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Accelerated ripening of a Saint-Paulin cheese variant by addition of heat-shocked lactobacillus suspensions

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Summary

Lactobacillus helveticus CNRZ 32 cultures were grown in MRS broth at 40 °C for 15 h at a constant pH of 5.85. The acidifying and proteolytic activities of suspensions of the lactobacilli in buffer solutions were tested after various heat-shock treatments. The thermoresistance of the enzyme system for acid formation and of proteinase and aminopeptidase activities differed considerably. A heat treatment for 18 s at 64 °C proved to be most suitable for accelerated cheese ripening: it inhibited 89 % of the initial acidifying activity whereas 40 % of the cell wall proteinase activity and 90 % of the aminopeptidase activity remained unaffected. Reduction in viable cells was approximately 99.5 %. Increasing the treatment temperature by 2 °C drastically inactivated the proteinase and aminopeptidase systems. A variant of Saint-Paulin cheese made with an extra inoculum of 1 % (v/v) heat-shocked cell suspension accelerated the production of free amino acids during ripening. The cheese developed an increased mature flavour and overall quality after 15 or 30 days of ripening and showed no texture modification, no bitterness or other flavour defect. Cheeses made with the same amount of untreated cells developed considerable flavour and texture defects.

1 Introduction

Acceleration of cheese ripening is of great economic importance. Several methods have been proposed to achieve this objective (1,2). One possible approach is to increase the amount of enzyme systems involved in the breakdown of casein into different soluble peptides and amino acids which serve as cheese flavour precursors.

Nowadays, to incorporate and slowly liberate these enzyme systems in cheese, two techniques are utilized: the addition of enzymes entrapped in lipo-

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somes or fat capsules (3-6) and the incorporation of heat-shocked cells of lactic acid bacteria. For the latter technique, lactobacilli are considered appropriate because of their high proteinase and peptidase activities when compared to other lactic acid bacteria (7-10). In order to prevent too fast a production of lactic acid during cheese making, their acidifying activity needs to be diminished selectively. A heat-shock treatment appeared to be the most suitable means of achieving this (11).

The concept of heat-shocked cells was developed by Pettersson & Sjöström (12) and is based on the greater heat lability of the lactose-fermenting system of cells compared to that of their proteolytic systems. With heat-shocked cells, a fairly well-balanced mixture of cell-indigenous proteolytic enzymes is added to the milk, and the cells are largely entrapped in the curd. The combination of active starter and heat-treated cells permits the acidification of cheese to proceed at a normal rate. In relation to proteolysis, maximum effects of heat-shocked cells are to be expected when the heat treatment does not affect the proteinase and peptidase systems of the cells.

Since then, other workers have approached the subject (11, 13-16) and succeeded in speeding up proteolysis and production of amino acids in different types of cheese through the addition of concentrated suspensions of heat-shocked lactic acid bacteria. Although proteolysis was apparently increased, the results were not always consistent with respect to acceleration of taste or flavour development. In spite of much research, the mechanisms of aroma formation in cheese are not well understood. This, and the production cost of heat-treated bacterial suspensions, have probably prevented these methods becoming widely applied in industry.

The present study is another attempt to investigate technological aspects and possibilities for industrial application of heat-shocked cells of *Lactobacillus helveticus (Lb. delbrueckii* subsp. *helveticus*) with high residual proteolytic activity after heating. The results deal with the characterisation of suspensions of the cells as well as with the effect of the incorporation of the cells in Saint-Paulin type cheese.

2 Materials and methods

2.1 Lactic starter cultures

Lactobacillus helveticus strain CNRZ 32 was selected because of its high aminopeptidase and proteinase activity. A lyophilised culture was resuspended in MRS broth (17) and after incubation transferred into aliquots of the same medium which were immediately frozen and stored at −30 °C. Before experimental use, a frozen culture was thawed, precultured at 37 °C for 16 h

and subcultured twice in MRS broth at 42 °C for 4.5 h (1 % inoculation).

