A. Cristina Freitas · Ana E. Pintado
Manuela E. Pintado · F. Xavier Malcata

Organic acids produced by lactobacilli, enterococci and yeasts isolated from Picante cheese

Abstract Four species of bacteria (*Enterococcus faecium* and *Enterococcus faecalis, Lactobacillus plantarum* and *Lactobacillus paracasei*) and three species of yeasts (*Debaryomyces hansenii, Yarrowia lipolytica* and *Cryptococcus laurentii*), previously isolated from Picante cheese, were cultured in ovine and in caprine milk and assayed for sugar and organic acids metabolism for 6 days. The results indicated that both milk types can be coagulated by the four strains of lactic acid bacteria. *Lb. paracasei* led to a faster and greater reduction in pH. Production of lactic acid correlated to lactose degradation, and was highest for *Lb. paracasei* followed by *E. faecium*; citrate metabolism was apparent for *E. faecalis* and, to a lesser extent, for *E. faecium, Lb. plantarum* and *Lb. paracasei.* Relatively high contents of formic acid were found when inoculation was with *Enterococcus* and with *Lb. plantarum*.

Key words Microflora · Ovine milk · Caprine milk · Glycolysis · Organic acids

Introduction

Picante da Beira Baixa cheese (or simply Picante), a traditional cheese manufactured in Portugal from mixtures of ovine and caprine milk, originates from an *Appéllation d'Origine Controllée* region in Portugal, defined in 1988. This variety of cheese, which is characterised by unique hardness, saltiness and spiciness, is manufactured manually, at the farm level only, and ripened for a minimum period of 120 days.

The inconsistency of milk quality and of its microflora, together with the lack of standardised processing, contribute considerably to high variations in physicochemical and biochemical cheese characteristics. Although Picante cheese is manufactured in the absence of starter/non-starter cultures, it is well known that the achievement and maintenance of high organoleptic and safety standards for this variety of cheese will eventually require production (and subsequent use) of microbial additives based on adventitious microflora. The major families present throughout ripening of Picante cheese are lactic acid bacteria and yeasts [1], with viable numbers above 10^7 cfu/g $_{\text{cheese}}$; the most abundant species of the former are enterococci (*Enterococcus faecium*, *Enterococcus faecalis* and *Enterococcus durans*) and lactobacilli (*Lactobacillus plantarum* and *Lactobacillus paracasei*), whereas those of the latter are *Debaryomyces hansenii* and *Yarrowia lipolytica* [2]. Smaller viable numbers were also reported for Enterobacteriaceae (which ranged from ca. 10^7 cfu/g $_{\text{cheese}}$ in fresh cheese to less than 10^2 cfu/g $_{\text{cheese}}$ by 80 days of ripening) and staphylococci (from ca. 10^6 cfu/g $_{\text{cheese}}$ in fresh cheese to 10^4 cfu/g $_{\text{cheese}}$ by 180 days of ripening) [1, 2].

A few organic acids (e.g. lactic, propionic, succinic and acetic acids) have been shown to correlate to flavour characteristics of such cheeses as Cheddar, Swiss, Emmental, Tilsit and Edam [3]. The purpose of this research effort was to investigate the effects of production of organic acids by lactobacilli, enterococci and yeasts, previously isolated from Picante cheese, on ovine and caprine milk in order to assess their tentative role in glycolysis.

Materials and methods

Preparation of microbial cultures. Dominant species, previously isolated from Picante cheese and identified according to Freitas et al. [2], were used in the present study. Four species of bacteria and three species of yeasts were isolated, namely two *Lactobacillus* strains (*Lb. plantarum* and *Lb. paracasei*) and two *Enterococcus* strains (*E. faecalis* and *E. faecium*) for the first microbial group, and *D. hansenii*, *C. laurentii* and *Y. lipolytica* for the second microbial group. Experimental inocula of lactobacilli and enterococci for milk and curdled milk experiments were obtained after growth for 18 h (which was the average time necessary to

A.C. Freitas · A.E. Pintado · M.E. Pintado · F.X. Malcata (✉)
Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, P-4200-072 Porto, Portugal

reach the exponential phase) in de Man, Rogosa & Sharpe (MRS) broth (Lab M, Bury, UK) at 30 °C; inocula of yeasts were obtained after growth for 18 h in the case of *Y. lipolytica*, and for 48 h in the case of *D. hansenii* and *C. laurentii*, in Yeast Morphology (YM) broth (Difco, Detroit Mich., USA) at 30 °C. Viable counts were determined for each species after the proper incubation times as detailed.

