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Food Chemistry 69 (2000) 73–79

Food Chemistry

www.elsevier.com/locate/foodchem

A procedure for the manufacture of goat milk cheese with controlled-microflora by means of high hydrostatic pressure

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Received 26 July 1999; received in revised form 16 September 1999; accepted 16 September 1999

Abstract

Cheesemaking techniques (including a high pressure treatment) suitable for the production of controlled-microflora cheese, are described. Starter-free and starter and rennet-free cheeses were made and a high pressure treatment was applied to some of them. Starter-free cheeses and their pressurized counterparts had higher moisture but lower pH than control cheeses. Under the same conditions pressurized cheeses had higher water-holding capacities but similar degrees of proteolysis, as show by Kjeldhal analyses of water-soluble nitrogen fractions and the electrophoretic patterns, and similar plasmin contents as unpressurized cheeses, suggesting that the pressure conditions used did not affect the cheese enzyme activities. The high pressure treatment step, after cheese salting, allowed cheese production with very low initial counts and without strict aseptic conditions during cheesemaking.

Keywords: Goat cheese; Controlled-microflora; High pressure treatment

1. Introduction

The conversion of fresh cheese curd into ripening cheese is largely determined by proteolysis. Other events, such as lipolysis and glycolysis may also play a more or less important role in some varieties. These primary reactions are mainly responsible for textural changes and basic flavour development during cheese ripening.

Proteolytic agents in cheese originate from four or five sources: milk coagulant (rennet or rennet substitutes), endogenous milk enzymes (plasmin, cathepsin), starter microorganisms and their enzymes, non-starter microorganisms and secondary inocula, in certain varieties only (Fox, 1989).

There has been recent interest in devising a model system that would permit quantitation of the contribution of each of these agents to the primary aspects of cheese ripening and to the secondary reactions. The techniques developed eliminate one or more of the above agents, thereby, permitting a good estimation of the role of each of the principal agents during cheese ripening.

A number of techniques have been developed to obtain a rennet-free cheese (RFC), starter-free cheese (SFC) by means of chemical acidification (e.g. gluconic acid-δ-lactone, GDL) to simulate the normal pH profile produced by the starter, and starter and rennet-free cheese (SRFC) by combining these techniques (Fox, 1989). However, the major problem in model cheese production is the presence of non-starter bacteria, e.g. organisms that either survive pasteurization of the cheese milk or gain access to the pasteurized milk or curd from the environment, and may grow prolifically. On death, these cells lyze and release enzymes.

Non-starter bacteria in cheese milk can be excluded by an aseptic milking technique (Reiter, Sorokin, Pickering & Hall, 1969) and the use of selected animals screened for the bacteriological quality of their milk (Kleter & de Vries, 1974; O'Keeffe, Fox & Daly, 1976; Perry & McGillivray, 1964).

After having collected low-count milk, a heating step is usually employed to further reduce bacterial counts. Chapman, Mabbitt and Sharpe (1966) did not use an aseptic milking technique, thereby employing directly, pasteurization to obtain a low-count milk. Many other authors have utilized high-temperature short-time pasteurization to obtain high microbiological quality milk (Kleter, 1976; LeBars, Desmazeaud, Gripon & Bergere, 1975; Reiter, Fryer, Pickering, Chapman, Lawrence & Sharpe, 1967; Visser, 1976).

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0308-8146/00/$ - see front matter
PII: S0308-8146(99)00242-3

Antibiotics are usually included in model systems, being probably necessary, especially if the cheese curd is to be chemically acidified to inhibit the growth of any remaining or contaminating bacteria in the production of SFC. Starter bacteria produce a range of antibiotics, which are very effective in inhibiting the growth of non-starter bacteria. Previous authors (LeBars et al.; Mabbit, Chapman & Sharpe, 1959; O'Keeffe et al., 1976; Reiter et al., 1967) have used antibiotics (nisin, penicillin and streptomycin) in the manufacture of controlled-microflora cheeses.