2.2 Mass cultivation

The culture was inoculated at the 1 % v/v level in MRS broth and incubated for 15 h at 40 °C at pH 5.85, kept constant by addition of NaOH with moderate agitation. Growth was assessed by measurement of optical density and stopped at the beginning of the stationary phase by cooling at 4-7 °C. Cells were harvested by centrifugation (10 min at 8500 *g*), washed once with 0.1M sodium phosphate buffer (pH 5.85) and resuspended in this buffer to half of their initial volume in MRS. Suspensions were submitted to various heat treatments and stored for 16 h at 4-7 °C before cheese making.

2.3 Heating experiments

Heat-shock assays were performed by a continuous flow pasteurisation method. Bacterial suspensions were heat-shocked in a laboratory pasteurisation apparatus (Fig. 1), consisting of:

A. A peristaltic pump (Watson-Marlow, type MHRE).
B. A plate heat exchanger of stainless steel (18), heated in counter current with hot water from a temperature-controlled bath (F).
C. A tubular heat exchanger (holding cell) made of two pyrex glass tubes mounted in parallel, and immersed in a temperature-controlled water bath. The tubes had different lengths and their volumes amounted to

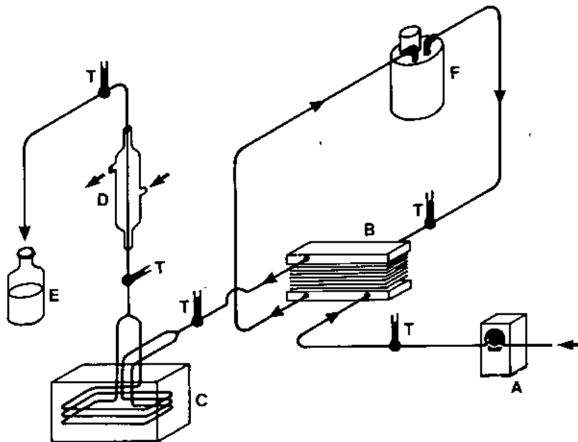

Fig. 1. Diagram of the continuous-flow pasteurisation apparatus: A) peristaltic pump; B) heating unit; C) holding cell; D) cooler; E) sterile receiver for collecting the suspension; F) controlled temperature bath; T) thermometers.

0.0554 and 0.0995 l respectively. One of the tubes was chosen according to the holding time needed. Loss of heat was prevented by covering the unit with insulating material.

D. A tubular glass condenser cooled by tap water (10-15 °C).

E. A receiver for collecting the suspension cooled in ice.

Flow rates of the suspension, obtained by the peristaltic pump, were from 5 to 25 l/h. Holding time in the exchangers ranged from 8 to 200 s. Temperature was checked in all sections of the apparatus. Before each treatment the equipment was autoclaved at 120 °C for 30 min.

A capillary tube method was also used for determination of the thermoresistance of lactobacillus suspensions (determination of D values). Suspensions were aseptically transferred into calibrated borosilicate capillary tubes of 100 μl volume (wall thickness 0.25 mm, inner diameter 1.4 mm; Vitrex, Herley, Denmark), which were heat sealed. Heating was carried out by complete immersion of the tubes in a temperature-controlled water bath. Holding times varied from 30 s to 30 min. Adjustment of the desired temperature was considered to be instantaneous.

Viable cell counts of suspensions before and after heat treatments were performed on MRS agar plates, incubated anaerobically (Anaerobic systems BBL, Gaspak, H_2 + CO_2) for 2 days at 42 °C.