Preparation of milk feedstocks. Ovine milk from the Frízia breed and caprine milk from the Charnequeira breed were thermally processed at 110 °C for 10 min; this heat treatment does not damage the casein micellar structure or the fat globule network [4, 5]. Milk sterility was confirmed as absence of microorganisms on plate count agar (PCA) (Lab M) incubated at 30 °C for 5 days at a 1/10 dilution rate.

Experiments with milk. Milk portions of 40 ml were sterilised in 100 ml flasks using the procedure described. Then, 0.1 ml of each single-strain culture and 0.1 ml of a mixed inoculum were added to the sterilised milk and incubated at 30 °C; the mixed inoculum (denoted hereafter as tentative starter) had been prepared previously with a 2 ml inoculum of *E. faecium*, a 2 ml inoculum of *Lb. plantarum* and a 2 ml inoculum of *D. hansenii*. Sterile milk samples, incubated under identical experimental conditions, were used as controls. Microbiological counts, pH and the type of coagulation (strong curd, fine and grainy curd, or no curd at all) were determined at 0, 1, 3 and 6 days of incubation. To check for possible contamination, the microbiological counts were obtained simultaneously on PCA and on MRS agar for lactic acid bacteria, and on PCA and potato dextrose agar (PDA) for yeasts. Furthermore, microscopic observation using Gram staining for bacteria, as well as plain observation for yeasts was carried out; the catalase test was also performed for bacteria.

Sugars and organic acids were quantified by HPLC via an ion exchange column using the following separation conditions: the flow rate was 6 ml/min of 5 mM H_2SO_4 at 60 °C; the injection volume was 50 μl of sample; and the detection methods were refractive index at 30 °C for sugars and UV detection at 60 °C for organic acids. Samples were prepared according to Macedo and Malcata [6] by precipitation of 5.0 g of sample with 20.0 g of 1.0 M perchloric acid, standing overnight at 4 °C, centrifugation of 1.0 ml of supernatant for 15 min at 4000 rpm and filtration through a 0.45 μm membrane filter.

Assessment of statistical significance of results. Analysis of variance and Scheffé's *F*-test (at the 5% significance level) were carried out on the data obtained throughout incubation, for the different single strains and types of milk. Linear relationships between organic acids and pH for each strain and type of milk were statistically evaluated. All statistical analyses were performed using the StatView v.4.01 Computer Software (Abacus, Berkeley Calif., USA).

Results and discussion

The compositional characteristics of the caprine and ovine milk used in this set of experiments are tabulated in Table 1. The evolutions of the microflora viable numbers, pH and type of coagulation (if any) in sterile caprine and ovine milk inoculated with each selected strain of bacterium or yeast (and the corresponding controls) are shown in Table 2. It should be emphasised that a slight reduction in pH was observed after sterilisation and throughout the 6 days of incubation at 30 °C in the control samples. This can probably be accounted

Table 1 Composition of caprine and ovine milks

Characteristic	Caprine milk	Ovine milk
pH (fresh milk)	6.78	6.63
pH (sterilised milk)	6.65	6.55
Total solids (%, w/w)	13.50	16.65
Total protein (%, w/w)	4.03	6.17
Total fat (%, w/w)	4.92	6.02

Table 2 Changes in microflora viable numbers, pH and aspect of casein matrix in caprine and ovine milk inoculated with strains isolated from Picante cheese and incubated at 30 °C

