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ScienceDirect
Food Chemistry 101 (2007) 1446–1450
Food Chemistry
www.elsevier.com/locate/foodchem

Esterase activities of indigenous lactic acid bacteria from Argentinean goats' milk and cheeses

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Received 4 November 2005; received in revised form 31 January 2006; accepted 31 March 2006

Abstract

Esterase activities of indigenous lactic acid bacteria isolated from goats' milk and cheese were investigated. All of the strains exhibited esterase activity on α-naphthyl derivative of fatty acids of 2–6 carbon atoms. *Lactobacillus fermentum* ETC1 and *L. bulgaricus* ETC2 showed the highest specific activity on α-naphthyl acetate; *L. rhamnosus* ETC14 presented the highest specific activity on α-naphthyl butyrate and caproate. All enterococci strains presented the highest specific activities on α-naphthyl propionate, butyrate and caproate, and the lowest specific activities on α-naphthyl acetate. *Pedicoccus pentocaseus* ETC5 only had esterase activity on α-naphthyl acetate. The electrophoretic zymogram showed for each strain an individual enzyme profile on α-naphthyl acetate and revealed the presence of more than one esterase. *L. plantarum* ETC11 and *Enterococcus faecium* ETC9 showed four and five bands of esterase activity, respectively. The strains evaluated in this work showed different esterase activities, which were species and strain specific.

Keywords: Lactic acid bacteria; Goats' milk cheese; Esterase activity; Flavour compounds

1. Introduction

The semi hard goats' milk cheeses from northwest Argentina are manufactured with raw milk. These cheeses posses a particular flavour due to the lipid fraction and the primary products of its degradation, e.g., free volatile fatty acids or esters. The enzymatic activities responsible for formation of these flavour compounds are esterases and lipases. Esterases from lactic acid bacteria, yeasts, and *Pseudomonas* may be involved in the development of fruity flavours in foods (Bardi, Dell'Oro, & Delfini, 1992; McKay, 1993), and pregastric lipase and esterases are essential for the development of typical flavour in Italian cheese (Fox & Stepaniak, 1993). Microbial esterases may improve quality or accelerate the maturation of cheeses, cured bacon, and fermented sausages (Lee & Lee, 1990). Essentially nothing is known about the contribution of bacterial esterases to flavour formation in Cheddar, Dutch, and Swiss cheeses (Fox & Stepaniak, 1993).

Indigenous lactic acid bacteria isolated from goats' milk and artisanal goats' milk cheeses from the provinces of northwest Argentina were identified as lactobacilli (60%), enterococci (35%) and pediococci (5%). The species isolated were *Lactobacillus plantarum* (36 isolates), *L. rhamnosus* (15 isolates), *L. delbrueckii* subsp. *bulgaricus* (5 isolates), *L. fermentum* (5 isolates), *Enterococcus faecium* (36 isolates), and *Pediococcus pentosaceus* (5 isolates) (Oliszewski, González, & Pérez Chaia, 2004).

Several authors have studied the free fatty acid profile of goats' milk cheese and the changes on individual free fatty acids (FFA) during cheese ripening (Buffa, Guamis, Pavia, & Trujillo, 2001; Franco, Prieto, Bernardo, González

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doi:10.1016/j.foodchem.2006.03.053

Prieto, & Carballo, 2003; Georgala et al., 2005; Mallatou, Pappa, & Massouras, 2003; Saldo et al., 2003). However, there is little information about the contribution of esterases from lactic acid bacteria (LAB) to the formation of flavour in cheese manufactured with caprine milk (Vafopoulou-Mastrojiannaki, Litopoulou-Tzanetaki, & Tzanetakis, 1994).

In this study, the esterase activities of LAB isolated from goats' milk and cheese were investigated, to determine the esterolytic systems of those microorganisms present in goats' milk cheese.

2. Materials and methods

2.1. Microorganisms and growth conditions

Lactic acid bacteria strains used in this study were: *L. fermentum* ETC1, *L. bulgaricus* ETC2, *L. plantarum* ETC8, ETC11, ETC13, ETC15, ETC16, ETC19 and ETC20; *L. rhamnosus* ETC14, ETC17 and ETC18, *E. faecium* ETC3, ETC4, ETC6, ETC7, ETC9, ETC10, ETC12 and *P. pentosaceus* ETC5 were isolated from goats' milk and cheeses (Oliszewski et al., 2004). Lactobacilli were grown in MRS broth (De Man, Rogosa, & Sharpe, 1960), enterococci and pediococci were grown in LAPTg broth (Raibaud, Caulet, Galpin, & Mocquot, 1961) for 16 h. The cultures were incubated at 30 °C (*Pediococcus*) or 37 °C (*Lactobacillus* and *Enterococcus*).