2.4 Determination of proteolytic activities

Proteinase activity of whole cells was determined by incubating cell suspensions with ([^{14}C] methylated casein. The substrate was prepared as described by Donnelly et al. (19); its specific activity was 1609 KBq/mmol. Aliquots (100 μl) of the suspension were incubated at 37 °C for various times (0, 20, 40, 60 min) with 100 μl 0.1 % [^{14}C] casein in 0.1M sodium phosphate buffer, pH 8.0. The reaction was stopped with 200 μl 12 % trichloroacetic acid (TCA) and the mixtures were cooled at 4 °C. After 2 h, they were centrifuged for 20 min at 4600 *g* and the supernatants were filtered through 0.45 μm Millipore filters. Radioactivity was measured in the filtrates with a scintillation counter (Beckman, LS 1800).

One unit of proteinase activity was defined as the amount of enzyme liberating 1 % of initial casein radioactivity in 6 % TCA-soluble form after 30 min of hydrolysis (20).

For determination of aminopeptidase activity, bacterial suspensions were incubated for 2 h at 37 °C with a solution of lysozyme (1 mg/ml) in 0.1M sodium phosphate buffer at pH 7.0. Preliminary experiments showed that intracellular lactate dehydrogenase activity was maximal under these conditions. Lysed cells were centrifuged for 10 min at 8500 *g*. Aminopeptidase

activity was determined in the supernatant, using L-leucine p-nitroanilide as substrate according to the method of Rabier & Desmazeaud (21). One unit of aminopeptidase activity was defined as the amount of enzyme producing one μmole of p-nitroanilide per minute under the assay conditions.

2.5 Acidifying activity

A 100 μl aliquot of bacterial suspension was incubated at 42 °C for 4.5 h in 15 ml sterilized reconstituted skim milk powder (10 g/100 ml of distilled water). Developed acidity was measured according to Accolas & Auclair (22).

2.6 Examination of bacterial cells by electron microscopy

Cells were examined by electron microscopy to determine morphological damage caused by heat treatment. They were successively fixed in 2.5 % glutaraldehyde at pH 7.2 for 24 h at 4 °C and in 1 % osmium tetroxide at pH 7.2 for 15 h at 4 °C. They were then dehydrated in acetone and embedded in Epon 810. Sections were made with a Reichert ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Zeiss-EM10 electron microscope at 80 kV.

2.7 Cheese-making

Small Saint-Paulin cheeses (250 g) were manufactured in three parallel pilot-plant vats according to the procedure usually followed in the experimental dairy plant (5). To two of the cheese vats an extra inoculum (1 %, v/v) of heat-shocked (S) or untreated (U) cell suspension was added, while the third one was simultaneously run as a control (C).

Milk was pasteurized at 72 °C for 15 s and inoculated with 2 % of a starter composed of a *Lactococcus lactis* subsp. *lactis* CNRZ 1075 (Prt^-) culture (95 parts) and a *L. lactis* subsp. *lactis* CNRZ 1076 (Prt^+) culture (5 parts), both grown in autoclaved skim milk. Under these conditions, Prt^+ cells constituted 20 % of the total lactococcus cells inoculated in the cheese milk.

Renneting was carried out at 33 °C using 0.015 % (v/v) liquid rennet Ets Boll (Hansen, France; 520 mg active chymosin/l). The extra inoculum of *Lactobacillus helveticus* suspension was added immediately before the rennet. One per cent (v/v) of phosphate buffer was added to the control vat.

Curd was partially delactosized by addition of water after removing of 30 % of the whey. One day after salting in saturated brine for 4 h, the cheeses were waxed.

The cheeses were stored for 60 days at 12-13 °C and 95 % relative humidity. The product thus obtained contained 52 % dry matter and had a fat/dry matter ratio of 45 %.

2.8 Chemical and microbial analysis of cheese

The pH of the cheese was measured by means of a penetration electrode (Ingold type 406). Dry matter was determined by drying at 102 °C for 16 h.