Microorganism	Milk type	Viable numbers (log (cfu/g))					pH					Coagulation[c]			
		0 day	1 day	3 days	6 days	SEM[a]	0 day	1 day	3 days	6 days	SEM[a]	0 day	1 day	3 days	6 days
E.	Caprine	6.30	8.76	8.78	9.19	0.10	6.65	6.09	5.43	4.96	0.03	−	−	+	++
faecium	Ovine	6.23	9.27	9.62	8.90	0.08	6.55	5.59	4.73	4.57	0.02	−	+	++	++
E.	Caprine	6.03	9.70	9.80	9.30	0.03	6.65	6.21	5.79	5.50	0.02	−	−	+	++
faecalis	Ovine	6.23	8.94	9.41	8.74	0.07	6.55	6.28	5.51	5.35	0.02	−	−	++	++
Lb.	Caprine	6.85	8.45	9.05	8.35	0.06	6.65	5.72	5.46	5.31	0.03	−	+	+	++
plantarum	Ovine	6.92	8.64	8.72	8.57	0.10	6.55	5.68	4.78	4.68	0.03	−	+	++	++
Lb.	Caprine	6.24	9.52	9.90	11.32	0.05	6.65	4.84	3.74	3.75	0.04	−	++	++	++
paracasei	Ovine	6.08	9.37	9.45	9.18	0.06	6.55	4.93	3.63	3.62	0.02	−	++	++	++
D.	Caprine	3.76	5.00	4.86	6.20	0.06	6.65	6.76	6.65	6.17	0.01	−	−	−	−
hansenii	Ovine	3.35	4.63	6.85	7.32	0.10	6.55	6.69	6.41	6.18	0.02	−	−	−	−
Y.	Caprine	6.17	6.90	7.81	7.40	0.08	6.65	6.72	6.37	6.23	0.02	−	−	−	−
lipolytica	Ovine	4.35	7.03	7.11	6.25	0.03	6.55	6.66	5.18	5.10	0.01	−	−	−	−
C.	Caprine	0.04	0.04	3.94	4.48	0.02	6.65	6.76	6.56	6.64	0.02	−	−	−	−
laurentii	Ovine	0.04	0.04	0.04[b]	0.04[b]	0.00	6.55	6.64	6.45[b]	5.93[b]	0.03	−	−	−	−
Tentative	Caprine	6.65	8.95	8.80	8.88	0.09	6.65	5.93	5.18	4.82	0.02	−	+	++	++
starter	Ovine	6.36	9.17	9.04	9.12	0.09	6.55	5.45	4.78	3.97	0.01	−	+	++	++
Control	Caprine	0	0	0	0	0	6.65	6.52	6.50	6.46	0.02	−	−	−	−
	Ovine	0	0	0	−[b]	0	6.55	6.66	6.40	5.97[b]	0.01	−	−	−	+

[a] Standard error of the mean
[b] Contamination detected
[c] ++ Strong curd, + fine and grainy curd, − no curd

for by thermostable enzymes, possibly from *Pseudomonas* spp., that were not destroyed by the heat treatment used [5], and/or by plasmin action upon β-caseins; although plasmin is inactivated by sterilisation at 120 °C for 15 min [7], the thermal treatment used in our experiment (i.e. 110 °C for 10 min) probably did not inactivate the enzyme, an argument supported by Driessen and van der Walls [7], who claimed that this enzyme can remain partially active in milk after heat processing at 142 °C for 3 s.

Both milk types were coagulated by the four strains of lactic acid bacteria but, as expected, different behaviours throughout the incubation time were observed depending on the strain. *Lb. paracasei* produced a faster and greater reduction in pH than the other strains, whereas *E. faecium* and *Lb. plantarum* produced similar pH values, especially by 3 days and 6 days. According to Scheffé's *F*-test, significant differences in terms of pH were found between the actions of *E. faecium* and *E. faecalis*, but not between those of *E. faecium* and *Lb. plantarum*. *Lb. paracasei* was responsible for a lower pH value than *Lb. plantarum*, which is in agreement with results presented by Macedo and Malcata [5] in a somewhat similar experiment. The tentative starter (composed of *E. faecium*, *Lb. plantarum* and *D. hansenii*) showed statistically significant differences when compared to each of the aforementioned strains independently, suggesting synergisms between these strains. In terms of pH in milk inoculated with yeasts, variations with time were slight except for contaminated samples (which are obviously of no value); ovine milk inoculated with *C. laurentii* was already contaminated by 3 days and 6 days of incubation.