2.2. Preparation of cell free extracts

Cells were harvested after 16 h by centrifugation at 10,000*g* for 10 min at 4 °C, washed twice with 50 mM sodium phosphate buffer, pH 7.0, and resuspended at 50% (w/v) in the same buffer. The suspension was disrupted adding 1–2 g of glass beads (No. 31/14: diameter, 0.10–0.11 mm, B. Braun Biotech International, Germany) and using a cell disruptor (B. Braun Melsungen AG, Germany). Five mixing sequences (speed, 6.5/s) each of 1 min were successively applied under CO_2 atmosphere. Samples were cooled in ice for 1 min between each mixing sequence. Cellular debris was removed by centrifugation (20,000*g* for 30 min at 4 °C) and the supernatant was used as a cell free extract (CFE).

2.3. Esterase activity

The esterase activity of CFE was determined on α-naphthyl derivatives of fatty acids of 2–10 carbon atoms as substrate (Sigma, St. Louis, MO, USA) (Medina, Katz, González, & Oliver, 2004a). The assay mixture contained 160 μl of 100 mM sodium phosphate buffer, pH 7.0, 20 μl of α-naphthyl substrate (10 mM in ethanol) and 100 μl of CFE. After incubation for 1 h at 37 °C, colour was developed by adding 0.6 ml of Fast Garnet GBC (Sigma, St. Louis, MO, USA) solution (5 mg/ml in 10% SDS) and further incubation at room temperature for 15 min. The absorbance was measured at 560 nm CECIL 2021 spectrophotometer (Cambridge, England). A standard curve was prepared using α-naphthol.

A unit of esterase activity was defined as the amount of enzyme that released 1 μmol of α-naphthol per min. Specific esterase activity was defined as units per milligram of protein.

2.4. Post-electrophoretic detection of esterases

The CFE was subjected to PAGE using 12% (w/v) acrylamide gels without SDS in 25 mM Tris-0.19 M glycine buffer, pH 8.3 and 4% (w/v) stacking gel (Laemmli, 1970) in a Mini Protean 3 electrophoresis cell (Bio-Rad, Hercules, CA). The PAGE was conducted at 60 V for 2 h. Substrates used were α-naphthyl derivates of fatty acids of 2–4 carbon atoms. Gels were incubated at 37 °C in 0.1 M sodium phosphate buffer, pH 7.0, containing the substrate (1% w/v in acetone) and 0.5 mg/ml Fast Red TR (Sigma, St. Louis, MO, USA). Activities were identified as coloured bands on the gels (Medina, Katz, & González, 2004b).

2.5. Protein determination

Concentrations of proteins were determined by the method of Bradford (1976), using bovine serum albumin (Sigma, St. Louis, MO, USA) as standard.

2.6. Statistical analysis

All experiments were repeated three times. Enzymatic activities of CFE were submitted to one-way analysis of variance using Minitab® Release 14 Statistical Software (2003, Minitab Inc).

3. Results and discussion

3.1. Esterase activity

Esterase specific activity was determined on cell free extracts using α-naphthyl (NA) derivatives of fatty acids of 2–10 carbon atoms as substrates. Esterase activity was present in the cell free extracts of all the strains assayed (Table 1). Among *Lactobacillus* strains, *L. fermentum* ETC1 showed the highest specific activity (32.47 ± 0.50 U/mg) on α-NA acetate and this strain also hydrolyzed α-NA butyrate, propionate and caproate. *L. bulgaricus* ETC2 showed high specific activity on α-NA acetate and low specific activity on α-NA propionate and caproate. No esterase activity was observed in this strain on α-NA butyrate. *Lactobacillus casei* ETC19 had esterase activity on α-NA acetate, propionate, butyrate and caproate.