Samples for total nitrogen (TN), pH 4.6 soluble nitrogen (SN) and 2.5 % phosphotungstic acid soluble nitrogen (AN), were prepared according to Gripon et al. (23). Nitrogen determination of solutions was performed after mineralization by colorimetric measurement according to Koops et al. (24). Free amino acids were determined in the AN fraction adjusted to pH 2.2 with 10M NaOH, with a Biotronic LC 5000 amino acid analyser (Biotronic, Munich, FRG).

Lactobacilli in cheese were enumerated as described in Section 2.3. A number of colonies equal to the square root of the total number of colonies present in petri dishes was transferred to litmus milk and the nature of the microorganisms was confirmed by microscopic examination after incubation at 37 °C for 16 h.

2.9 Rheological analysis

Cheese texture was studied by compression tests performed with an Instron Universal Testing machine (Instron Ltd, Bucks, England). Cylindrical samples taken from the centre of the cheese (24 mm diameter, 24 mm height) were compressed at a constant rate of 25 mm/min, to 20 % of their original height. Assays were ran in duplicate in a controlled temperature room at 20 °C.

2.10 Sensory evaluation

Cheese characteristics were scored from 0 to 4 by a trained taste panel of 12 members from INRA for flavour (clean, sweet and mature), texture (firmness, elasticity and body), overall quality and flavour defects. Experimental and control cheeses of the same trial were tasted simultaneously. Statistical analysis was performed with the Wilcoxon signed ranks test using BMDP 3S nonparametric statistics.

3 Results

3.1 Heat treatment and characterisation of Lactobacillus suspensions

3.1.1 Characteristics of Lactobacillus helveticus CNRZ 32 during growth. Growth of the bacterium in MRS broth at constant pH 5.85 and 40 °C stopped after 10.5 h, due to lack of glucose. Under these conditions, the maximal bacterial concentration obtained was 1.10^9 cfu/ml.

Table 1. Viable cell counts and acidifying, proteinase and aminopeptidase activities during growth of *Lb. helveticus* CNRZ 32 incubated in MRS broth at a constant pH of 5.85, 40 °C.

	Unit	Measured on	Incubation time (h)			
			10.5	13	15	29
Viable cell count	cfu/ml	MRS culture	1×10^9	1×10^9	7.2×10^8	8.5×10^6
Viable cell count	cfu/ml	suspension[1]	1.8×10^9	1.5×10^9	1.4×10^9	1.4×10^7
Acidifying activity[2]	%	suspension	0.40	0.45	0.32	0.06
Proteinase activity[3]	a.u.	suspension	5.8	6.7	7.8	8.9
Aminopept. activ.[4]	a.u.	suspension	7.5	8.7	8.7	6.0

[1] Resuspended in phosphate buffer 0.1M pH 5.85 (half of original MRS volume) according to Section 2.2.
[2] % of lactic acid in milk.
[3] Arbitrary unit of proteinase activity (Section 2.4).
[4] Arbitrary unit of aminopeptidase activity (Section 2.4).

The physiological state of the culture influenced the acid forming and proteolytic activities. Table 1 shows viable cell counts and acidifying, caseinolytic and aminopeptidase activities during different stages of growth. The acidifying activity reached a maximum during the early stationary phase (13 h) and then declined as a consequence of the decreasing numbers of viable cells. The aminopeptidase activity increased to reach a maximum between 13 and 15 h of cultivation and then decreased at the end of the stationary phase. The effect of the pH of the medium on the cellular activity was determined by comparing the results of a culture at a controlled pH of 5.85 with those of a culture without pH control, during 15 h at 40 °C. The viable cell count and acidifying activity were similar under both experimental conditions but the proteinase and aminopeptidase activities were 70 % and 38 % lower in the culture without pH control.