Tables 3–5 depict the concentrations of sugars and organic acids produced (or degraded) by the microbial strains tested; distinct profiles between strains and between caprine and ovine milks are apparent. Lactose content was higher in caprine than in ovine milk; lactose was actually metabolised by all bacterial strains, as concluded from the statistically significant value of Scheffé's *F*-test. Production of lactic acid, as expected, correlated negatively to lactose degradation and was, in general, highest for *Lb. paracasei* followed by *E. faecium*. Production of lactic acid by the tentative starter and by *E. faecium* were similar but differed from production by *Lb. plantarum* when considered independently, thus suggesting a greater contribution of *E. faecium* to the pH reduction; this fact was more evident in the experiment with caprine milk. The existence of citrate metabolism is apparent from inspection of the data obtained from *E. faecalis*; lower rates of metabolism were recorded for *E. faecium, Lb. plantarum* and *Lb. paracasei*, and according to Scheffé's *F*-test, no statistically significant difference was found between *Lb. paracasei* and the control. In terms of yeasts, low rates of citrate metabolism were observed for *D. hansenii* and for *Y. lipolytica*; Roostita and Fleet [8] reported strong and weak utilisation of citric acid by *Candida lipolytica* and *D. hansenii*, respectively. Citrate utilisation appears to be a widespread feature among lactobacilli;. Fryer [9] reported that 19 out of 25 strains of *Lb. casei*, *Lb. plantarum* and *Lb. brevis* utilise citrate in the presence of a fermentable carbohydrate. According to the same author, several species of mesophilic lactobacilli metabolise citrate with concomitant production of diacetyl and formic acids; Fox et al. [10] have further reported that the presence of lactose influences the amount of formic acid formed. Relatively high contents of formic acid were found for both *Enterococcus* strains, for *Lb. plantarum* and for *Y. lipolytica*. *Lb. pa-*

Table 3 Evolution of concentrations of citric acid and lactose in caprine and ovine milk inoculated with strains isolated from Picante cheese and incubated at 30 °C. *TS* Total solids

Microorganism	Milk type	Citric acid (mg/100g$_{TS}$)					Lactose (mg/100g$_{TS}$)				
		0 day	1 day	3 days	6 days	SEM[a]	0 day	1 day	3 days	6 days	SEM[a]
E.	Caprine	24.63	15.97	19.88	16.95	0.20	387.5	367.2	372.9	329.0	3.32
faecium	Ovine	29.22	17.61	16.45	12.43	0.18	325.4	290.2	309.9	288.1	4.95
E.	Caprine	24.63	7.18	2.54	2.20	1.67	387.5	380.8	356.5	361.8	3.92
faecalis	Ovine	29.22	19.93	4.93	7.28	0.08	325.4	312.9	313.1	308.9	2.86
Lb.	Caprine	24.63	21.63	15.22	18.49	0.11	387.5	367.1	339.7	337.1	2.59
plantarum	Ovine	29.22	29.18	19.62	12.12	0.06	325.4	286.7	270.9	255.3	0.98
Lb.	Caprine	24.63	22.34	20.02	17.84	0.17	387.5	340.1	290.8	271.7	6.01
paracasei	Ovine	29.22	27.92	26.16	16.21	0.07	325.4	255.1	214.4	190.3	2.18
D.	Caprine	24.63	22.69	22.94	20.37	0.13	387.5	380.8	381.5	364.2	2.62
hansenii	Ovine	29.22	29.39	22.69	24.87	1.52	325.4	320.4	336.1	305.9	6.03
Y.	Caprine	24.63	22.46	28.65	20.00	1.02	387.5	378.0	376.0	359.3	5.04
lipolytica	Ovine	29.22	29.33	20.99	12.81	0.12	325.4	328.8	323.2	272.5	4.60
C.	Caprine	24.63	21.08	20.22	24.01	0.18	387.5	379.9	385.2	347.4	3.60
laurentii	Ovine	29.22	28.44	28.25[b]	6.42[b]	0.15	325.4	313.6	320.1[b]	297.4[b]	0.47
Tentative	Caprine	24.63	15.62	11.75	11.73	0.76	387.5	369.2	340.6	296.7	3.29
starter	Ovine	29.22	15.97	12.61	12.33	0.04	325.4	278.0	252.6	204.6	0.14
Control	Caprine	24.63	20.66	21.76	21.85	0.20	387.5	389.4	401.3	372.6	2.57
	Ovine	29.22	25.38	23.38	13.99[b]	0.61	325.4	328.8	315.5	257.9[b]	0.87