Contamination of the milk in cheese manufacturing can be avoided by using aseptic systems as enclosed vats (Mabbit et al., 1959), aseptic rooms (LeBars et al., 1975) or vats in a laminar air-flow unit (McSweeney, Walsh, Fox, Cogan, Drinan & Castelo-González, 1994; O'Keeffe et al., 1976).

High hydrostatic pressure technology (100–1000 MPa) is of increasing interest as an alternative method for food preservation, primarily because it permits microbial inactivation at low or moderate temperatures (Cheftel, 1995). Numerous investigations have been reported about the application of high pressure to milk (see Trujillo, Ferragut, Gervilla, Capellas & Guamis, 1997). High pressure treatment (HPT) causes the inactivation of vegetative microbial cells, without altering the sensorial quality of food. Pressure induces unfolding, aggregation and gelation of food proteins, and enzyme inactivation in some pressurization conditions.

The techniques described above in manufacturing controlled-microflora cheeses are very time-consuming in preparation and suffer a number of limitations, principally difficulties in performing cheesemaking operations under strict aseptic conditions. It was, therefore, decided to evaluate the use of HPT to obtain model cheeses with controlled microflora that would permit quantitation of the contribution of individual proteolytic agents to cheese ripening.

2. Materials and methods

2.1. Cheese manufacture

Cheesemaking was performed in triplicate within a 2-week period. Cheeses were prepared from pasteurized (72°C, 15 s) goat milk from the Experimental Farm of Universitat Autònoma of Barcelona (Bellaterra, Spain).

Control cheeses (CC) were prepared using 2% starter (AM Larbus S.A., Madrid, Spain: *Lactococcus lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris*), $CaCl_2$ (0.006%, w/v) and calf rennet (0.02%, v/v). Coagulation occurred in 40 min at 30°C. The curd was cut and stirred for 10 min at 30°C up to 38°C. The curd was pre-pressed (1.2 kPa for 15 min) and moulded. The cheeses were pressed for 1 h (using 1.2 kPa for the first 30 min and at 7 kPa after that) and salted (19% NaCl solution) for 30 min at 15°C (Fig. 1). SFC were made under controlled bacteriological conditions, essentially as described by O'Keeffe et al. (1976) using lactic acid and solid GDL as acidulants (Fig. 1).

SRFCs were prepared from porcine pepsin-coagulated curd by the modification number 2 of O'Keeffe, Fox and Daly (1977). After cutting the renneted milk gel, the pH of curd-whey mixture was increased to about 7 and the temperature rapidly raised to 38°C to inactivate pepsin (Fig. 1). Chemically-acidified cheeses were obtained using lactic acid and GDL as above.

In both SFC and SRFC, vats and other cheesemaking material were steam-sterilized or rinsed in a sterilizing solution. All the additives used in the cheese manufacture were sterile: calf rennet and porcine pepsin were filter-sterilized, the brine steam-sterilized and GDL UV-sterilized. No other precautions in the cheesemaking processes (moulding, pressing, vacuum packaging) were adopted to ensure asepsis.

2.2. High-pressure processing

Samples of SFC and SRFC were vacuum-packed and pressurized (PSFC and PSRFC, respectively) by using discontinuous high hydrostatic pressure equipment (Alstom, Nantes, France), with a pressure chamber of 2 l able to reach 500 MPa in 4 min. Cheeses were submerged

