Use of Heat-Shocked Mesophilic Lactic Acid Bacteria in Low-Fat Goat's Milk Cheesemaking

C. Asensio, L. Parra, C. Peláez, and R. Gómez*

Instituto del Frio (CSIC), Ciudad Universitaria s/n, 28040 Madrid, Spain

Heat-treated cultures (50 °C, 15 s) of mesophilic lactic acid bacteria, *Lactococcus lactis*, *Lactobacillus casei*, or *Lactobacillus plantarum*, were each added at a level of 1.25% as an adjunct to the starter in the manufacture of low-fat goat's milk cheese. This addition did not affect the cheesemaking process and the overall composition characteristics of the cheeses but led to an increase of free aminopeptidase activity and the amine nitrogen over the ripening period. This improved the flavor intensity and body characteristics of the cheeses. The ratio of hydrophobic to hydrophilic peptides of MW < 10 000 in the water-soluble fraction was lower in cheeses made with heat-shocked cells than in the control cheeses at 30 days of ripening. General acceptability of cheeses containing treated cells was greater than of the others, scoring low for bitter and acid taste.

Keywords: *Mesophilic lactic acid bacteria; heat treated; low-fat goat's cheese*

INTRODUCTION

In recent years there has been growing interest in the development of a new range of cheeses, similar to conventional cheeses but with a substantially reduced fat content.

Developments in low-fat cheese have tended to be in the field of soft and hard varieties of ripened cow's milk cheeses, such as Cheddar (Muir *et al*., 1992) or Ras (El Neshawy *et al*., 1986). Little work has been done, however, on low-fat goat's milk cheese. Low-fat cheeses in general tend to lack flavor and have a significantly different texture from full-fat cheeses (Banks *et al*., 1989). A number of techniques have been tried to improve the flavor and texture of these cheeses, most of them entailing acceleration of proteolysis during ripening, by adding enzyme concentrates, in the case of Cheddar-type cheeses (Muir *et al*., 1992), or *Micrococcus* or *Pediococcus* cells (Bhowmik *et al*., 1990) or else by incorporating freeze-shocked (El Soda *et al*., 1991) or heat-shocked (Ardo *et al*., 1989) cultures to the starter.

Other researchers have tried to improve flavor and body characteristics by adding whey proteins, emulsifying salts, or carrageenan (Lazaridis and Rosenau, 1980; McGregor and White, 1990).

In previous experiments at our laboratory, mesophilic lactococci and lactobacilli isolated from goat's milk cheese were treated by heat-shock (50 °C, 15 s), resulting in substantial reductions in viability and acidifying activity without adversely affecting aminopeptidase activity (Asensio *et al*., 1995).

The object of the present research was to improve the flavor of low-fat goat's milk cheeses by incorporating these same heat-shocked mesophilic lactococci and lactobacilli to the starter.

MATERIALS AND METHODS

Microorganisms. The starter culture used to make the cheese for this study was one previously developed at our laboratory by Requena *et al*. (1992). A mixture of *Lactococcus lactis* subsp. *lactis* IFPL 359 (80%), *Lactobacillus casei* subsp. *casei* IFPL 731 (5%), *Lactobacillus plantarum* IFPL 935 (5%), *Leuconostoc mesenteroides* subsp. *dextranicum* IFPL 709 (5%), and *Leuconostoc paramesenteroides* IFPL 705 (5%) was inoculated in sterile skimmed milk at a final level of 1% ($\sim 10^7$ cfu/mL). Then the culture was incubated at 22 °C until a pH between 4.6 and 4.7 was attained (~16 h). The starter obtained was used to inoculate the cheese vat.

Heat-shocked microorganisms added with the starter were *L. lactis* subsp. *lactis* IFPL 359 and IFPL 60, *L. casei* subsp. *casei* IFPL 731, and *L. plantarum* IFPL 3. All strains have been previously isolated from artisanal cheese made with raw goat's milk and belong to the collection of the Instituto del Frío.

Heat was applied for 15 s at 50 °C. The method used for culturing bacteria and preparation of heat-shocked cells was as described by Asensio *et al*. (1995).