[a] Standard error of the mean

[b] Contamination detected

Table 4 Evolution of concentrations of succinic and lactic acids in caprine and ovine milk inoculated with strains isolated from Picante cheese and incubated at 30 °C. *TS* Total solids

Microorganism	Milk type	Succinic acid (mg/100g_{TS})					Lactic acid (mg/100g_{TS})				
		0 day	1 day	3 days	6 days	SEM[a]	0 day	1 day	3 days	6 days	SEM[a]
E. faecium	Caprine	16.02	0.00	0.00	1.26	0.34	0.00	9.49	17.32	36.86	0.07
	Ovine	8.52	0.00	0.57	3.99	0.02	1.04	22.80	47.75	51.82	0.54
E. faecalis	Caprine	16.02	12.55	3.32	19.90	0.13	0.00	1.54	4.43	11.58	0.04
	Ovine	8.52	18.33	2.32	7.34	0.06	1.04	1.32	4.77	10.29	0.01
Lb. plantarum	Caprine	16.02	0.00	0.79	1.95	0.14	0.00	12.25	22.12	20.32	0.08
	Ovine	8.52	0.00	0.00	0.00	0.05	1.04	15.46	41.69	43.82	0.12
Lb. paracasei	Caprine	16.02	24.45	23.93	24.02	0.64	0.00	40.82	92.21	88.93	0.14
	Ovine	8.52	22.29	29.93	26.18	0.56	1.04	42.11	99.86	102.35	0.16
D. hansenii	Caprine	16.02	16.60	16.79	19.42	1.19	0.00	0.64	0.62	0.66	0.00
	Ovine	8.52	8.61	9.05	7.29	0.02	1.04	1.14	1.39	1.31	0.01
Y. lipolytica	Caprine	16.02	13.71	11.91	19.20	0.12	0.00	0.00	0.63	0.00	0.00
	Ovine	8.52	4.34	8.20	13.15	0.04	1.04	0.00	11.22	0.00	0.07
C. laurentii	Caprine	16.02	16.29	19.87	16.15	0.17	0.00	0.65	0.62	1.01	0.00
	Ovine	8.52	7.93	0.09[b]	2.68[b]	0.11	1.04	1.32	1.10[b]	6.00[b]	0.11
Tentative starter	Caprine	16.02	0.00	0.00	0.00	0.12	0.00	13.36	26.57	35.13	0.16
	Ovine	8.52	0.00	0.00	0.00	0.02	1.04	29.23	43.71	81.75	0.55
Control	Caprine	16.02	19.58	20.04	19.08	0.20	0.00	0.65	0.00	0.00	0.00
	Ovine	8.52	7.47	9.40	2.56[b]	0.09	1.04	2.73	0.00	5.41[b]	0.05

[a] Standard error of the mean

[b] Contamination detected

Table 5 Evolution of concentrations of formic and acetic acids and acetoin in caprine and ovine milk inoculated with strains isolated from Picante cheese and incubated at 30 °C. *TS* Total solids