L. plantarum strains ETC17 and ETC20 showed the highest specific activity on α-NA butyrate and caproate, and *L. plantarum* ETC11 showed the highest specific activity on α-NA acetate. Of all lactobacilli studied,

Table 1
Specific esterase activity[a] in cell free extract of lactic acid bacteria isolated from goat's milk and artisanal cheese of northwest Argentine

Source of enzyme	Substrate α-napthyl derivative			
	Acetate	Propionate	Butyrate	Caproate
L. fermentum ETC1	32.47 ± 0.50^{A}	22.97 ± 1.23^{A}	27.87 ± 2.34^{A}	14.93 ± 0.12^{A}
L. delbrueckii subsp. *bulgaricus* ETC2	30.45 ± 0.89^{B}	3.65 ± 0.89^{B}	n.d.	0.58 ± 0.12^{B}
L. casei ETC19	11.80 ± 0.23^{FI}	3.80 ± 0.77^{B}	7.82 ± 0.48^{E}	4.25 ± 0.34^{F}
L. plantarum ETC8	18.23 ± 0.35^{C}	7.23 ± 0.15^{C}	1.07 ± 0.23^{B}	1.41 ± 0.45^{C}
L. plantarum ETC11	26.11 ± 1.05^{D}	16.11 ± 0.98^{D}	2.83 ± 0.34^{C}	2.86 ± 0.23^{D}
L. plantarum ETC13	15.04 ± 0.45^{CE}	5.04 ± 0.67^{BEH}	1.60 ± 0.26^{D}	1.01 ± 0.43^{C}
L. plantarum ETC15	16.0 ± 0.76^{C}	6.0 ± 0.59^{E}	0.91 ± 0.34^{B}	1.18 ± 0.68^{C}
L. plantarum ETC16	13.93 ± 0.45^{EF}	3.73 ± 0.12^{B}	2.79 ± 0.66^{C}	1.96 ± 0.12^{E}
L. plantarum ETC20	9.49 ± 0.12^{F}	7.49 ± 0.14^{F}	15.11 ± 0.09^{F}	11.85 ± 0.16^{G}
L. rhamnosus ETC17	22.80 ± 0.83^{H}	12.80 ± 0.24^{G}	15.99 ± 0.25^{H}	14.90 ± 0.14^{A}
L. rhamnosus ETC14	6.80 ± 0.08^{G}	16.80 ± 0.15^{D}	22.56 ± 0.23^{G}	19.84 ± 0.35^{H}
L. rhamnosus ETC18	24.66 ± 0.52^{D}	4.66 ± 0.08^{H}	2.26 ± 0.14^{C}	1.25 ± 0.21^{C}
E. faecium ETC3	6.33 ± 0.12^{G}	36.56 ± 1.34^{I}	31.58 ± 0.91^{I}	32.61 ± 0.83^{I}
E. faecium ETC4	12.51 ± 0.22^{I}	23.81 ± 1.10^{A}	3.74 ± 0.14^{J}	20.00 ± 0.35^{H}
E. faecium ETC6	4.71 ± 0.21^{GJ}	35.91 ± 1.23^{I}	39.11 ± 1.13^{L}	32.39 ± 1.32^{K}
E. faecium ETC7	3.47 ± 0.08^{J}	30.72 ± 1.17^{J}	4.77 ± 0.09^{M}	30.61 ± 1.13^{K}
E. faecium ETC9	11.23 ± 0.21^{I}	21.97 ± 0.97^{A}	5.84 ± 0.12^{M}	21.30 ± 1.05^{H}
E. faecium ETC10	7.22 ± 0.23^{G}	17.43 ± 0.45^{K}	2.66 ± 0.07^{C}	17.97 ± 0.14^{L}
E. faecium ETC12	13.65 ± 0.34	28.20 ± 0.67^{L}	n.d.	25.51 ± 0.25^{M}
P. pentosaceus ETC5	10.54 ± 0.21^{F}	n.d.	n.d.	n.d

$^{A–M}$: Values in the same column with different superscript letters differ significantly ($P < 0.05$).
n.d.: not detected.
[a] U/mg of protein. Mean ± SD.

L. rhamnosus ETC14 showed the highest specific acitivity on α-NA butyrate and caproate.

All enterococci strains showed the highest specific activities on α-NA propionate, butyrate and caproate, and the lowest specific activities on α-NA acetate. *E. faecium* strains, ETC3 and ETC6 showed highest activity with α-NA derivatives of fatty acids of 3, 4 and 6 carbon atoms as substrates. Sarantinopoulos et al. (2001) reported that *E. faecium* were more esterolytic than *Enterococcus faecalis* and *Enterococcus durans*.