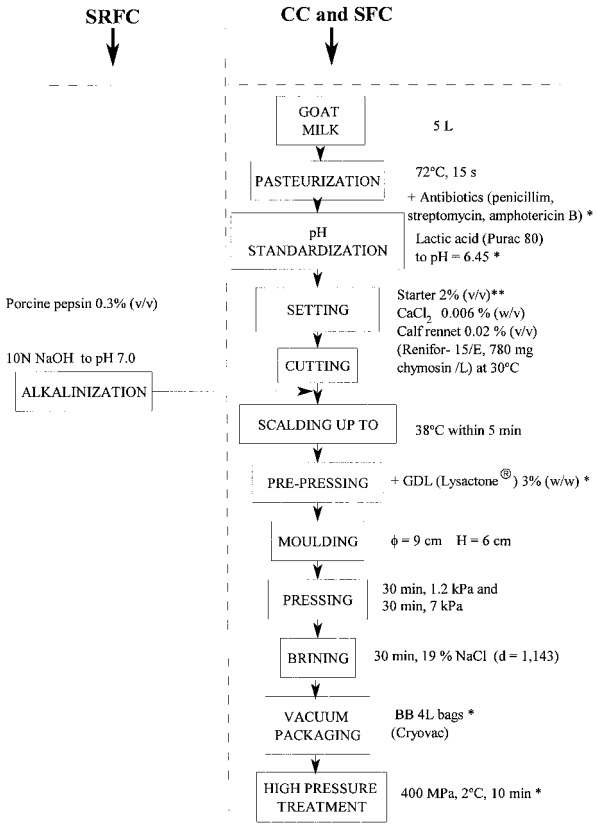

Fig. 1. Manufacture protocol for control cheese (CC), starter-free cheese (SFC) and starter and rennet-free cheese (SRFC). *Only in SFC and SRFC. **Only in CC.

in water, which acted as the hydrostatic fluid medium, and pressurized at 400 MPa for 10 min at 2°C. The chamber temperature was determined by means of a thermoregulating system which circulated cooling fluid (ethanol-water mixture) within the walls of the vessel. Samples were kept for 5–10 min at atmospheric pressure in the chamber until temperature equilibrium was established.

2.3. Bacteriological and chemical analyses

Total mesophilic and enterobacteria in raw and cheese milks, and cheeses were quantified on plate count agar (PCA) and violet red bile glucose (VRBG) agar (ICMSF, 1983), respectively. Milks were analyzed for total solids, fat, nitrogen fractions and pH, and cheeses were analyzed for fat, total nitrogen (TN), total solids, NaCl, pH, calcium and phosphorus according to the International Dairy Federation standards. Proteolysis was monitored by determining pH 4.6 water-soluble nitrogen (WSN) and by urea-polyacrylamide gel electrophoresis (PAGE) as Guamis, Trujillo, Ferragut, Chiralt, Andres and Fito (1997) have described.

2.4. Enzyme activities quantification

In order to assess the pressure effect on rennet enzymes, samples of commercial calf rennet (Renifor 15/E, Lamirsa, Barcelona, Spain) containing 780 mg of chymosin/l and 565 mg of pepsin/l, pure chymosin (Aniren 880, Sanofi Bio-Ind, Beaune Cedex, France) with a declared activity of 880 mg of chymosin/l, and pure bovine pepsin (Bovipep 1700, Sanofi Bio-Ind) containing 1700 mg of active pepsin/l, were pressurized (400, 450 and 500 MPa) at 2 and 10°C for 10 min. Quantification of enzyme activity was achieved by measuring the coagulation time by the method of Berridge modified by Collin, Grappin and Legraet (1977).

Plasmin activities in milk and cheese, and rennet activity in cheese were quantified by the Richardson and Pearce (1981) and Singh and Creamer (1990) procedures, respectively.

2.5. Statistical analysis

Composition results were analyzed by an analysis of variance (ANOVA) using the general linear models procedure of Statistical Analysis System (SAS). Evaluations were based on a 5% significance level.

3. Results and discussion

3.1. Cheese milk treatment

Raw milk used in the process contained a maximum of 50,000 total bacteria and less than 500 enterobacteria per millilitre. After heat-treatment total count decreased to <100 CFU$\times$ml^{-1} and absence of enterobacteria in all cases. The presence of post-pasteurization remaining microorganisms (including those that gain access to the pasteurized milk or curd from the environment) did not represent a problem, since the HPT eliminated it almost completely.

Antibiotics were added to cheese milk to inhibit development of different pasteurization and pressurization-resistant forms such as spores. In a complementary experiment, we checked the possible inhibitor effect of antibiotics on coagulant enzymes and plasmin and proved that the doses used in the cheese milk do not affect the enzyme activities.

Compositions of pasteurized milks used for cheesemaking are shown in Table 1.

