 affinés en été et 2 autres affinés en automne). L'analyse du lait a révélé un nombre élevé de microorganismes appartenant aux différents groupes étudiés ($\log_{10}$ ufc/g: Germes aérobies mésophiles totaux 6,72–7,55; lactocoques présumés 6,53–7,40; lactobacilles présumés 4,84–5,05; leuconostocs présumés 5,47–5,68; entérocoques présumés 2,94–4,29; micrococcaceae présumés 4,47–4,66; moisissures et levures 4,59–4,66). Pour la majorité des groupes microbiens étudiés, les niveaux de population les plus élevés ont été trouvés dans le fromage âgé d'une semaine. À partir de ce moment, la charge microbienne a progressivement diminué jusqu'à la fin de l'affinage. Nous avons pu constater que les fromages affinés en été n'ont présenté des dénombrements significativement inférieurs à ceux affinés en automne que pendant la seizième semaine d'affinage. Les bactéries lactiques ont constitué la flore la plus abondante durant les phases de fabrication et d'affinage du fromage; 1120 souches ont été isolées et identifiées à partir du lait, du caillé et du fromage pendant l'affinage. Les lactocoques (*Lactococcus lactis* subsp *lactis* et *Lactococcus lactis* subsp *cremoris*) ont formé le groupe majoritaire du lait (50% des souches isolées dans le milieu M17), du caillé (70% des souches isolées dans le milieu M17) et du fromage d'une semaine (70% des souches isolées dans le milieu M17), tandis que les lactobacilles (surtout *Lactobacillus casei* subsp *casei* et *Lactobacillus plantarum*) furent les plus abondants durant la dernière période de la maturation (92,5%, 95% et 77,5% des souches isolées dans le milieu Rogosa respectivement du fromage de 4, 8 et 16 sem). La sélectivité du milieu M17 s'est révélée modérée pour dénombrer les lactocoques (45,71% des souches isolées dans ce milieu ont été classées comme lactocoques); celle du milieu MSE (pour les leuconostocs) est encore plus faible (seulement 16,42% des souches isolées dans ce milieu ont été classées comme leuconostocs). En revanche, les milieux Rogosa et KAA sont apparus très sélectifs pour isoler respectivement les lactobacilles et les entérocoques (86,42% des souches isolées dans le milieu Rogosa ont été classées comme lactobacilles et 91,85% des souches isolées dans le milieu KAA ont été classées comme entérocoques). Nous avons aussi déterminé les valeurs de certains paramètres physicochimiques (pH, a_w , teneurs en sel et humidité), ainsi que les coefficients de corrélation entre les valeurs de ces paramètres et le dénombrement des différents groupes microbiens tout au long de l'affinage. Le pH a chuté de 6,6 dans le lait à 5,1 dans le caillé, a atteint sa valeur minimale dans les fromages âgés d'une à 4 sem (4,3 à 4,6) puis s'est stabilisé autour de 5,0 en fin d'affinage. Les valeurs moyennes de la teneur en humidité et de l'activité de l'eau atteintes en fin d'affinage se sont situées à respectivement 21,1% et 0,902. Les teneurs en NaCl ont montré une variation importante d'un fromage à l'autre, du fait du procédé de salage mis en oeuvre. La diminution de l'ensemble des groupes microbiens étudiés semble être essentiellement due à la chute des valeurs d'activité, de l'eau ainsi qu'à l'abaissement du pH. Le salage ne semble pas en revanche avoir influencé les niveaux de population microbienne.

fromage Armada / fromage de chèvre / évolution de la flore bactérienne / bactérie lactique

INTRODUCTION

Spain produces a large quantity of goat's milk (about 400 million litres/year) and approximately 40% is used in cheesemaking. Although 28 varieties of goat cheeses (MAPA, 1990) are listed in this country, they are not very well distributed on the market, and only 1 variety (Majorero cheese) is produced on an industrial scale. This situation is mainly due to a lack of information on production methods and biochemical and microbiological characteristics, which prevents manufacture under controlled conditions.

Among the Spanish goat's milk cheeses, only Valdeteja (Gutiérrez *et al.*, 1988), Majorero (Gómez *et al.*, 1989; Fontecha *et al.*, 1990), Ibore (Mas Mayoral *et al.*, 1991; Mas and González-Crespo, 1992), Cendrat del Montsec (Mor-Mur *et al.*, 1992) and Gredos (Medina *et al.*, 1992) have been extensively studied for their microbiological characteristics. There is a lack of studies on the bacterial flora of other varieties.