Microorganism	Milk type	Formic acid (mg/100g_{TS})					Acetic acid (mg/100g_{TS})					Acetoin (mg/100g_{TS})				
		0 day	1 day	3 days	6 days	SEM[a]	0 day	1 day	3 days	6 days	SEM[a]	0 day	1 day	3 days	6 days	SEM[a]
E. faecium	Caprine	11.02	16.03	15.45	16.49	0.20	0.06	3.50	2.51	3.98	0.00	0.00	5.17	6.90	4.00	0.06
	Ovine	8.44	19.75	20.34	12.21	0.16	0.13	4.12	4.02	8.70	0.07	0.00	0.00	0.37	2.34	0.08
E. faecalis	Caprine	11.02	13.45	14.08	13.02	0.20	0.06	5.07	6.36	7.41	0.02	0.00	19.31	38.00	37.52	0.11
	Ovine	8.44	9.22	10.87	11.69	0.03	0.13	2.07	8.82	14.06	0.03	0.00	9.37	46.25	62.07	0.05
Lb. plantarum	Caprine	11.02	13.60	17.20	16.22	0.20	0.06	1.71	3.73	2.28	0.04	0.00	6.79	4.38	5.73	0.02
	Ovine	8.44	10.00	12.61	12.30	0.05	0.13	6.61	4.19	5.68	0.01	0.00	0.00	0.00	0.00	0.00
Lb. paracasei	Caprine	11.02	13.49	5.89	5.03	0.20	0.06	2.11	1.52	3.06	0.02	0.00	3.41	16.59	13.03	0.08
	Ovine	8.44	8.33	2.49	2.30	0.02	0.13	0.92	1.65	3.15	0.01	0.00	10.55	24.49	26.71	0.23
D. hansenii	Caprine	11.02	11.97	12.30	13.00	0.20	0.06	0.09	0.08	1.99	0.02	0.00	0.00	0.00	0.00	0.00
	Ovine	8.44	8.63	8.73	8.57	0.02	0.13	0.06	0.29	2.90	0.01	0.00	0.00	0.00	0.00	0.00
Y. lipolytica	Caprine	11.02	12.61	15.61	13.56	0.20	0.06	0.30	0.41	1.90	0.03	0.00	0.00	0.00	0.00	0.00
	Ovine	8.44	8.55	13.43	11.23	0.12	0.13	0.03	5.94	10.54	0.03	0.00	0.74	2.79	16.35	0.26
C. laurentii	Caprine	11.02	11.87	13.00	11.39	0.20	0.06	0.12	2.15	0.43	0.01	0.00	0.00	0.00	0.00	0.00
	Ovine	8.44	8.63	8.05[b]	10.45[b]	0.07	0.13	0.12	0.15[b]	7.73[b]	0.04	0.00	4.00	0.35[b]	74.33[b]	0.10
Tentative starter	Caprine	11.02	14.72	14.82	12.52	0.21	0.06	3.03	6.04	4.47	0.01	0.00	4.23	6.45	3.77	0.04
	Ovine	8.44	11.16	8.94	3.31	0.10	0.13	4.26	4.45	8.38	0.04	0.00	0.04	1.07	0.00	0.13
Control	Caprine	11.02	12.95	13.69	11.54	0.21	0.06	2.24	1.60	0.23	0.09	0.00	0.00	0.00	0.00	0.00
	Ovine	8.44	7.93	7.90	3.58[b]	0.14	0.13	0.20	0.07	5.25[b]	0.01	0.00	0.00	0.00	7.53[b]	0.02

[a] Standard error of the mean

[b] Contamination detected

racasei led to the lowest concentrations of formic acid, which correlated to the lowest levels of lactose. The tentative starter generated lower values of this acid than did the two strains of lactic acid bacteria included in its formulation.

Production of succinic acid was clearly detected for *Lb. paracasei* but was not so clear for *E. faecalis*, whereas degradation occurred in milk incubated with *E. faecium* and *Lb. plantarum* strains, as well as in milk incubated with the tentative starter. Ocando et al. [11] reported that succinic acid was produced to some extent by strains of *Lb. brevis*, *Lb. lactis* and *E. faecalis* in sterile skimmed milk. Production of acetic acid and acetoin was measured for the *Enterococcus* and *Lactobacillus* strains tested, but the source of these organic acids can vary; Ocando et al. [11] suggested that a substrate other than lactose was actually used, and the possibility that acetic acid was formed from amino acids was not completely ruled out, whilst Nakae and Elliot [12] reported that some lactobacilli can actively produce acetic acid from alanine, glycine and serine, but Fryer et al. [13] claimed that this organic acid may be a

product of glycolysis. In addition to acetic acid, acetoin [14] is produced via citrate metabolism by lactobacilli, although both these products are among those that can derive from lactose metabolism [15]. Among the lactic acid bacteria tested, *Lb. paracasei* and *E. faecalis* are undoubtedly the strongest producers of acetoin.