Cheesemaking. Goat's milk cheeses were manufactured at the laboratory. Ten liters of low-fat goat's milk (1.5% fat content) was pasteurized (30 min at 65 °C). After cooling at 30 °C, the milk was inoculated with 1% (v/v) starter culture. After 30 min, an extra inoculum (1.25% final concentration) of unheated or heated bacterial cultures (50 °C, 15 s) was added immediately before the rennet. These inocula had previously been grown in milk for 17 h at 30 °C and constant pH 6.5, attaining a concentration of 10^9 cfu/mL. Then 0.2 g/L $CaCl_2$ and 0.025 g/L animal rennet (Chr. Hansen, Copenhagen, Denmark) at 30 °C were added. The strength of rennet was 1:40000 (w/v), measured at 32 °C and pH 6.50. After approximately 1 h of coagulation time, the curd was cut, reheated to 37 °C, and scooped into cylindrical molds. After pressing (5 kg, 1 h), the cheeses were kept at room temperature until the pH dropped to 5.4. The curd was then salted in brine (20° Baume NaCl) at 10 °C for 2.5 h. The finished cheeses, weighing around 500 g, were covered with paraffin and stored at 10−12 °C and 85−90% relative humidity for 1 month of ripening. Samples were taken at 2, 15, and 30 days.

Skim milk cheeses with starter only were made as controls.

Microbiological, Physical, and Chemical Analysis. Sampling and dilutions were performed in accordance with International Dairy Federation (IDF) standards (IDF, 1985). Total viable microorganism counts were run on PCA incubated for 48 h at 30 °C.

Fat, pH, total solids, NaCl, total N (TN), and non-casein nitrogen (NCN) were determined according to the methods of Fontecha *et al*. (1990). Amine nitrogen (NNH_2) was determined in the soluble fraction in 12% TCA, using the *o*-phthaldialdehyde spectrophotometric assay (OPA) (Church *et al*., 1983).

* Author to whom correspondence should be addressed (telephone +34-1-5492300/5445607; fax +34-1-5493627; e-mail rgomez@fresno.csic.es).

S0021-8561(95)00617-0 CCC: $12.00

Table 1. Effect of Addition of Heat-Treated or Unheated *L. lactis* IFPL 359 and IFPL 60, *L. casei* IFPL 731, and *L. plantarum* IFPL 3 on Proteolysis in Low-Fat Goat's Milk Cheese

	NCN/TN			NNH_2/NCN		
	2 days	15 days	30 days	2 days	15 days	30 days
L. lactis IFPL 359						
unheated cells	6.0	9.9	12.0	3.3	7.7	9.5
heated cells	5.3	10.5	12.3	3.7	8.1	11.6
L. lactis IFPL 60						
unheated cells	4.9	11.3	14.5	4.5	5.3	6.0
heated cells	4.3	9.4	12.5	4.9	6.2	9.2
L. casei IFPL 731						
unheated cells	5.5	10.7	12.4	4.2	5.2	7.7
heated cells	5.6	9.7	12.1	3.7	6.2	8.0
L. plantarum IFPL 3						
unheated cells	5.2	12.5	14.6	4.4	5.5	6.5
heated cells	5.4	12.2	12.8	3.8	7.1	7.8
control	4.9	9.7	12	3.9	4.9	6.5

The casein fraction was studied by sodium dodecyl sulfate polyacrylamide gel electrophoresis using Phastgel homogeneous 20 gels. Electrophoresis gels were developed at 250 V and 4 mA for 35 min on a Phastsystem Model AB apparatus (Pharmacia LKB Biotechnology, Uppsala, Sweden). Quantitative analysis of the bands present in the gels was carried out using a 3CX image analyzer (Millipore).

Peptide Profile Analysis. Profile analysis was run on peptides of MW <10000 in control and experimental cheeses at 2 and 30 days of ripening by reversed-phase high-resolution liquid chromatography of the water-soluble nitrogen fraction, obtained according to the procedure of Kuchroo and Fox (1982). The resulting supernatant liquid was ultrafiltered through an Amicon membrane with 10 000-Da cutoff.