3.2. Starter-free cheeses

In the manufacture of SFC we have employed the chemical acidulation technique using GDL suggested by Mabbitt, Chapman and Berridge (1955) and modified later by O'Keeffe, Fox and Daly (1975) to simulate the pH development pattern of starter in Cheddar cheese. However, the technique had to be adapted to goat cheese technology. Therefore, a number of experiments were done to assay several quantities of GDL. Concentrations of 1, 2, 3, 4, 8 and 12% (w/w) were added on the pre-pressed cheese curd. Then the pH of these cheeses at 24 h were compared with these obtained from curd with added starter. As shown in Table 2, the almost similar pH reached by chemical acidification to that obtained by biological acidification was achieved by using 3% GDL. These results are comparable to those described by O'Keeffe et al. (1975) for Cheddar cheese.

Fig. 2 shows the pH development of cheese acidified with GDL and of starter cheese or CC. The final values of both cheeses were very similar after 24 h. However, the routes by which they were attained were different. Approximately 4 h post-acidification, cheeses with added GDL had reached the final pH, whereas those curds produced with starter took more that 10 h to

Table 1
Composition of milk used for cheesemaking

pH	6.57[a]
	(0.09)
Total solids (%)	13.74
	(0.62)
Fat (%)	5.08
	(0.23)
True Protein[b](%)	3.13
	(0.09)

[a] Mean and standard deviation of three replicates.
[b] Defined as 6.38$\times$(total nitrogen-non-protein nitrogen).

Table 2
pH values in cheeses acidified with starter or different glucono-δ-lactone (GDL) concentrations at 15°C for 24 h

Acidification	pH
Starter 2%	5.33
GDL 1%	6.07
GDL 2%	5.65
GDL 3%	5.35
GDL 4%	5.09
GDL 8%	4.42
GDL 12%	4.11

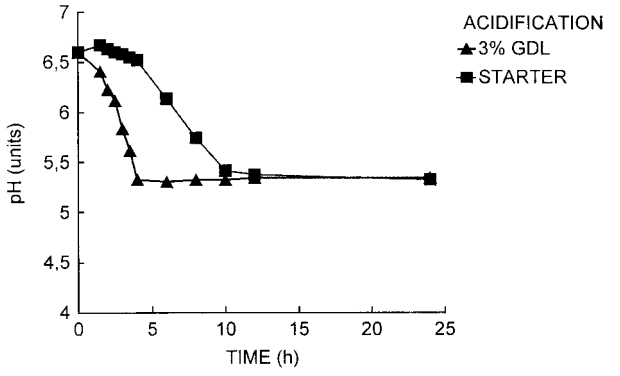

Fig. 2. pH development profiles in cheese acidified by starter and lactic acid plus 3% (w/w) glucono-δ-lactone (GDL).

Table 3
Composition of control cheeses (CC), starter-free cheeses (SFC), starter and rennet-free cheese (SRFC) and pressurized cheeses (PSFC and PSRFC) at 24 h

	Cheeses				
Variables	CC	SFC	PSFC	SRFC	PSRFC
pH	5.22[ab]	5.19[b]	5.18[b]	5.18[b]	5.21[b]
	(0.12)	(0.12)	(0.17)	(0.16)	(0.20)
Moisture (%)	51.54[d]	52.98[c]	54.24[b]	54.12[b]	55.47[b]
	(2.5)	(2.44)	(1.91)	(2.13)	(1.77)
Fat (%)	26.51[b]	26.31[b]	26.42[b]	26.15[b]	26.32[b]
	(1.69)	(1.81)	(1.81)	(2.13)	(2.13)
TN (%)	3.24[b]	3.11	3.06[d]	3.18[b]	3.00[e]
	(0.08)	(0.09)	(0.11)	(0.09)	(0.18)
NaCl (%)	0.81[bc]	0.79[c]	0.85[bb]	0.83[b]	0.87[b]
	(0.19)	(0.24)	(0.11)	(0.19)	(0.07)
WSN (%)	0.23[b]	0.21[b]	0.19[c]	0.18[c]	0.16[d]
	(0.04)	(0.04)	(0.02)	(0.04)	(0.03)
Rennet activity[c]	15.3[b]	17.07[b]	13.8[c]	ND[e]	ND
	(4.82)	(3.05)	(2.19)		
Plasmin activity[d]	2.72[b]	2.81[b]	2.82[b]	2.87[b]	2.85[b]
	(0.19)	(0.13)	(0.13)	(0.22)	(0.12)