The Armada cheese, a hard variety made in the north of Spain, is included in the group not yet studied. This cheese is made from raw goat's milk, without addition of starter cultures, using artisanal procedures so its quality is very variable, which makes difficult its diffusion in the national and international markets. The results published on this cheese refer only to the study of Enterobacteriaceae throughout the manufacturing and ripening process (Tornadillo *et al.*, 1993).

The aim of this work was to study the groups of microorganisms which are the most relevant in ripening, throughout the ripening process of Armada cheese. It constitutes a preliminary study in order to elaborate an adequate starter culture which would permit the manufacturing on an industrial scale of a uniform product, and preserve the quality characteristics of the artisanal product as much as possible.

MATERIALS AND METHODS

Manufacturing of cheeses and sampling

Four batches of Armada cheese (2 ripened in summer and 2 ripened in autumn) were made by experienced cheesemakers following the traditional methods. Raw whole goat's milk was coagulated at 30°C by adding about 15 ml of commercial calf rennet (strength 1/10 000) (Productos Nievi, Bilbao, Spain) for each 100 l of milk. One hour later, the curd, compact at this stage, was cut and transferred to cheesecloths. These were formed in bundles and hung for 48 h to drain the whey. The bundles were then opened and the curd given a rigorous kneading ("sobado"). It was then transferred to clean cheesecloths and hung for another 3 d after which it was kneaded and hand-moulded to give the characteristic square form. The cheeses were then wrapped again in cheesecloths and hung from the ceilings where the ripening process took place at a temperature of 10–15°C and a relative humidity of 70–85% depending on the time of year. Salting was carried out by adding solid salt during the kneading operations.

Milk, curd (6 h after placing in the cheesecloths), and 1-, 2-, 4-, 8- and 16-week-old cheese (each sample was constituted of 1 whole cheese) samples were taken from each batch. Samples were transported to the laboratory under refrigeration (below 5°C) and analyzed on arrival.

Microbiological analysis

Fifty g of each sample (after discarding the rind of the cheeses) were homogenized with 200 ml of a sterile solution of 2% sodium citrate at 40–45°C for 1 min in a Stomacher 400 Lab Blender (Seward Medical, London, England), thus making a 1/5 dilution. Consecutive decimal dilutions were prepared by mixing 10 ml with 90 ml of 0.1% sterile peptone water. Aerobic mesophilic bacteria were enumerated in standard plate count agar (Oxoid, Ltd, Basingstoke, England) after incubation at 30°C for 48 h (ICMSF, 1978), presumptive lactococci in M17 agar (Biorad Diagnostics, Beauvais, France) (Terzaghi and Sandine, 1975) after incubation at 30°C for 18 h, presumptive lactobacilli in Rogosa agar (Oxoid) (Rogosa *et*

al, 1951) after incubation at 30°C for 5 d and presumptive leuconostocs in MSE agar (Biokar) (Mayeux *et al*, 1962) after incubation at 22°C for 4 d. Presumptive enterococci were determined on KAA agar (Oxoid) (Mossel *et al*, 1978) incubated for 24 h at 37°C, presumptive Micrococcaceae on Mannitol salt agar (Oxoid) (Chapman, 1945) incubated for 48 h at 30°C, and moulds and yeasts on OGYE agar (Oxoid) (Mossel *et al*, 1970) incubated for 5 d at 22°C. In standard plate count agar, Rogosa agar, KAA agar and OGYE agar, 1 ml volumes of each dilution were inoculated in duplicate and mixed before solidification. Plates of Rogosa agar were covered with a layer of the same medium before incubation. In MSE agar, M17 agar and Mannitol salt agar, 0.1 ml volumes of each dilution were surface plated in duplicate.

After the end of the incubation period, from the M17 agar, MSE agar, Rogosa agar and KAA agar plates, 10 colonies were randomly taken from each sampling point and from each medium with the aid of a Harrison disc (Harrigan and McCance, 1976a). The isolates from M17 agar, MSE agar and Rogosa agar were purified by 4 alternate subcultures in MRS agar and MRS broth (Oxoid). Isolates from KAA agar were purified in nutrient agar - nutrient broth (Oxoid). The isolated strains were, afterwards, maintained in Robertson's cooked meat medium (Harrigan and McCance, 1976b) below 4°C.

Homofermentative, Gram-positive, catalase-negative cocci, which were capable of growing at 10 and 40°C but not at 45°C or at 6.5% salt or at pH 9.6, were considered as lactococci and were classified according to the methods and criteria of Sharpe (1979) and Mundt (1986b). The following tests were carried out on each isolate: hydrolysis of arginine; growth in 4.5% NaCl broth; acetoin production; utilization of L-arabinose, D-xylose, sucrose, trehalose, mannitol, salicin, raffinose, inulin, glycerol, sorbitol, rhamnose, ribose and maltose.