Of all the strains evaluated in this work, the enterococci showed the highest activities on α-NA derivatives of fatty acids of 4 and 6 carbon atoms. These results are in agreement with Katz, Medina, González, and Oliver (2002), who observed that enterococci isolated from ewes' milk and cheese had higher esterase activity on α-NA butyrate and caproate than lactobacilli strains.

Pedicoccus pentosaseus ETC5 only had esterase activity on α-NA acetate (10.54 U/mg). Vafopoulou-Mastrojiannaki et al. (1994) reported for *P. pentosaceus* isolated from traditional Greek cheeses a low esterase activity using 4-nitrophenyl butyrate as substrate.

None of the strains hydrolyzed α-naphthyl derivatives of fatty acids of 8 or 10 carbon atoms. Preferential or exclusive hydrolysis of esters containing C2–C6 of α-NA fatty acid derivatives has been reported for other lactic acid bacteria (Gobbetti, Fox, & Stepaniak, 1996; Katz et al., 2002).

Esterase activity was not observed with the extracellular fractions of the strains assayed. Several authors (El Soda, Abd El Wahab, Ezzat, Desazeaud, & Ismail, 1986; Katz et al., 2002) have reported that esterase activity is primarily intracellular.

Significant differences ($p < 0.05$) were observed with respect to specific esterase activity among studied strains. These strains would improve the organoleptic characteristics of goats' milk cheese.

3.2. Electrophoretic zymogram

The electrophoretic zymogram of strains studied is shown in Table 2. Based on electrophoretic mobility (R_f values), each strain gave an individual enzyme profile on α-NA acetate and butyrate. *L. fermentum* ETC1 presented three bands with esterase activity, E_1, E_2 and E_3 on α-NA acetate. E_3 band also showed activity on α-NA butyrate. *L. delbrueckii* subsp. *bulgaricus* ETC2 exhibited three bands with esterase activity, E_1, E_2 and E_3 on α-NA acetate. *L. casei* ETC19 contained two bands, E_1 and E_2 with activity on α-NA acetate. Among *L. plantarum* strains, *L. plantarum* ETC13 and ETC15 showed only one band with esterase activity on α-NA butyrate. The strains ETC8 and ETC16 showed two bands only with activity on α-NA acetate. *L. plantarum* ETC11 showed four bands with esterase activity. E_1, E_3 and E_4 bands had activity on α-NA butyrate. E_2 band only had activity on α-NA acetate. This strain possessed a very complex intracellular esterolytic system, producing four enzymes that hydrolyze carboxyl ester linkages with different specificity.

Table 2
Esterase electrophoretic patterns[a] of in cell extract of lactic acid bacteria

Strains	Intensity of esterase activity[b] with substrate			
	Bands	R_f	Specific activity esterase	
			α-Acetate	α-Butyrate
L. fermentum ETC1	E1	0.27	+	–
	E2	0.33	+	–
	E3	0.44	++++	++++
L. delbruckii subsp. *bulgaricus* ETC2	E1	0.40	–	+
	E2	0.55	++	–
	E3	0.84	++	–
L. casei ETC19	E1	0.37	++	–
	E2	0.54	+	+
L. plantarum ETC8	E1	0.37	++	–
	E2	0.82	+	–
L. plantarum ETC11	E1	0.27	–	++
	E2	0.44	+	–
	E3	0.58	–	+
	E4	0.62	–	+
L. plantarum ETC13	E1	0.20	–	+++
L. plantarum ETC15	E1	0.24	–	+++
L. plantarum ETC16	E1	0.37	+	–
	E2	0.55	+	–
L. plantarum ETC20	E1	0.52	++++	+++
	E2	0.74	+	–
L. rhamnosus ETC17	E1	0.22	–	+
	E2	0.37	+	–
	E3	0.54	++++	++++
L. rhamnosus ETC14	E1	0.22	–	++
	E2	0.55	++++	++++
L. rhamnosus ETC18	E1	0.40	+	–
	E2	0.55	++	–
E. faecium ETC3	E1	0.33	+++	++++
	E2	0.38	+	–
	E3	0.56	–	++++
E. faecium ETC4	E1	0.23	++++	–
	E2	0.51	+++	–
E. faecium ETC6	E1	0.33	+++	++
	E2	0.42	++++	++++
	E3	0.58	–	++
E. faecium ETC7	E1	0.19	+++	–
	E2	0.30	–	+++
	E3	0.47	+	–
	E4	1.80	–	++
E. faecium ETC9	E1	0.19	++	–
	E2	0.30	–	+++
	E3	0.36	–	+
	E4	0.47	++	–
	E5	0.60	+	–
E. faecium ETC10	E1	0.23	++++	–
	E2	0.36	–	++
	E3	0.54	++++	+++
E. faecium ETC12	E1	0.30	–	++
	E2	1.80	–	+
P. pentosaceus ETC5	E1	0.23	+++	–
	E2	0.51	+++	–

[a] Substrate used: α-naphthyl derivatives of acetate and butyrate.
[b] Activity was expressed from W (weak) to ++++: from a very thin to very deep coloured band. (–) absence of activity.