[a] Mean (and standard deviation of three replicates).
[b] Means with the same letters in the same row do not differ at $P < 0.05$.
[c] Rennet units $\times$ g^{-1}.
[d] U$\times$ g^{-1}.
[e] Not detected.

reach it. The possible problems that we could find in the manufacture of curd acidified with GDL would theoretically consist of:

1. differences in cheese composition,
2. a higher retention of rennet in curd due to a more sudden pH decrease,
3. an increased proteolytic activity of rennet because the theoretical optimum pH of these enzymes is reached quicker, and
4. an increased susceptibility of the micellar caseins to proteolysis due to curd demineralization.

The composition of the CC and SFC cheeses after 24 h is shown in Table 3. The small variations observed may be due to added GDL and to the remaining lactose, both of which would tend to increase the moisture content. These results agree with those of O'Keeffe et al. (1975) for Cheddar cheese.

A lower than normal pH at draining causes greater retention of rennet in the curd (Creamer, Lawrence & Gilles, 1985). Since lactic acid was used to standardize the pH that milk reached in CC by the starter, and since the draining process was quickly achieved, the pH values of both curds at draining (CC and SFC) were practically identical (pH 6.4). Residual coagulant values in our curds corresponded to ~9% of added rennet. Differences observed in coagulant retention between CC and SFC may be due to the higher moisture in the SFC. The liquid fraction retained in the cheese may contain an amount of rennet similar to that of the whey. WSN values, together with electrophoretic analysis (Fig. 3, lanes 1 and 2) showed that SFC did not suffer excessive proteolysis, those values being significantly lower ($P < 0.05$) than those obtained in CC. Differences observed in CC may be due to the presence of lactic bacteria collaborating in the production of these nitrogen fractions. Fox (1970) showed that micellar caseins prepared with a low concentration of colloidal calcium phosphate complex were more susceptible to proteolysis than intact micelles. This also occurs in the acid cheese manufacture, which leads to partial curd demineralization. Values obtained for calcium and phosphorus (data not shown) in both types of cheeses from some batches were similar, showing that micelles did not suffer any demineralization. These results confirm that the coagulant enzyme activities in both cheese curds were approximately the same and that SFC did not suffer demineralization phenomena favouring proteolysis. The electrophoretic pattern (Fig. 3, lanes 1 and 2) obtained in both cheeses showed that β- and α_s-caseins appeared almost intact, as corresponds to fresh cheese after salting.

3.3. Starter and rennet-free cheeses

Table 3 shows composition of SRFC in comparison with CC. Again, as it occurred for SFC, differences in

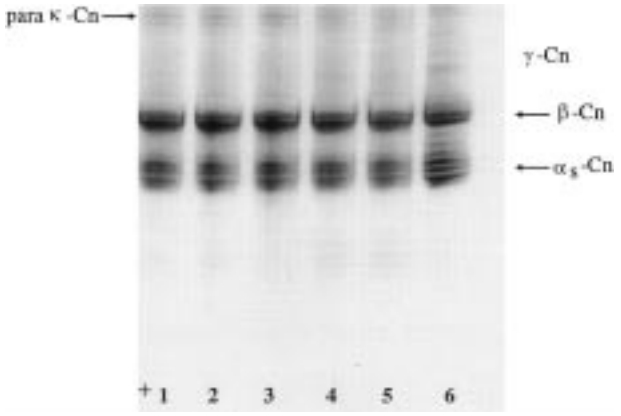

Fig. 3. Urea-PAGE electrophoregrams obtained from control cheeses (CC), starter-free cheeses (SFC), starter and rennet-free cheese (SRFC) and pressurized cheeses (PSFC and PSRFC) at 24 h (lanes 1, 2, 4, 3 and 5, respectively). Lane 6: whole casein from goat milk.

moisture ($P < 0.05$) could be seen between the cheeses, probably due to GDL and residual lactose. However, this phenomenon was emphasized in SRFC in comparison with SFC, probably due to the use of porcine pepsin that produces low-consistent gel and loss of protein and fat in the whey. These results are in agreement with those of Green & Foster (1974).