3.1.2 Effect of heat treatment on enzymatic activities. Heat treatments caused different degrees of inactivation of the enzymatic systems studied. Fig. 2 shows the effects of various heat treatments on lactic acid production, proteinase and aminopeptidase activities, expressed as a percentage of initial activity. The proteolytic systems were more stable than the acid-forming system. From the data, residual activities after a heat treatment for 18 s at different temperatures were calculated. Leucine aminopeptidase was more stable than extracellular proteinase. A treatment for 18 s at 66 °C drastically inactivated both proteinase and aminopeptidase activities. The most suitable condition for the treatment of lactobacillus suspensions proved to be 18 s at 64 °C. This

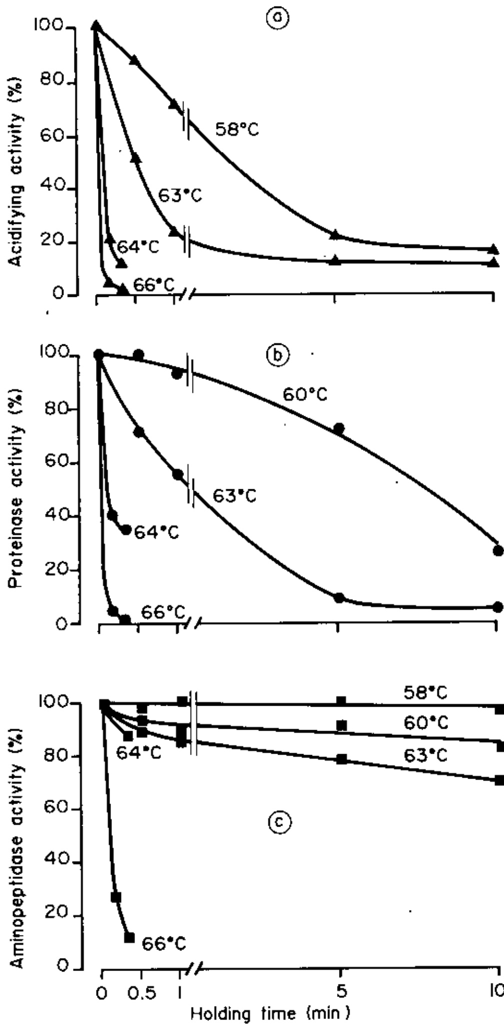

Fig. 2. Effect of heat treatment on: a) acid producing activity; b) cell wall proteinase activity; c) aminopeptidase activity.

treatment inhibited 89 % of the original acidifying activity whereas 40 and 90 % respectively, of the proteinase and aminopeptidase activities were unaffected. The D value (determined by capillary-tube and continuous-flow methods) was 7.8 s at 64 °C; the viability of the bacterial population thus decreased by about 2 log units in 18 s.

3.1.3 Damage of treated cells. Electron microscopic examination showed that the cytoplasm of treated cells contained fewer ribosomes after heat treatment for 18 s at 64 °C, and aggregation of chromosomes was frequently observed. Prolonged heat treatment (64 °C, 200 s) produced more pronounced modifications. Damage of membrane or cell wall, however, was not observed. The micrographs did not show lysed cells as a result of heat treatment.

3.2 Cheese making experiments

Four comparative Saint-Paulin cheese trials were performed from 18, 120, 120 and 180 l of milk, respectively. Effects of additions to the cheese vats of a 1 % extra inoculum of a *Lb.helveticus* CNRZ 32 suspension (heat-shocked or not) were evaluated. Residual acidifying activity of the heat-shocked suspensions was 12, 11, 17 and 15 % of the initial activity for cheese trials E1, E2, E3, and E4 respectively, and the residual aminopeptidase activity was 7.8, 7.8, 5.8 and 8.8 units, respectively, for the same trials.

Parameters associated with cheesemaking were not altered by the addition of cells.

3.2.1 Physico-chemical and microbiological cheese properties. The fat and water contents of cheeses made with addition of S and U suspensions showed no difference from those in control (C-)cheeses, results being reproducible for the four trials. The average water content was 48.1 %.