The Gram-positive, catalase-negative cocci, grouped in pairs or short chains, homofermentative, which grew at 10, 37, 40 and generally at 45°C, generally survived after heating at 60°C after 30 min, grew in a 6.5% salt, at a pH 9.6 and in 40% bile, and formed red or pink colonies generally with a yellowish halo in KF Streptococcus agar (Difco Laboratories, Detroit, MI, USA), were considered as enterococci and were classified according to the methods and criteria described by Sharpe (1979), Collins *et al* (1984), Colman

and Ball (1984), Mundt (1986b) and Devriese *et al* (1987). The following tests were carried out on each isolate: growth at 50°C; hydrolysis of arginine; reduction of 0.1% methylene blue milk; growth in 0.04% potassium tellurite broth; utilization of L-arabinose, arbutin, melezitose, melibiose, sorbitol, sorbose, rhamnose, raffinose, starch and sucrose.

The Gram-positive, catalase-negative, heterofermentative cocci which did not hydrolyze arginine (leuconostoc) were characterized according to the methods and criteria of Garvie (1984, 1986). The following tests were carried out on each isolate: growth at 37°C and in 4 and 6.5% NaCl broth; dextran production; utilization of L-arabinose, cellobiose, fructose, glucose, lactose, maltose, melibiose, sucrose, salicin and trehalose.

Gram-positive, catalase-negative rods (lactobacilli) were characterized according to the methods and criteria of Sharpe (1979) and Kandler and Weiss (1986). The following tests were carried out on each isolate: growth at 15 and 45°C; gas production from glucose; hydrolysis of arginine; utilization of L-arabinose, cellobiose, lactose, melezitose, melibiose, raffinose, salicin, trehalose, D-xylose, mannose, sucrose, inulin, rhamnose, sorbitol and sorbose.

Chemical analysis

Moisture and NaCl contents in curd and cheese were determined according to the IDF standards 4A (1982) and 17A (1972), respectively. The pH values in curd and cheese were measured according to the 14022 AOAC (1975) method. Moisture content in milk was determined according to the IDF standard 21B (1987). NaCl content in milk was determined according to the French standard (Norme Francaise Homologuée) V 04-212 (1969). The pH of milk was measured directly with a pH meter PHM 82 Standard (Radiometer, Copenhagen, Denmark). Water activity (a_w) was measured with a Decagon CX-1 Water Activity System apparatus (Decagon Devices, Pullman, WA, USA).

Statistical analysis

Analysis of variance was carried out to investigate the differences in the counts of the different

microbial groups between the batches ripened in summer and in autumn.

Correlations between counts of the microbial groups and physicochemical parameters were determined with the aid of the computer programme SPSS/PC+ version 3.1 (SPSS Inc, Chicago, IL, USA).

RESULTS AND DISCUSSION

Changes in the microbial groups throughout ripening

Tables I and II show the evolution of the counts of the investigated microbial groups throughout the maturation of the batches ripened in summer and autumn, respectively. The milk used in the manufacture of the cheeses had high microbial counts ($\log_{10}$ counts of aerobic mesophilic flora 6.72–7.55/g). Although similar counts have been observed in goat's milk used in the manufacture of other varieties of cheese (Fatichenti *et al*, 1979; Gutiérrez *et al*, 1988; Fontecha *et al*, 1990; Mas Mayoral *et al*, 1991), these values were very much higher than those reported by other authors (Espie and Mullan, 1987; Tirard-Collet *et al*, 1991; Medina *et al*, 1992) and they were the consequence of the hand milking in unhygienic conditions and of the absence of refrigeration after milking. The counts of the other microbial groups in milk were also high but within the range of those observed in milk used in the manufacture of other goat cheeses (Fatichenti *et al*, 1979; Gutiérrez *et al*, 1988; Fontecha *et al*, 1990; Mas Mayoral *et al*, 1991).

The counts reached in curd by the investigated microbial groups were, on average, 1 $\log_{10}$ unit higher than those observed in milk. The increase of the microbial counts in curd is usual in cheese manufacture and is partly due to the microbial multiplication and partly to the physical retention of the microorganisms in the coagulum during the

whey drainage. The increase observed in Armada curd was similar to that described by Medina *et al* (1992) in Gredos cheese and by Gutiérrez *et al* (1988) in Valdeteja cheese. However, Pouillet *et al* (1991) in Casar de Cáceres ewe cheese observed increases notably higher (3 $\log_{10}$ units). Gómez *et al* (1989) in Majorero cheese observed even higher increases in total viable counts (3–5 $\log_{10}$ units); however, it must be taken into account that in this instance pasteurized milk was used to which a starter culture was added before coagulation. The low increases in the counts in Armada cheese curd can be explained by the short period of time between milk and curd sample taking (approximately 7 h), in which the microbial multiplication was minimal. This possibility is supported by other authors (Tatini *et al*, 1971) who conclude that the physical retention of the bacteria in the curd is responsible for the increase in 1 $\log_{10}$ unit counts, the remaining increase being due to microbial multiplication.