L. rhamnosus strains C18 and C14 presented two bands with activity. Various authors (El Soda et al., 1986; Gobbetti, Fox, & Stepaniak, 1997; Katz et al., 2002; Lee & Lee, 1990) have observed that lactobacilli possess more than one intracellular esterase.

E. faecium ETC4 and ET12 possessed two active esterolytic bands, *E. faecium* ETC3, ETC6 and ETC10 possessed three, *E. faecium* ETC7 showed four bands and *E. faecium* ETC9 five active esterolytic bands. Sarantinopoulos et al. (2001) reported for *E. faecium* strains five active esterolytic bands with a maximum of two bands per strain.

In *P. pentosacens* only ETC5 possessed two bands with esterase activity on acetate. There is little information about the esterolytic system of the *Pediococcus* genus.

Our results show that esterases from lactic acid bacteria strains tested released preferentially short-chain fatty acids and that several strains possessed more than one esterase. Multiple esterases were also found in other lactic acid bacteria (Gobbetti et al., 1997; Katz et al., 2002; Sarantinopoulos et al., 2001; Tsakalidou, Zoidou, & Kalantzopoulos, 1992).

Franco et al. (2003) quantified the major free fatty acids during ripening of traditional Babia-Laciana cheese. Despite the variation in free fatty acids concentrations and proportions, these cheeses contained high levels of C_4–C_{10} free fatty acids, which contribute to cheese flavour.

4. Conclusion

Enterococcus and *Lactobacillus* were the major LAB found in goats' milk and artisanal Argentine cheeses. These bacteria play a role in fat breakdown. Both genera of lactic acid bacteria tested in this study exhibited esterase activities that may release C_2–C_6 fatty acids during ripening and therefore, would actively contribute to flavour development in goats' milk cheese. Short-chain fatty acids such as butanoic and hexanoic acids are potent flavour compounds at concentrations below 10 mg/ml (Brennand, Ha, & Lindsay, 1989; Fenster, Rankin, & Steele, 2003). Esterases have the capacity for both hydrolyzing and synthesizing esters by esterification of fatty acids and ethanol (Liu, Holland, & Crow, 2004). These esters, which are potent flavour compounds at less than 5 ppm, are important for development of the characteristic "fruity" type

flavours such as ethyl butyrate and ethyl hexanoate (Liu et al., 2004).

The LAB strains evaluated in this work showed different esterase activities, which were species and strain specific. Wide variations in activity between strains highlight the need for selecting appropriate starters.

Acknowledgements

This work was supported by grants from FONCYT: PICT: 09-12378 and Concejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT) D-348.

References

Bardi, L., Dell'Oro, V., & Delfini, C. (1992). Esterase activity of wine yeasts. *Rivista di Viticoltura e di Enologia, 45*, 17–19.

Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–255.

Brennand, C. P., Ha, J. K., & Lindsay, R. C. (1989). Aroma properties and other thresholds of some branched-chain and other minor volatile fatty acids occurring in milkfat and meat lipids. *Journal Sensory Studies, 4*, 105–150.

Buffa, M., Guamis, B., Pavia, M., & Trujillo, A. J. (2001). Lipolysis in cheese made from raw, pasteurized or high-pressure treated goats' milk. *International Dairy Journal, 11*, 175–179.

De Man, J. C., Rogosa, M., & Sharpe, E. (1960). A medium for the cultivation of lactobacilli. *Journal of Applied Bacteriology, 23*, 130–155.

El Soda, M., Abd El Wahab, H., Ezzat, N., Desazeaud, M. J., & Ismail, A. (1986). The esterolytic and lipolytic activities of the Lactobacilli. *Le Lait, 66*, 431–433.

Fenster, K. M., Rankin, S. A., & Steele, J. L. (2003). Accumulation of short n-chain ethyl esters by esterases of lactic acid bacteria under conditions simulating ripening Parmesan cheese. *Journal of Dairy Science, 86*, 2818–2825.