In all trials, the cheeses made with S cells showed slightly lower (by 0.03 to 0.05 unit) pH values at the end of the manufacturing process and after 48 h than the C-cheeses. This effect was reversed after the first 2 weeks of ripening, and at 60 days, the pH of S-cheeses was about 0.08 to 0.19 unit higher than that of the C-cheeses. Average pH values in C-cheese were respectively 5.12, 5.12, 5.15, 5.17, 5.16 after 1, 4, 15, 30 and 60 days of ripening.

Throughout the ripening period, the pH of U-cheeses was 0.1 to 0.2 unit lower than that of control cheeses. U-cheeses also showed a markedly crumbly texture.

Viable counts of thermophilic organisms in the cheese showed that the lactobacilli found originated exclusively from the added cell suspensions, since a very low number was encountered in pasteurized cheese milk. This flora remained nearly constant up to 60 days. Table 2 shows the results obtained for trial E2.

3.2.2 Protein breakdown during ripening. The presence of heat-shocked and untreated lactobacilli increased the soluble nitrogen fraction (SN) over that of the control. Fig. 3 shows the changes in SN and AN fractions in trial E2; at

Table 2. Viable counts of thermophilic organisms (cfu/g) in cheese milk and cheese at 15, 45 and 60 days of ripening for trial E2.

	Viable count of thermophilic organisms (cfu/g)			
	cheese milk after the addition of 1 % *Lb. helveticus* suspension	Saint-Paulin cheese at		
		15 days	30 days	60 days
Control cheeses		$<10^2$	$<10^2$	1×10^2
S-cheeses	4.1×10^4	3.1×10^6	2.9×10^6	3.8×10^6
U-cheeses	1.0×10^7	8.8×10^7	8.7×10^7	7.2×10^7

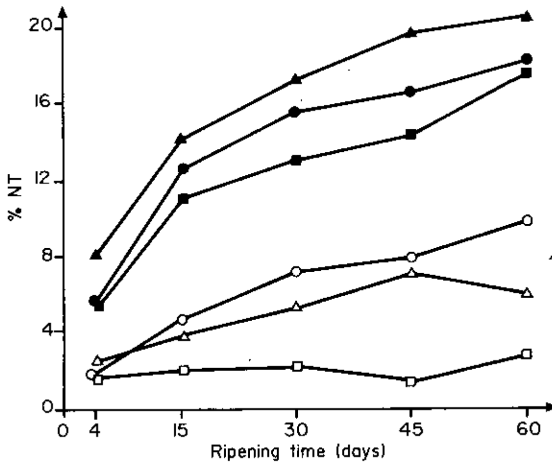

Fig. 3. Development of soluble nitrogen (■, ●, ▲) and phosphotungstic acid soluble nitrogen (□, ○, Δ) during ripening of Saint-Paulin cheese (trial E2). Control (■, □); S-cheese (●, ○); U-cheese (▲, Δ). SN and AN are expressed as % of total nitrogen.

30 days the increase of SN over that of the control was 2 and 4 % (of total nitrogen) for S-cheeses and U-cheeses, respectively. Differences in SN between S-cheeses and the C-cheeses were maximal between 15 and 30 days of ripening, diminishing slightly thereafter.

The increase of AN in S-cheeses was high and superior to that of U-cheeses. It became clearly detectable after 15 days of ripening and persisted during the entire ripening period. Fig. 4 shows the concentrations of leucine in C-, S- and U-cheeses in trial E2.

3.2.3 Sensory evaluation and texture. Sensory evaluation of cheese was carried out at 15, 30 and 60 days of ripening. S-cheeses showed a significant in-