Peptide separation in the permeate was carried out according to a modified version of the method described by Cliffe and Law (1990) on a HPLC (TSP, FL,) using a RP C_{18} Delta-Pak column (Waters) (100 A, 5 μm; size 3.9 × 150 mm). Flow rate was 1 mL/min. For mobile phase A, 0.1% trifluoroacetic acid (TFA) in water was used, and for mobile phase B, 0.1% TFA in acetonitrile/water (1:1). The gradient was 0% A to 100% B for the first 50 min and then 100% B to 100% A for the next 10 min. The resulting chromatograms were used to examine the ratio of hydrophobic to hydrophilic peptides.

Free Leucine Aminopeptidase Activity in Cheese. Activity was determined following the method of Ardo *et al.* (1989). Two grams of cheese was stir-homogenized for 2 h at 4 °C in 10 mL of 20 mM bis-Tris propane buffer, pH 7. Aminopeptidase activity was determined versus 1 mM leucine *p*-nitroanilide substrate (410 nm, 10 min) in the supernatant after centrifugation (5000 rpm, 15 min, 4 °C) and filtration through Millipore filters (0.22 μm). One unit of activity was arbitrarily defined as the variation of 0.1 unit of optic density in 1 min.

Sensory Analysis. Sensory characteristics of cheeses were assessed at 30 days of ripening following the recommendations in IDF standards (IDF, 1987) by a panel composed of 15 tasters from the laboratory. Bitterness and acidity of cheeses were also evaluated.

RESULTS

Gross Chemical and Microbiological Composition. At the end of ripening, the composition characteristics of the experimental cheeses were similar to those of the control. These were as follows: dry matter, 44.6−46.2%; fat, 10−12%; and protein, 26.6−28.6%.

The rate of decrease of pH in the first hours after manufacturing of cheeses made with unheated cells of any of the strains was greater than in the control or the cheeses with heat-shocked cells. While the controls took 6 h to reach pH 5.4, cheeses with heat-shocked cells took 4 h and those with unheated cells 2 h. However, after 30 days of ripening, pH in all cheeses was around 4.9−5.1.

As to microbiological characteristics, maximum viable microorganisms level was reached in all cheeses at 2 days of ripening, thereafter either remaining stable or decreasing slightly (results not shown). No differences were found between the control cheese and those made with heat-shocked cells over this period. However, after 30 days of ripening, the number of microorganisms in the cheeses with unheated cells was slightly higher (0.5 log cfu/mL).

Proteolysis. NCN/TN and NNH_2/NCN values in controls and in cheeses made with treated and untreated cells are shown in Table 1. There was practically no difference between NCN/TN values in experimental and control cheeses at 30 days of ripening with the exception of those made with unheated cells of *L. lactis* IFPL 60 and *L. plantarum* IFPL 3, in which the level was higher than in the control. Electrophoretic analysis of caseins showed no differences between the control and the experimental cheeses; in all cases there was more hydrolysis of α_s-casein than of β-casein, which exhibited little degradation (results not shown).

As Table 1 also shows, when unheated or heated cells of any of the strains were added, NNH_2/NCN values during ripening were higher than those of the control. The values of this ripening indicator were higher in the cheeses made with heat-shocked cells. Addition of *L. lactis* IFPL 359 or IFPL 60 produced higher levels of NNH_2/NCN, which after 30 days of ripening were 78.5% and 41.5% higher, respectively, than in the control. Levels of ripening in cheeses made with heated cells at 15 days were generally higher than those found in the control at 30 days.

The water-soluble extracts taken from the control and experimental cheeses after 2 and 30 days of ripening were fractionated by HPLC. The peptide profiles from the control cheese and cheeses with heated and unheated cells of any of the strains were all similar. Figure 1 shows the chromatograms and the ratios of hydrophobic to hydrophilic peptides at 2 and 30 days of ripening of cheeses containing unheated or heated cells of *L. lactis* IFPL 359. These were complex chromatograms which resolved peptides with a wide range of polarity. Peptide numbers and concentration, which were very low at 2 days of ripening, had increased considerably by 30 days. The peptide profile of cheeses at the end of ripening, determined by detection at 254 nm and after injection of a standard amino acid solution (results not shown), showed numerous peaks and high aromatic amino acid content. It was found that the retention times of phenylalanine and tryptophan over-