Acid production is an important phenomenon associated with cheese manufacture and ripening, and hence with final cheese composition and quality [10]. Good linear correlations between pH and total acid development were found ($r>0.90$), with exception of *E. faecium* in caprine milk ($r=0.89$) and tentative starter in ovine milk ($r=0.85$). Very good correlations ($r>0.95$) in both types of milk for individual compounds and pH were found in milk samples for lactic acid versus pH for *E. faecium*, *Lb. plantarum, Lb. paracasei* and tentative starter; for citric acid versus pH and acetic acid versus pH for *E. faecalis*; for acetoin versus pH for *E. faecalis* and *Lb. paracasei*; and for succinic acid versus pH and formic acid versus pH for *Lb. plantarum*.

Conclusions

Among the lactic acid bacteria studied, *Lb. paracasei* was the dominant producer of lactic acid, so in terms of glycolysis it should be considered as the most suitable strain to be part of a starter tailored for Picante cheese. Although conclusions about the final pH of cheese should be drawn carefully when based upon acid production in milk, because sugars become more limited in cheese, strains of enterococci and *Lb. plantarum* gave rise to pH values most similar to Picante cheese, which is characterised by values of ca. 5 at the early stages of ripening [16].

The existence of citrate metabolism by *E. faecalis* and, at lesser rates, by *E. faecium*, *L. plantarum* and *L. paracasei* points at the importance of this phenomenon in Picante cheese production as these strains, especially the enterococci, are present in Picante cheese throughout the whole ripening period [2].

Acknowledgements The authors are grateful to the members of the technical board of the Governmental Directorate of Agriculture for the Region of Beira Interior (DRABI, Portugal) for their cooperation in supervising the milk collection and in transporting the milk to the ESB premises for analysis. Financial support for author A.C.F. was provided by Ph.D. fellowships, BD/2111/92-IF and BD/5364/95-IF, under the auspices of programs CIENCIA and PRAXIS XXI, respectively (administered by JNICT and FCT, Portugal). Partial financial support for the research program was provided by project PROTOLACTIS: *PROdução, por Tecnologias Optimizadas, de LACticínios TradicionaIS*, administered by PAMAF (INIA, Portugal).

References

1. Freitas AC, Sousa M, Malcata FX (1995) Ital J Food Sci 7:361–377
2. Freitas AC, Pais C, Malcata FX, Hogg TA (1996) J Food Prot 59:155–160
3. Lansgrud R, Reinbold G (1973) J Milk Food Technol 36:593–609
4. Gomes AMP, Malcata FX (1998) J Appl Microbiol 85:839–848
5. Macedo AC, Malcata FX (1997) Z Lebensm Unters Forsch A 205:25–30
6. Macedo AC, Malcata FX (1996) Z Lebensm Unters Forsch A 74:409–415
7. Driessen FM, van der Walls CB (1978) Neth Milk Dairy J 32:245–251
8. Roostita R, Fleet GH (1996) Int J Food Microbiol 31:205–219
9. Fryer TF (1970) J Dairy Res 37:9–15
10. Fox PF, Lucey JA, Cogan TM (1990) Food Sci Nutr 29:237–252
11. Ocando AF, Granados A, Basanta Y, Gutierrez B, Cabrera L (1993) Food Microbiol 10:1–7
12. Nakae T, Elliot JA (1965) J Dairy Sci 48:287–292
13. Fryer TF, Sharpe ME, Reiter B (1970) J Dairy Res 37:17–28
14. El-Gendy SM, Abdel-Galil H, Shahim Y, Hegazi FZ (1983) J Milk Food Technol 35:242–244
15. Fryer TF (1969) Dairy Sci Abstr 31:471–490
16. Freitas AC, Fresno JB, Prieto B, Malcata FX, Carballo FJ (1997) Food Chem 2:219–229