Quantification of residual coagulant in SRFC showed that porcine pepsin was completely inactivated under these conditions. Porcine pepsin is highly unstable at pH higher than 6.0, and, therefore, less stable than the calf rennet enzymes.

The electrophoretic study carried out in urea-PAGE (Fig. 3, lanes 1 and 4) of SRFC showed a very similar pattern to CC.

3.4. Pressurization effect on SFC and SRFC

Pressurization conditions (temperature, pressure and time) were chosen from previous experiences. These conditions had to ensure an important decrease of the number of microorganisms but, on the other hand, should not affect enzyme activities, especially those of the coagulant enzymes and plasmin.

Fig. 4 shows previous experiences in which calf rennet, bovine chymosin and pepsin were pressurized at different pressure conditions (400, 450 and 500 MPa) at 2 and 10°C for 10 min. Graphics showed that HPT did not affect pepsin activity under the conditions used. Chymosin activity was affected from 450 MPa at 10°C and 500 MPa at 2°C. Calf rennet was the enzyme preparation most affected by pressure at both temperatures. However, at 400 MPa and 2°C little difference was observed between non-pressurized and pressurized samples. It seems that HPT has a limited effect on isolated pepsin and chymosin, although when both enzymes are pressurized jointly, some relationship or reaction must occur between them since the enzyme preparation loses activity.

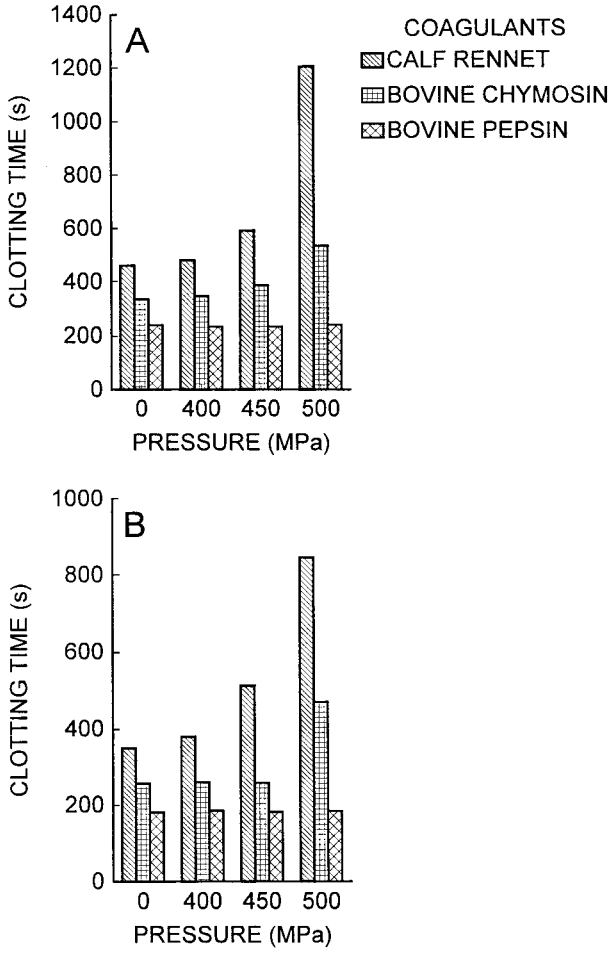

Fig. 4. High pressure effect on the clotting activity from different coagulants at 2°C (A) and 10°C (B) for 10 min.

On the other hand, plasmin activity was evaluated in goat milk after pressurization at 400 and 500 MPa at 2°C for 10 min in two independent experiments. Under those conditions, plasmin activity was unaltered and, therefore, conditions of 400 MPa, 2°C and 10 min were utilized subsequently to pressurize the cheese.