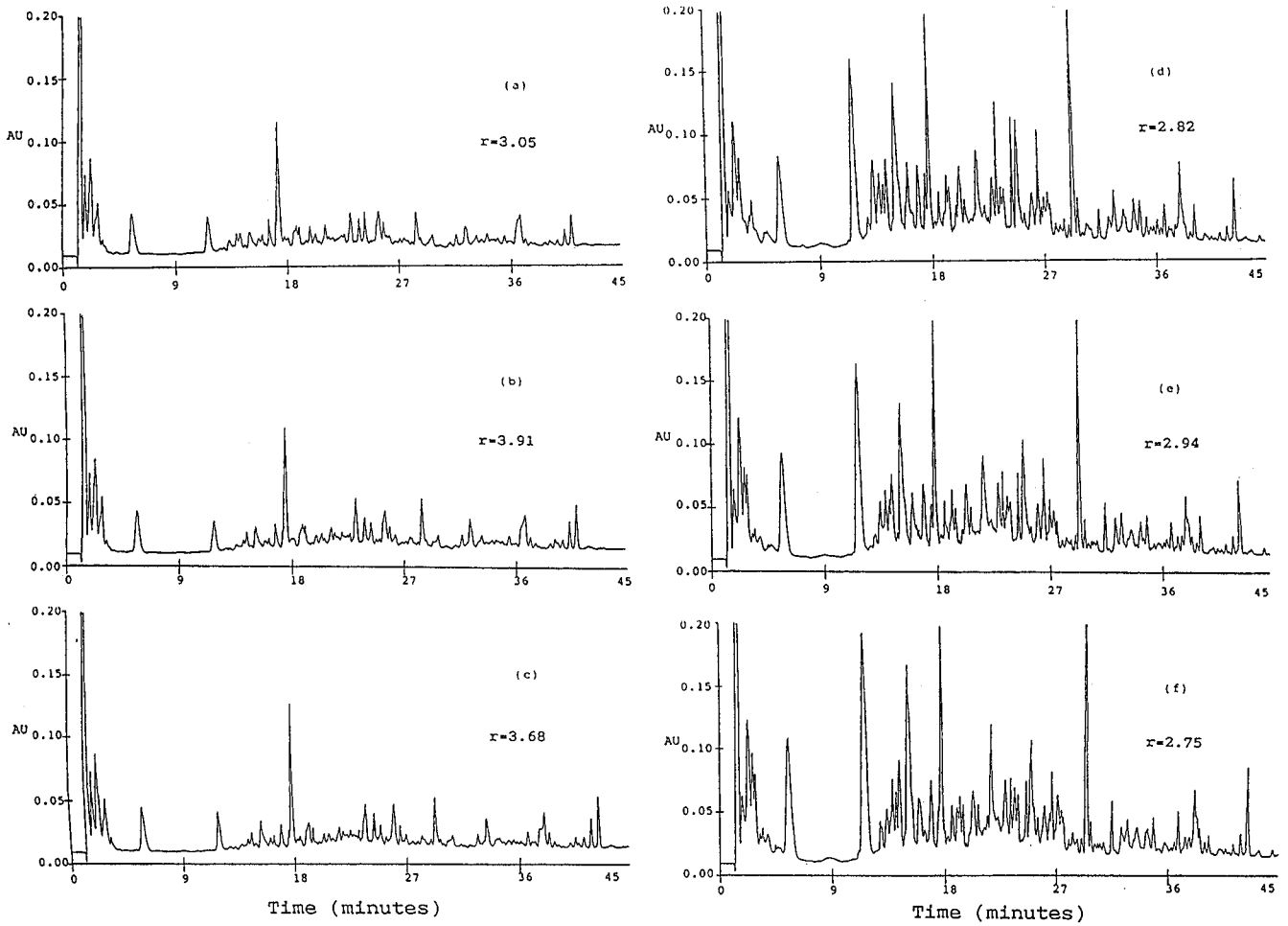

Figure 1. RP-HPLC profiles and ratio of hydrophobic to hydrophilic peptides (r = A 21−43/A 11−20) of water-soluble extract from cheeses: control (a, d), with unheated cells (b, e) or heated cells (c, f) of *L. lactis* IFPL 359; (a−c) 2 days of ripening; (d−f) 30 days of ripening.

lapped with the major peaks. Respective retention times were 9.9 and 16.9 min.