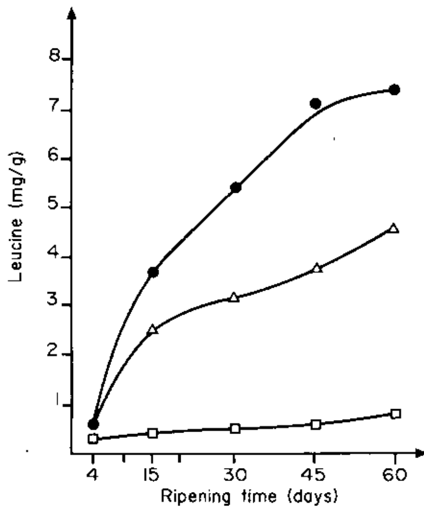

Fig. 4. Release of free leucine during ripening of Saint-Paulin cheese (trial E2) (in mg/g of cheese). Control (□); S-cheese (●); U-cheese (Δ).

crease of the flavour score (although this flavour was not really typical of a Saint-Paulin, due in part to a more developed sweet taste) and a better overall quality without changes in the textural characteristics. The difference observed between C- and S-cheese was most obvious in the mature flavour. Table 3 shows data obtained for trials E2, E3 and E4. Neither bitter flavour nor other flavour defects were found.

Objective analysis of texture by means of the Instron Testing Machine

Table 3. Sensory evaluation of Saint-Paulin cheese manufactured with addition of heat-shocked *Lb. helveticus* CNRZ 32 suspensions. C: control; S: S-cheese. Scale 0-4.

Trial	Ripening time (days)	Overall quality		Flavour						Texture					
				clean		mature		sweet		firmness		elasticity		body	
		C	S	C	S	C	S	C	S	C	S	C	S	C	S
E2	15			1.80	2.30*	1.25	2.30**	2.20	2.40	1.60	2.20*	1.40	1.80	1.50	1.80
	30	1.85	2.90**	2.20	2.40	1.35	2.70**	1.60	2.10	1.40	2.00	1.60	1.70	0.90	1.30
	60	1.80	3.05***	1.55	2.60**	0.81	2.45***	1.30	2,30**	1.90	1.90	1.25	1.50	1.40	1.15
E3	15	1.87	2.31	2.25	2.12	1.50	2.13	1.86	2.57	2.05	1.90	1.60	1.60	1.65	1.60
	30	2.06	2.94	2.00	2.57	1.06	2.63*	1.35	2.00	2.00	1.87	1.50	1.50	1.75	1.62
	60	1.75	3.25**	1.37	2.06*	0.81	3.00**	1.12	2.12*	1.30	1.20	1.20	1.30	0.90	1.00
E4	15	2.25	2.25			1.50	2.00	1.62	1.69	2.19	2.06	1.50	1.81	1.62	1.56
	30	1.56	2.87**			0.75	2.62**	1.37	2.19	1.67	2.11	2.11	2.56	1.33	1.39

*, **, ***: significantly different at $P<0.05$, 0.02 and 0.01 respectively.

showed that values of the slope at the origin, percentage deformation and force at rupture were equivalent in S- and C-cheeses.

On the other hand, U-cheeses developed considerable flavour and texture defects. An enhanced acid taste was found and a slightly bitter flavour was recognized. The texture was crumblier and harder and the colour was whiter than those of the C-cheeses.

4 Discussion

Aminopeptidase activity in *Lb. helveticus* CNRZ 32 was maximal between 13 and 15 h of culture and then decreased at the end of the stationary phase. These results agree with those of Ezzat et al. (8) for *Lb. helveticus* CNRZ 303 grown in MRS medium without pH control. Heat treatments of *Lb. helveticus* suspensions decreased the acidifying and the proteolytic activity to a different degree. Heating for 18 s at 64 °C abolished about 90 % of the acid-forming activity, but only 60 % of the cell wall proteinase activity and 10 % of the leucine aminopeptidase activity. Heating at 66 °C drastically inactivated both proteolytic activities. These results agree with those of Pettersson & Sjöström (12) and Bartels et al. (11) for *Lb. helveticus* CNRZ 32 and with those of Ardö & Pettersson (16) for *Lb. helveticus* CNRZ 303, although all these authors applied more severe conditions for shocking the cells, 15 s at 69 °C, 18 s at 70 °C and 10 s at 67 °C, respectively. The differences are probably due to the degree of protection of the cells during heating, which depends on the medium composition, e.g. skim milk versus phosphate buffer in our investigation. The reduction in number of viable cells was approximately 99.5 % under our conditions. Electron microscopy did not show damage of the cell wall, although its occurrence was suggested by Frey et al. (14).