Cheese milk contained a maximum of 100 CFU×ml^{-1} after pasteurization. Moreover, experimental cheeses after processing and before pressurization contained between 1300 and 1500 CFU×g^{-1} (Table 4). Increase of the number of microorganisms may be due to the concentration effect made during the transformation from milk to cheese and, also, to some kind of external contamination that may take place at different steps of the process. After HPT, microbiological analyses obtained from all cheeses samples at 10^{-1} dilution were never higher than 10 CFU×g^{-1} when plated on PCA and < 1 on VRBG media. Although no identification or characterization of the colonies was carried out, they should belong to sporulated and baroresistant forms. Results obtained in our laboratory (Capellas, Mor-Mur, Sendra, Pla & Guamis, 1996) showed that these pressurization conditions are able to reduce microbiological

Table 4
Microbiological counts of starter-free cheeses (SFC), starter and rennet-free cheeses (SRFC) and pressurized cheeses (PSFC and PSRFC) as CFU × g^{-1}[c]

	Media	
Cheeses	PCA[a]	VRBG[b]
SFC	1465 (50)	ND[d]
PSFC	64 (12)	ND
SRFC	1332 (90)	ND
PSRFC	70 (28)	ND

[a] Plate count agar medium.
[b] Violet Red Bile Glucose medium.
[c] Mean and standard deviation of three replicates.
[d] No colonies observed.

counts, made on VRBG, of a fresh cheese innoculated with *E. coli* at a level of 10^7–10^8 CFU×g^{-1} to undetectable levels, and on PCA to 10^2 CFU×g^{-1}, *Micrococcus* and *Lactobacillus* being the most representative genders resistant to pressurization conditions. The pressure resistance of microorganisms is very variable, those cells in exponential growing phase being the least resistant. Temperature, pH and medium composition have a significant influence on the resistance to pressure. Bacterial spores are much more resistant to pressure that vegetative cells, and pressurization does not destroy them significantly. It is known, for instance, that treatment of milk at 1030 MPa and 10°C for 10 min does not destroy the spores (Hoover, Metrick, Papineau, Farkas & Knorr, 1989).

Table 3 shows the results of composition of pressurized cheeses. In general, composition of pressurized cheeses was very similar to that of non-pressurized, but the moisture content showed differences in all cases. Pressurized cheeses had higher moisture contents ($P < 0.05$) than their non-pressurized counterparts. HPT increased the water-holding capacity of the pressure-treated cheeses which hold the liquid fraction better. Analysis of residual rennet was negative in PSRFC, as expected, and the rennet activity in PSFC was reduced with respect to SFC reaching, in this case, values of rennet activity very close to CC. In both SFC and SRFC, pressurization did not affect the plasmin activity. The electrophoretic patterns and WSN analysis of pressurized curds were similar to those of non-pressurized (Fig. 3, lanes 1, 3 and 5) showing that pressurization does not alter primary and secondary proteolysis processes, which indicates that HPT would not alter the proteolytic processes during ripening.

The technique described in this paper is an effective method in cheese production with controlled-microflora for experimental purposes. In the enclosed vat systems (Mabbit et al., 1959), manipulation of certain cheesemaking operations is restricted. The laminar air-flow system, suggested by O'Keeffe et al. (1976), overcomes most of the major difficulties found in the enclosed vats. However, it is very time-consuming and requires strict asepsis in all the cheesemaking operations, including moulding, pressing and salting. The HPT facilitates the cheesemaking process because it eliminates organisms that either survive pasteurization of the cheese milk or gain access to curd during manufacture. HP-treated cheeses had counts never higher than 10 CFU×g^{-1} when plated at a 10^{-1} dilution on PCA agar, and minimal compositional alterations. These results are comparable with those of other authors who have worked with similar experimental cheeses (Chapman et al., 1966; Green & Foster, 1974; LeBars et al., 1975; O'Keeffe et al., 1976). Research is ongoing, using these techniques, to assess the contribution of starter, rennet and indigenous milk enzymes during goat cheese ripening.

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