The ratio of hydrophobic to hydrophilic peptides of MW <10 000 in the water-soluble fraction of cheeses dropped during ripening in all cases. At 30 days, this value was higher than the control in cheeses made with unheated cells and lower in those containing heat-shocked cells, particularly where the strain used was *L. lactis* IFPL 359 (Figure 1).

Free Aminopeptidase Activity. Levels of free leucine aminopeptidase activity were higher in cheeses made with heated cells of any of the strains than in either controls or cheeses made with unheated cells (Table 2). Cheeses containing heat-shocked cells of *L. plantarum* IFPL 3 and *L. casei* IFPL 731 exhibited the highest levels of free aminopeptidase activity because of the greater leucine aminopeptidase activity of these microorganisms (Asensio *et al.*, 1995).

Organoleptic Assessment. Table 3 shows the results of sensory analysis of cheeses at 30 days of ripening.

Flavor, texture, and general acceptability of cheeses made with heat-shocked cells were generally superior to those of other cheeses. Cheeses made with heat-shocked cells of *L. lactis* IFPL 359, *L. casei* IFPL 731, and *L. lactis* IFPL 60 scored highest for general acceptability and low for bitter and acid taste. Cheeses made with unheated cells, on the other hand, were generally found to be slightly acidic and crumbly. Moreover, when unheated cells of *L. lactis* IFPL 60 or *L. plantarum* IFPL 3 were used, they were also found to be bitter-tasting. This defect was not found in cheeses containing unheated cells of other strains.

Table 2. Free Aminopeptidase Activity in Controls and Cheeses Made with Heated and Unheated Cells after 30 Days of Ripening

	leucine aminopeptidase activity[a]
L. lactis IFPL 359	
unheated cells	5.30
heated cells	7.10
L. lactis IFPL 60	
unheated cells	7.10
heated cells	8.30
L. casei IFPL 731	
unheated cells	5.40
heated cells	10.9
L. plantarum IFPL 3	
unheated cells	15.20
heated cells	21.20
control	0.69

[a] Units of activity per gram of cheese.

DISCUSSION

Addition of unheated or heat-shocked cells did not affect the cheesemaking process and scarcely affected overall composition characteristics. Abdel Baky *et al.* (1986) also found no changes in the composition of Ras cheese made with heated cells of *L. casei* and *Lactobacillus helveticus* with respect to a control.

Table 3. Sensory Characteristics of the Cheeses at the End of Ripening

	unheated cells				heated cells			
	IFPL 359	IFPL 60	IFPL 731	IFPL 3	IFPL 359	IFPL 60	IFPL 731	IFPL 3
general acceptance[a]	−0.1	0	−0.5	−0.6	0.6	0.5	0.6	0.2
flavor[a]	0.2	0.5	0.3	−0.1	0.5	0.6	0.7	0.3
texture[a]	0	0.1	0.4	−0.2	1	0.2	0.6	−0.5
bitterness[b]	0	30	0	10	0	0	0	0

[a] 0, similar to control; 0−±2 characteristic variation with respect to the control. [b] Percentage of tasters who found defects.

The decrease in acidification rate in cheeses made with treated as opposed to untreated cells of any of the experimental strains is consistent with the findings of Ardo *et al.* (1989), who reported that pH was lower (−0.05) after 5 h of processing in cheeses made with untreated cells; and again, after 24 h, they found no difference in pH between the control and cheeses made with heated cells of *L. helveticus*. The reason for the differences in the rate of acidification when heated as opposed to unheated cells were added is that, as shown in a previous paper (Asensio *et al.*, 1995), heat-shocking causes a drop in microorganism viability and also, to a lesser extent, in proteolytic, aminopeptidase, and dipeptidase activity.

The present work shows that when heated cells of mesophilic lactococci and lactobacilli are added to the starter culture in low-fat cheesemaking, amine nitrogen content is higher from the outset of ripening and likewise free aminopeptidase activity is higher at the end of ripening than in either controls or cheeses made with unheated cells. These effects have also been reported by Bartels *et al.* (1987) and Castañeda *et al.* (1990), in research on acceleration of ripening in Gouda and Saint Paulin cheeses respectively, and by El Soda *et al.* (1991) and Ardo *et al.* (1989), in low-fat cheeses. The latter two groups of authors found free aminopeptidase activity to be approximately 7 times greater than the control in cheeses made with heated cells of *L. helveticus*. According to El Soda and Ardo, the increase in amine nitrogen is caused by the release of microbial intracellular exopeptidases into the medium when these microorganisms undergo lysis as a result of heat-shocking.