The highest counts of all the microbial groups were reached, generally, in 1-week-old cheese. The increase in count during the first week of ripening was accompanied by a sharp drop in pH values (about 2 units) (fig 1) which is a consequence of the production of acid by the microorganisms. This sharp drop in pH is logical if one takes into account that the lactococci (main producers of lactic acid) were the dominant microbial group in milk, curd and 1-week-old cheese.

After the first week of ripening, the counts of all the microbial groups fell slowly and irregularly, reaching, after 16 weeks of ripening, in the batches ripened in autumn, counts on average 1 $\log_{10}$ unit lower than those shown in 1-week-old cheese (table II). The decrease undergone by the lactococci in these 2 batches (2.45 $\log_{10}$ units) was noticeable. In the batches ripened in summer (table I), the decrease in the counts throughout ripening was higher (the counts

Table I. Changes in log₁₀ cfu/g of main microbial groups of Armada cheese ripened in summer.
Évolution des comptages (log ufc/g) des principaux groupes microbiens du fromage Armada affiné en été.

Microbial group	Milk	Curd	Ripening time (weeks)				
			1	2	4	8	16
Aerobic mesophilic flora	6.72 ± 0.15	8.29 ± 0.14	9.37 ± 0.55	8.65 ± 0.35	8.69 ± 0.55	7.42 ± 0.17	4.39 ± 0.03
Presumptive lactococci	6.53 ± 0.07	7.89 ± 0.40	9.48 ± 0.56	8.41 ± 0.26	7.14 ± 0.40	6.80 ± 0.04	4.17 ± 0.40
Presumptive lactobacilli	4.84 ± 0.08	5.76 ± 0.16	8.40 ± 0.14	8.50 ± 0.16	8.41 ± 0.07	7.68 ± 0.15	4.19 ± 0.41
Presumptive leuconostocs	5.47 ± 0.21	6.95 ± 0.66	8.60 ± 0.71	8.21 ± 0.01	8.23 ± 0.17	7.08 ± 0.11	3.38 ± 0.02
Presumptive enterococci	4.29 ± 0.44	5.42 ± 0.31	6.80 ± 0.51	6.89 ± 0.55	6.26 ± 0.63	3.08 ± 0.50	0.80 ± 1.13
Presumptive Micrococcaceae	4.47 ± 0.17	5.21 ± 0.52	4.74 ± 0.19	3.89 ± 0.30	4.03 ± 1.17	4.00 ± 2.83	2.58 ± 0.05
Moulds and yeasts	4.59 ± 0.06	5.26 ± 0.81	6.79 ± 0.25	6.20 ± 0.35	6.16 ± 0.79	5.73 ± 0.32	2.94 ± 1.75

Data are the average ± standard deviation values of 2 batches.
Les données sont la moyenne ± l'écart type des valeurs de 2 fabrications.

Table II. Changes in log₁₀ cfu/g of main microbial groups of Armada cheese ripened in autumn.
Évolution des comptages (log ufc/g) des principaux groupes microbiens du fromage Armada affiné en automne.

Microbial group	Milk	Curd	Ripening time (weeks)				
			1	2	4	8	16
Aerobic mesophilic flora	7.55 ± 0.44	8.46 ± 0.07	9.21 ± 0.97	8.84 ± 0.15	8.37 ± 0.25	8.75 ± 0.44	8.14 ± 0.10
Presumptive lactococci	7.40 ± 0.76	8.47 ± 0.26	8.83 ± 0.53	8.55 ± 0.12	7.50 ± 0.10	7.05 ± 0.37	6.38 ± 0.71
Presumptive lactobacilli	5.05 ± 0.00	6.11 ± 0.76	8.42 ± 0.56	8.50 ± 0.01	8.25 ± 0.50	8.11 ± 0.57	8.09 ± 0.34
Presumptive leuconostocs	5.68 ± 0.40	7.39 ± 0.02	8.49 ± 0.03	8.53 ± 0.04	8.06 ± 0.23	8.05 ± 0.53	7.58 ± 0.12
Presumptive enterococci	2.94 ± 0.32	4.96 ± 0.38	5.85 ± 0.61	5.47 ± 0.00	5.71 ± 0.20	5.34 ± 0.02	3.94 ± 1.70
Presumptive Micrococcaceae	4.66 ± 0.04	5.27 ± 0.23	5.37 ± 0.42	5.06 ± 0.13	5.39 ± 0.67	3.85 ± 0.65	4.20 ± 0.56
Moulds and yeasts	4.66 ± 0.56	5.54 ± 0.16	6.60 ± 0.00	6.60 ± 0.19	6.63 ± 0.17	5.60 ± 0.20	5.58 ± 0.30