Fox, P. F., & Stepaniak, L. (1993). Application of enzymes in cheese technology. *International Dairy Journal, 3*, 509–536.

Franco, I., Prieto, B., Bernardo, A., González Prieto, J., & Carballo, J. (2003). Biochemical changes throughout the ripening of a traditional Spanish goat cheese variety (Babia-Laciana). *International Dairy Journal, 13*, 221–230.

Georgala, A., Moschopoulou, E., Aktypis, A., Massouras, T., Zoidou, E., Kandarakis, I., et al. (2005). Evolution of lipolysis during the ripening of traditional Feta cheese. *Food Chemistry, 93*, 73–80.

Gobbetti, M., Fox, P. F., & Stepaniak, L. (1996). Esterolytic and lipolytic activities of mesophilic and thermophilic lactobacilli. *Italalian Journal of Food Science, 2*, 127–135.

Gobbetti, M., Fox, P. F., & Stepaniak, L. (1997). Isolation and characterization of a tributyrin esterase from *Lactobacillus plantarum* 2739. *Journal of Dairy Science, 80*, 3099–3106.

Katz, M., Medina, R., González, S., & Oliver, G. (2002). Esterolytic and lipolytic activities of lactic acid bacteria isolated from ewe's milk and cheese. *Journal of Food Protection, 65*, 1997–2001.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 277*, 680–685.

Lee, S. Y., & Lee, B. H. (1990). Esterolytic and lipolytic activities of *Lactobacillus casei* subsp. *casei* LLG. *Journal of Food Science, 55*, 119–122.

Liu, S. Q., Holland, R., & Crow, V. L. (2004). Esters and their biosynthesis in fermented dairy products: a review. *International Dairy Journal, 14*, 923–945.

Mallatou, H., Pappa, E., & Massouras, T. (2003). Changes in free fatty acids during ripening of Teleme cheese made with ewes', goats', cows' or a mixture of ewes' and goats' milk. *International Dairy Journal, 13*, 211–219.

McKay, A. M. (1993). Microbial carboxylic ester hydrolases (EC 3.1.1) in food biotechnology. *Letters in Applied Microbiology, 16*, 1–6.

Medina, R., Katz, M., & González, S. (2004b). Differentiation of lactic acid bacteria strains by post-electrophoretic detection of esterases. In Spencer & Ragout de Spencer (Eds.), *Methods in molecular biology, public health microbiology: Methods and protocols* (pp. 459–463). Totowa, NJ: Humana Press.

Medina, R., Katz, M., González, S., & Oliver, G. (2004a). Methods to determine esterolytic and lipolytic activities of lactic acid bacteria. In Spencer & Ragout de Spencer (Eds.), *Methods in molecular biology, public health microbiology: Methods and protocols* (pp. 437–441). Totowa, NJ: Humana Press.

Oliszewski, R., González, S.N., & Pérez Chaia, A. (2004). Evaluation of regional starters in goat cheeses manufacture. In: *Third International congress in marketing and cheeses technology*. Buenos Aires, Argentine.

Raibaud, P., Caulet, M., Galpin, J. V., & Mocquot, G. (1961). Studies on the bacterial flora of the alimentary tract of pigs II. Stretococci: selective enumeration and differentiation of the dominant group. *Journal of Applied Bacteriology, 24*, 285–291.

Saldo, J., Fernández, A., Sendra, E., Butz, P., Tauscher, B., & Guamis, B. (2003). High pressure treatment decelerates the lipolysis in a caprine cheese. *Food Research International, 36*, 1061–1068.

Sarantinopoulos, P., Andrighetto, C., Georgalaki, M. D., Rea, M. C., Lombardi, A., Cogan, T. M., et al. (2001). Biochemical properties of enterococci relevant to their technological performance. *International Dairy Journal, 11*, 621–647.

Tsakalidou, E., Zoidou, E., & Kalantzopoulos, G. (1992). Esterase activities of cell-free extracts from strains of *Lactococcus lactis* subsp. *lactis* isolated from traditional Greek cheese. *The Journal of Dairy Research, 59*, 111–113.

Vafopoulou-Mastrojiannaki, A., Litopoulou-Tzanetaki, E., & Tzanetakis, N. (1994). Proteinase, peptidase ans esterase activity of crude cell-free extracts of *Pediococcus pentosaceus* isolated from cheese. *Lebensmittel-Wissenschaft und-Technologie, 27*, 342–346.