The highest levels of free aminopeptidase activity were found in cheeses made with heated cells of *L. casei* IFPL 731 or *L. plantarum* IFPL 3. In cheeses made with heated cells of *L. lactis* IFPL 359 or IFPL 60, for which NNH_2/NCN levels were much higher than the control, aminopeptidase activity was lower than in those containing *L. casei* IFPL 731 or *L. plantarum* IFPL 3, which suggests that other peptidases also had a part in proteolysis of the cheese.

Addition of heat-shocked cells did not increase initial casein hydrolysis, there being no observable rise in NCN/TN with respect to the controls. These results are consistent with the findings of Law and Wigmore (1983), using free extracts of *Lactococcus* cells to accelerate ripening of Cheddar, and of El Soda *et al.* (1991) using freeze-shocked microorganisms to make low-fat Cheddar. The lack of any contribution to primary casein hydrolysis by heat-shocked cells was confirmed by electrophoresis, which showed degradation of casein fractions to be similar in all cases. There was greater degradation of α_s-casein than of β-casein, which is generally the case in semihard cheeses (Fox, 1989). In previous work at our laboratory on characterization of semihard goat's milk cheese, degradation was also found to be greater in α_s-casein than in β-casein (Fontecha *et al.*, 1990; Requena *et al.*, 1992).

The fall in the ratio of hydrophobic to hydrophilic peptides during ripening and the higher increase of NNH_2/NCN and free aminopeptidase activity when heat-shocked cells were added confirm the assumption that more microbial intracellular peptidase activity is released as a consequence of heat-shocking and the theory of the way that this contributes to hydrolysis of medium-sized hydrophobic peptides. The higher NCN/TN values found in cheeses made with unheated cells of *L. lactis* IFPL 60 or *L. plantarum* IFPL 3 coincided with a higher concentration of hydrophobic peptides (results not shown) and detection of a bitter taste in sensory analysis. This defect was not found in cheeses made with heat-shocked cells.

General acceptability of cheeses containing treated cells was greater than the rest, the highest scores being awarded to those made with *L. lactis* IFPL 359, *L. casei* IFPL 731, or *L. lactis* IFPL 60. The acidity noted by the tasting panel in cheeses with untreated cells reflects a sharper drop in pH during the early hours of cheesemaking, which upsets the final balance between flavor and aroma in the cheese. This may also have favored greater retention of rennet and, hence, an increased tendency to bitterness, as was found in these cheeses.

The results of the present research show that early peptidolysis induced in low-fat goat's milk cheese by addition of heat-shocked mesophilic microorganisms enhances flavor and reduces bitterness. This defect was noted when untreated microorganisms were added or when microbial enzymes or enzyme extracts were added directly, as reported by other authors. Moreover, the microorganisms that gave the best results in the present study, *L. lactis* subsp. *lactis* IFPL 359 and *L. casei* subsp. *casei* IFPL 731, were previously selected as specific starter cultures for goat's milk cheese and, therefore, possess the requisite technological properties for balanced development of flavor and aroma during ripening of this kind of cheese.

LITERATURE CITED

Abdel Baky, A. A.; El Neshawy, A. A.; Rabie, A. M.; Ashour, M. M. Heat shocked lactobacilli for accelerating flavour development of Ras cheese. *Food Chem.* **1986**, *21*, 301−313.

Ardo, Y.; Larsson, P. O.; Lindmark Mansson, H.; Hedenberg, A. Studies of peptidolysis during early maduration and its influence on low-fat cheese quality. *Milchwissenschaft* **1989**, *44*, 485−490.

Asensio, C.; Gómez, R.; Peláez, C. Effect of heat treatment on the proteolytic activity of mesophilic bacteria isolated from goat's milk cheese. *Lett. Appl. Microbiol.* **1995**, *21*, 25−30.