Data are the average ± standard deviation values of 2 batches.
Les données sont la moyenne ± l'écart type des valeurs de 2 fabrications.

in 16-week-old cheese were in the different microbial groups on average of $4.5 \log_{10}$ units lower than those recorded in 1-week-old cheese), the drop in the enterococci

being particularly marked ($6 \log_{10}$ units). The greatest drop in these batches occurred during the last 8 weeks of ripening (on average $2.80 \log_{10}$ units in the different groups).

Fig 1. Changes in a_w and pH values and in moisture and NaCl contents throughout the manufacturing and ripening of Armada cheese. Plots show the average of the values measured in the batches ripened in summer (●) and in autumn (▲).

Modifications des valeurs de a_w , pH, humidité et NaCl durant la fabrication et l'affinage du fromage Armada. Les graphiques montrent les valeurs moyennes des fromages affinés en été (●) et en automne (▲).

Judging from the correlation coefficients between the different microbial group counts and the physicochemical parameters (table III), the drop in the microbial groups during ripening may have been due to the decrease in water activity. This decrease in water activity was more evident in the batches ripened in summer (fig 1) due to the higher temperatures and lower relative humidity existing in the curing rooms in this season which lead to a greater loss in humidity because of the interchange with its environment. This large fall in a_w in batches ripened in summer was accompanied by a large decrease in the microbial counts in these batches.

From the correlation coefficients between a_w values and microbial counts, presumptive lactococci ($r = 0.79$) and presumptive enterococci ($r = 0.72$) seem to be the microbial groups the most affected by the drop in water activity. pH values in the range 4.3 to 4.8 during most of the ripening period could also influence the fall in microbial counts.

Salting, carried out during the kneading operations, did not seem to have much effect on the counts. Furthermore, the flora which better tolerates salt (*Micrococcaceae*) was the microbial group which increased least between the curd counts and 1-week-old cheese counts, even decreasing ($0.47 \log_{10}$ units) during this period in the batches ripened in summer. The weak growth of *Micrococcaceae* is probably related to the low pH values observed in this cheese. It must also be taken into account that this behaviour of *Micrococcaceae* may be explained by the fact that micrococci are aerobic bacteria which do not grow well at the low redox potential reached inside the cheeses.

It is important to point out that the salt content of the different cheeses is fairly irregular due to the salting technique used. The cheeses were individually salted adding solid salt during the first kneading operation. It is possible that the exact same quan-

tity of salt was not added to all the cheeses and it is also probable that the loss in salt content together with the whey, which still drop out from the curd while in cheesecloth before giving them their final shape, were also distinct in the different cheeses.

Overall, considering the 4 batches, the lactobacilli were the dominant microbial group in the last stages of ripening, which was corroborated by the identification of the isolates carried out in the different culture media and observed previously by other authors in different types of cheese (Núñez, 1978; De Giori *et al.*, 1983; Fernández del Pozo *et al.*, 1988; Mas Mayoral *et al.*, 1991; Medina *et al.*, 1992; Tzanetakis and Litopoulou-Tzanetaki, 1992). This circumstance could be due to the great facility the lactobacilli have to grow at low pH values (Núñez, 1976; McDonald *et al.*, 1990), corroborated by the high negative correlation established between the $\log_{10}$ counts of the presumptive lactobacilli and the pH values (table III). The high resistance of the lactobacilli to the salt (Sharpe, 1979) may also be the cause of their predominance in the last few stages of ripening.

It may be concluded that the lactic acid bacteria were the predominant flora during manufacturing and ripening of Armada cheese. *Micrococcaceae* showed initial low counts but they maintained their levels relatively constant during the ripening period ($4-5 \log_{10}$ cfu/g). Similar steadiness was observed in the yeast and mould counts (about $5-6 \log_{10}$ cfu/g). The activity of yeasts which metabolize lactic acid was possibly, as well as the liberation of alkaline compounds during breakdown of the proteins, responsible for the increase in pH after the first week of ripening (fig 1).