Addition of heat-shocked *Lb. helveticus* CNRZ 32 increased proteolysis and development of flavour of the cheese. This flavour was not really typical of a Saint-Paulin but panel members considered it a good cheese flavour. S-cheeses scored higher for mature flavour and overall quality after 15 or 30 days of ripening, and no texture differences were found with C-cheeses.

The slightly lower pH reached in S-cheeses during manufacturing and the first 48 h is probably due to the residual acidifying capacity of the added lactobacilli. Subsequent release of free amino acids and other hydrolysis products from casein might be responsible for the later increase of the pH.

The increased proteolysis we observed in S-cheeses is in agreement with the findings of other authors (11, 12, 13, 16). However, the increase in AN nitrogen was higher than that previously observed with mesophilic streptococci (13) or thermophilic lactobacilli (11).

A higher SN concentration was observed in U-cheeses than in S-cheeses. It has been reported that the contribution of the mesophilic starter proteinase to SN production is negligible and that chymosin is mainly responsible (25, 26). Therefore, an increased inclusion of chymosin, due to a lower pH during cheesemaking, could explain the higher SN concentration we observed. However, at the curd cutting (45 min after starter inoculation), the pH was almost identical in the C-, S- and U-cheese vats, so the retention of chymosin in the curd should not be very different between the cheeses. The higher proteinase activity of untreated lactobacillus cells in U-cheeses might also contribute to the increase of SN.

In our experiments, the AN content increased slowly during ripening in C-cheeses (from 1.5 % at 4 days to 2.7 % of TN at 60 days in trial E2). According to Stadhouders et al. (26), this could be due to too high a proportion of Prt^- cells in the lactic starter. Our cheeses were made with starters containing about 20 % of Prt^+ variants. Similar Saint-Paulin cheeses made with starters containing 100 % of Prt^+ cells (strain CNRZ 378; Vassal, unpublished results) showed an AN content, although being slightly higher than that in the present C-cheeses, that was clearly lower than that in U- and S-cheeses. The addition of the lactobacillus cells is therefore responsible for the high increase of AN, as has been described previously for cheddar cheeses by Hickey et al. (27). Although more peptides were formed in U- than in S-cheeses, release of free amino acids was highest in the S-cheeses. This may be due to the pH in the S-cheeses, which increased more rapidly than in U- and C-cheeses, thus creating more favourable conditions for aminopeptidase activity. Better access of aminopeptidase to its substrate, possibly due to modifications of the cell-envelope (which could not, however, be confirmed by electron microscopy) may also have contributed to this increase of free amino acids.

Saint-Paulin cheese with heat-shocked *Lb. helveticus* developed no bitterness throughout the whole ripening period. These results agree with those of some other authors (11, 16). The sweeter taste observed in S-cheeses was also mentioned by Lloyd et al. (28), who used a mixed starter containing *Lb. helveticus* subsp. *yogurti* in Cheddar cheese, and by Bartell et al. (11). A high concentration of free proline was found in our four trials (not shown), and this amino acid may possibly be responsible for the sweet flavour (27, 28).

In fact, a variety of cheese based on the technology normally applied for Saint-Paulin cheese has been manufactured and results from this study suggest that the ripening period of such a cheese can be shortened by the addition of heat-shocked *Lb. helveticus* cells. Cheeses made with the addition of these bacteria, which do not belong to the normal flora of Saint-Paulin cheese, developed good taste and flavour. However, these characteristics were not typi-

cal for Saint-Paulin, and it is probable that this cheese can no longer bear the name of Saint-Paulin.

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