Banks, J. M.; Brechany, E. Y.; Christie, W. W. The production of low fat Cheddar-type cheese. *J. Soc. Dairy Technol.* **1989**, *42*, 6−9.

Bartels, H. J.; Johnson, M. E.; Olson, N. F. Accelerated ripening of Gouda cheese. II. Effect of freeze shocked *Lactobacillus helveticus* on proteolysis and flavor development. *Milchwissenschaft* **1987**, *42*, 139−143.

Bhowmik, T.; Riesterer, R.; Van Boekel, M. A. J. S.; Marth, E. H. Characteristics of low fat Cheddar cheese made with

added *Micrococcus* and *Pediococcus* species. *Milchwissenschaft* **1990**, *45*, 230−235.

Castañeda, R.; Vassal, L.; Gripon, J.; Rosseau, M. Accelerated ripening of Saint-Paulin cheese variant by addition of heat-shocked *Lactobacillus* suspensions. *Neth. Milk Dairy J.* **1990**, *44*, 49−63.

Church, F. C.; Swaisgood, H. E.; Porter, D. H.; Catignani, G. L. Spectrophotometric assay using *o*-phthaldialdehyde for determination of proteolysis in milk and isolated milk proteins. *J. Dairy Sci.* **1983**, *66*, 1219−1227.

Cliffe, A. J.; Law, B. Peptide composition of enzyme treated Cheddar cheese slurries determined by reverse phase high performance liquid chromatography. *Food Chem.* **1990**, *36*, 73−80.

El Neshawy, A. A.; Adbel Baky, A. A.; Rabie, A. M.; Ashour, M. M. An attempt to produce low fat Cephalotyre (Ras) cheese of acceptable quality. *Food Chem.* **1986**, *22*, 123−137.

El Soda, M.; Chen, C.; Riesterer, B.; Olson, N. Acceleration of low-fat cheese ripening using liophilized extracts or freeze shocked cells of some cheese related microorganisms. *Milchwissenschaft* **1991**, *46*, 358−360.

Fontecha, J.; Peláez, C.; Requena, T.; Gómez, C.; Ramos, M. Biochemical and microbiological characteristics of artisanal hard goat's cheese. *J. Dairy Sci.* **1990**, *73*, 1150−1157.

Fox, P. F. Proteolysis during cheese manufacture and ripening. *J. Dairy Sci.* **1989**, *72*, 1379−1400.

IDF (International Dairy Federation). Norma FIL 50B, Brussels, 1985.

IDF (International Dairy Federation). Norma FIL 99A, Brussels, 1987.

Kuchroo, C. N.; Fox, P. F. Soluble nitrogen in Cheddar cheese. Comparison of extraction procedures. *Milchwissenschaft* **1982**, *37*, 331−335.

Law, B.; Wigmore, A. Accelerated ripening of Cheddar cheese made with a commercial proteinase and intracellular enzymes from starter streptococci. *J. Dairy Res.* **1983**, *50*, 519−525.

Lazaridis, H. N.; Rosenau, J. R. Effects of emulsifying salts and carragenan on rheological properties of cheese-like products prepared by direct acidification. *J. Food Sci.* **1980**, *45*, 595−597.

McGregor, J. U.; White, C. H. Optimizing ultrafiltration parameters for the development of a low fat Cheddar cheese. *J. Dairy Sci.* **1990**, *73*, 314−318.

Muir, D. D.; Banks, J. M.; Hunter, E. A. Sensory changes during maturation of fat-reduced Cheddar cheese: effect of addition of enzimatically active attenuated starter cultures. *Milchwissenschaft* **1992**, *47*, 218−222.

Requena, T.; de la Fuente, M. A.; Fernandez de Palencia, P.; Juárez, M.; Peláez, C. Evaluation of a specific starter for the production of semihard goat's milk cheese. *Lait* **1992**, *72*, 437−448.

Received for review September 14, 1995. Revised manuscript received June 10, 1996. Accepted June 19, 1996.[⊗] We acknowledge the financial support received from the following research projects: ALI 94-0735, granted by the Interministerial Commision of Science and Technology; ECLAIR AGRE-CT91-0064 (DTEE) and AAIR 3-CT93-1531, both granted by the European Community.

JF950617D

[⊗] Abstract published in *Advance ACS Abstracts,* August 1, 1996.