In the first 8 weeks of ripening no significant differences were observed in the counts of different microbial groups associated to the ripening season. Only in 16-week-old cheese (last sampling point) were the counts in the cheeses ripened in sum-

Table III. Correlation coefficients between the log₁₀ cfu/g of main microbial groups and some physicochemical parameters throughout the manufacturing and ripening of Armada cheese (4 batches).

Coefficients de corrélation entre les numérations des différents groupes microbiens et les valeurs de certains paramètres physicochimiques durant la fabrication et l'affinage du fromage Armada (4 fabrications).

	Aerobic mesophilic flora	Lactococci	Lactobacilli	Presumptive Leuconostocs	Enterococci	Micrococcaceae	Moulds & yeasts	Salt	Water activity	Moisture	pH
Aerobic mesophilic flora	1										
Presumptive Lactococci	0.86***	1									
Lactobacilli	0.78***	0.55**	1								
Leuconostocs	0.93***	0.77***	0.90***	1							
Enterococci	0.80***	0.79***	0.68***	0.83***	1						
Micrococcaceae	0.42*	0.57***	0.18NS	0.42*	0.39*	1					
Moulds and yeasts	0.80***	0.72***	0.83***	0.89***	0.78***	0.51**	1				
NaCl	-0.05NS	-0.21NS	0.42*	0.13NS	-0.03NS	-0.17NS	0.27NS	1			
a _w	0.62***	0.79***	0.21NS	0.55**	0.72***	0.45*	0.44*	-0.74***	1		
Moisture	-0.00NS	0.26NS	-0.44*	-0.19NS	0.04NS	0.31NS	-0.12NS	-0.70***	0.91***	1	
pH	-0.40*	-0.27NS	-0.66***	-0.55**	-0.44*	0.06NS	-0.50**	-0.59***	-0.07NS	0.75***	1

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; NS $P > 0.05$.

mer lower than those found in the cheeses ripened in autumn. These differences were particularly outstanding in aerobic mesophilic flora ($P < 0.001$), presumptive leuconostocs (counts in MSE agar) ($P < 0.001$) and presumptive lactobacilli (counts in Rogosa agar) ($P < 0.05$).

Species isolated throughout ripening

Table IV shows the species isolated in M17 agar during the ripening of Armada cheese. M17 agar showed a moderate selectivity for the isolation of lactococci in this cheese (only 45.71% of isolates obtained in this medium were classified as lactococci). The main lactococci species was *Lactococcus lactis* subsp. *lactis*. This species constituted 47.5% of the isolates in milk, its proportion increased in curd (62.5% of isolates) and even more in 1-week-old cheese (70% of isolates). Its proportion dropped from this sampling point reaching very low proportions in 8-week-old cheese (12.5% of isolates) and 16-week-old cheese (17.5% of isolates). The decrease in the number of lactococci took place probably because of the inhibitory effect of low pH and high NaCl in moisture concentrations that in 8- and 16-week-old cheese reached values of 9% on average. This decrease has previously been observed by other authors in different varieties of cheeses (Litopoulou-Tzanetaki and Tzanetakis, 1992; Mas and González-Crespo, 1992; Tzanetakis and Litopoulou-Tzanetaki, 1992).

Lactobacilli species, which were not isolated from M17 agar in noticeable percentages in the first stages of ripening, constituted the main species in 8-week-old cheese (57.5% of isolates) and 16-week-old cheese (32.5% of isolates). The isolation of enterococci after the fourth week of ripening was also noticeable, with *Enterococcus faecalis* being the main enterococcus isolated species in this medium (20% of isolates in 4-

week-old cheese, 10% of isolates in 8-week-old cheese and 17.5% of isolates at the last sampling point).

The number of isolates lost in this culture medium was very high, especially at the first 3 sampling points. It is worth noting that practically all the losses were produced during the purification phase of the strains (alternate cultures in MRS agar – MRS broth), which makes us suspect that the isolates lost were probably non-lactic acid bacteria.

Table V shows the species isolated from MSE agar during the ripening of this cheese. Despite its high contents in sucrose (100 g/l) and the presence of sodium azide (0.075 g/l), MSE agar showed a very low selectivity for leuconostoc in our case, with only 16.42% of the total isolates carried out in this medium representing the microorganisms of this genus. The leuconostoc species isolated in Armada cheese agree with those identified in other goat cheeses (Fatichenti *et al.*, 1979; Fontecha *et al.*, 1990; Litopoulou-Tzanetaki and Tzanetakis, 1992; Mas and González-Crespo, 1992). *Leuconostoc mesenteroides* subsp. *dextranicum* was the most isolated leuconostoc species, with its presence in 1-week-old cheese (25% of isolates), and 8-week-old cheese (17.5% of isolates) being particularly significant.

The low selectivity shown by MSE agar made the isolated species in this medium throughout ripening a reflection of the overall evolution of lactic acid flora throughout ripening. Lactococci isolates (*Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*) predominated in milk, curd and 1-week-old cheese, with the lactobacilli (*Lactobacillus casei* subsp. *casei*, *Lactobacillus plantarum* and, to a lesser extent, *Lactobacillus brevis*) being the most frequent species isolated in this medium at the last sampling points. The isolation of *Enterococcus malodoratus* in almost all sampling points, and in some of them (milk, 2-week-old and 4-week-old cheese) in high

Table IV. Changes in species isolated from M17 agar during the manufacturing and ripening of Armada cheese (4 batches).
Distribution des espèces isolées dans le milieu M17 durant la fabrication et l'affinage du fromage Armada (4 fabrications).

Species	Milk		Curd		Ripening time (weeks)									
					1		2		4		8		16	
	No of isolates	(%)	No of isolates	(%)	No of isolates	(%)	No of isolates	(%)	No of isolates	(%)	No of isolates	(%)	No of isolates	(%)
<i>Lactococcus lactis</i> subsp <i>lactis</i>	19	47.5	25	62.5	28	70	21	52.5	15	37.5	5	12.5	7	17.5
<i>Lactococcus lactis</i> subsp <i>lactis</i> biovar <i>diacetylactis</i>	–	–	2	5	–	–	–	–	–	–	–	–	–	–
<i>Lactococcus lactis</i> subsp <i>cremoris</i>	1	2.5	1	2.5	–	–	4	10	–	–	–	–	–	–
<i>Lactobacillus plantarum</i>	1	2.5	–	–	–	–	4	10	–	–	17	42.5	4	10
<i>Lactobacillus casei</i> subsp <i>casei</i>	1	2.5	–	–	1	2.5	5	12.5	6	15	4	10	3	7.5
<i>Lactobacillus brevis</i>	–	–	–	–	–	–	1	2.5	–	–	1	2.5	4	10
<i>Lactobacillus fermentum</i>	–	–	–	–	–	–	1	2.5	–	–	–	–	1	2.5
Unidentified lactobacilli	–	–	–	–	–	–	–	–	1	2.5	1	2.5	1	2.5

<i>Leuconostoc mesenteroides</i> subsp <i>mesenteroides</i>	–	–	–	–	–	–	1	2.5	–	–	–	–	–	–
<i>Leuconostoc mesenteroides</i> subsp <i>dextranicum</i>	–	–	–	–	2	5	–	–	–	–	–	–	1	2.5
<i>Enterococcus malodoratus</i>	–	–	–	–	–	–	1	2.5	2	5	1	2.5	1	2.5
<i>Enterococcus faecalis</i>	–	–	–	–	–	–	2	5	8	20	4	10	7	17.5
<i>Enterococcus faecium</i>	–	–	–	–	–	–	–	–	1	2.5	–	–	–	–
<i>Enterococcus</i> <i>inter faecalis-faecium</i>	–	–	–	–	–	–	–	–	3	7.5	–	–	–	–
Unidentified enterococci	–	–	1	2.5	–	–	–	–	–	–	–	–	1	2.5
Non lactic acid bacteria	4	10	1	2.5	–	–	–	–	2	5	3	7.5	3	7.5
Isolates lost	14	35	10	25	9	22.5	–	–	2	5	4	10	7	17.5
Total	40	100	40	100	40	100	40	100	40	100	40	100	40	100

Microflora of Armada goat's milk cheeses

Table V. Changes in species isolated from MSE agar during the manufacturing and ripening of Armada cheese (4 batches).
Distribution des espèces isolées dans le milieu MSE durant la fabrication et l'affinage du fromage Armada (4 fabrications).

Species	Milk		Curd		Ripening time (weeks)									
					1		2		4		8		16	
	No of isolates	(%)	No of isolates	(%)	No of isolates	(%)	No of isolates	(%)	No of isolates	(%)	No of isolates	(%)	No of isolates	(%)
<i>Lactococcus lactis</i> subsp <i>lactis</i>	10	25	13	32.5	13	32.5	2	5	–	–	–	–	–	–
<i>Lactococcus lactis</i> subsp <i>cremoris</i>	6	15	5	12.5	3	7.5	3	7.5	4	10	–	–	–	–
Unidentified lactococci	2	5	–	–	–	–	–	–	–	–	–	–	–	–
<i>Lactobacillus plantarum</i>	1	2.5	3	7.5	2	5	12	30	17	42.5	28	70	21	52.5
<i>Lactobacillus casei</i> subsp <i>casei</i>	–	–	–	–	–	–	3	7.5	3	7.5	2	5	10	25
<i>Lactobacillus brevis</i>	–	–	–	–	–	–	–	–	1	2.5	1	2.5	7	17.5
<i>Leuconostoc mesenteroides</i> subsp <i>mesenteroides</i>	2	5	1	2.5	3	7.5	4	10	2	5	–	–	–	–
<i>Leuconostoc mesenteroides</i> subsp <i>dextranicum</i>	1	2.5	3	7.5	10	25	4	10	4	10	7	17.5	–	–

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
<i>Leuconostoc paramesenteroides</i>	–	–	3	7.5	2	5	–	–	–	–	–	–	–	–
<i>Enterococcus malodoratus</i>	6	15	–	–	2	5	4	10	7	17.5	1	2.5	–	–
<i>Enterococcus faecalis</i>	1	2.5	3	7.5	–	–	–	–	–	–	–	–	–	–
<i>Enterococcus</i> <i>inter faecalis-faecium</i>	–	–	1	2.5	–	–	–	–	–	–	–	–	–	–
Unidentified enterococci	1	2.5	–	–	–	–	–	–	–	–	–	–	–	–
Non lactic acid bacteria	–	–	–	–	–	–	1	2.5	–	–	1	2.5	1	2.5
Isolates lost	10	25	8	20	5	12.5	7	17.5	2	5	–	–	1	2.5
Total	40	100	40	100	40	100	40	100	40	100	40	100	40	100

proportions, was also noticeable. The fact that this species, also described in Gouda cheese (Collins *et al.*, 1984), does not figure among the enterococci species isolated in KAA agar is unusual.

The number of isolates lost in this medium was also important, particularly at the first sampling points. As occurred in M17 agar, almost all the losses were produced when the strains were grown in MRS medium. This makes us suspect that these strains were yeasts and other non-lactic acid flora.

Table VI shows the evolution of the species isolated from Rogosa agar during ripening. Rogosa agar showed a high selectivity for lactobacilli, which made up 86.42% of all isolations carried out in this medium. The main species isolated were *Lactobacillus casei* subsp. *casei* and *Lactobacillus plantarum* in practically the same proportion and with a fairly equal distribution at all sampling points. These 2 species were also mainly isolated in other goat cheeses such as artisanal Majorero (Fontecha *et al.*, 1990), industrial Majorero (Gómez *et al.*, 1989), white-brined cheese (Litopoulou-Tzanetaki and Tzanetakis, 1992) and Ibores (Mas and González-Crespo, 1992). However, the predominance of *Lactobacillus plantarum* was very evident in almost all of these cases.

The only heterofermentative lactobacilli species isolated was *Lactobacillus brevis* only reaching important proportions in 16-week-old cheese (20% of isolates); its presence in milk (2.5% of isolates) and 2-week-old cheese (2.5% of isolates) was minimal. The isolation on Rogosa agar of other non-lactobacilli lactic acid bacteria was minimal (only 1 isolate was identified as *Lactococcus lactis* subsp. *lactis*, 4 identified as *Leuconostoc mesenteroides* subsp. *dextranicum* and 2 isolates of unidentified enterococci).

Table VII shows the evolution of the isolated species from KAA agar throughout ripening. From the identity of isolates, we

can conclude that this medium showed an almost absolute selectivity for enterococci. Only at the last sampling point were 6 non-enterococci strains isolated (1 identified as *Lactobacillus casei* subsp. *casei* and 5 identified as *Lactobacillus casei* subsp. *alactosus*).

Enterococcus faecalis was the predominant species, constituting 100% of the isolates carried out in 2-week-old cheese and a percentage higher than 60% at all the other sampling points. A considerable number of isolates (10) showed characteristics very close to *Enterococcus faecalis* without strictly fulfilling the biochemical and cultural standards of this species; for this reason we feel more correct in including them under the name of *Enterococcus* like-*faecalis*. Six isolates were identified as *Enterococcus durans* and only 1 as *Enterococcus faecium*. Finally, 18 isolates showed intermediate characteristics between *Enterococcus faecalis* and *Enterococcus faecium* and they were considered as *Enterococcus* inter *faecalis*-*faecium*.

Enterococcus faecalis has also been described as the most dominant enterococci species in other artisanal goat cheeses such as Sardinia (Fatichenti *et al.*, 1979), Majorero (Fontecha *et al.*, 1990) and Ibores (Mas and González-Crespo, 1992). Only in white-brined raw goat's milk cheese (Litopoulou-Tzanetaki and Tzanetakis, 1992) did *Enterococcus faecium* dominate over the other species of enterococci.

In the 4 culture media, no appreciable differences in the isolated species were observed related to the ripening season.

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