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Summary. Intracellular peptidases of *Lactobacillus helveticus* may play a major role in the proteolysis of Swiss cheeses, provided that they are released through bacterial lysis. Experimental Swiss cheeses were manufactured on a small scale from thermized and microfiltered milk using as starters (in addition to *Streptococcus thermophilus* and *Propionibacterium freudenreichii*) one of two *Lb. helveticus* strains, ITGLH1 and ITGLH77, which undergo lysis to different extents *in vitro*. All the cheeses were biochemically identical after pressing. The viability of *Lb. helveticus* ITGLH1 and ITGLH77 decreased to a similar extent (96–98%) while in the cold room, but the concomitant release of intracellular lactate dehydrogenase in cheeses made with strain ITGLH1 was 5–7-fold that in cheeses made with ITGLH77. Protein profiles and immunoblot detection of the dipeptidase PepD confirmed a greater degree of lysis of the ITGLH1 strain. Free active peptidases were detected in aqueous extracts of cheese for both strains, and proteolysis occurred principally in the warm room. Reversed-phase HPLC revealed a more extensive peptide hydrolysis for ITGLH1, which was confirmed by the greater release of free NH_2 groups (+33%) and free amino acids (+75%) compared with ITGLH77. As the intracellular peptidase activities of ITGLH1 and ITGLH77 have previously been shown to be similar, our results indicated that the extent of lysis of *Lb. helveticus* could have a direct impact on the degree of proteolysis in Swiss cheeses.

Autolysis of starters is now regarded as a necessary part of cheese ripening, allowing cytoplasmic peptidases to be released into the curd to participate actively in further proteolysis (Crow *et al.* 1993, 1995*a*). Autolysis of *Lactococcus lactis* has been clearly established in semi-hard cheeses such as Cheddar and Saint-Paulin (Chapot-Chartier *et al.* 1994; Wilkinson *et al.* 1994*a*, *b*) and the extent of lysis was shown to be strain dependent and to have a positive influence on secondary proteolysis (O'Donovan *et al.* 1996). The relationship between lysis of the lactococci and the extent of proteolysis was further demonstrated by provoking various levels of autolysis of the same strain, either by adding small quantities of phages (Crow *et al.* 1995*b*) or by using a lytic bacteriocin (Fox & McSweeney, 1996).

Less information is available on the autolysis of thermophilic lactobacilli (Lortal *et al.* 1997). *Lactobacillus helveticus* is an essential species in Swiss cheesemaking and

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its intracellular peptidases were estimated to be particularly effective compared with those of other lactic acid bacteria (El Soda *et al.* 1991). Its autolysis was studied in buffered solutions or 'cheese-like' media (Ohmiya & Sato, 1970; Bie & Söjström, 1975) and a preliminary characterization conducted of the autolysins involved (Lortal *et al.* 1991; Valence & Lortal, 1995). However, the autolysis of *Lb. helveticus* was only recently demonstrated in experimental and commercial Swiss cheeses using a species-specific lysis marker, the dipeptidase PepD (Valence *et al.* 1998).

In the present study, a new series of Swiss cheese experiments was undertaken with two industrial strains of *Lb. helveticus*, ITGLH77 and ITGLH1, in order to confirm the early autolysis of *Lb. helveticus*, to compare its extent for two different strains and to assess its impact on the secondary proteolysis. Moreover, as salt is often cited as a potential lysis inducer (Vegarud *et al.* 1983; Yabu & Kaneda, 1995) some experimental cheese assays were conducted without brining.

materials and methods

Organisms and culture conditions

Lb. helveticus ITGLH77 (LH77) and ITGLH1 (LH1) and *Propionibacterium freudenreichii* ITGP23 (P23) were commercial starters from the Institut Technique Français des Fromages (ITFF, formerly Institut Technique du Gruyère) collection (F-35062 Rennes, France). *Streptococcus thermophilus* was a direct-to-vat commercial starter (TA060) from Texel (F-86220 Dangé Saint-Romain, France). The *Lb. helveticus* strains were selected for their different autolysis efficiency *in vitro* according to laboratory screening (Lortal *et al.* 1997). The extent of autolysis in 0·1 M-potassium phosphate buffer, pH 5·8 after 16 h at 40 °C for LH77 and LH1 was 62 and 34 % respectively. *Lb. helveticus* was stored in MRS broth (Difco, Detroit, MI 48232, USA) and *Prop. freudenreichii* strains in yeast extract–lactate broth (Malik *et al.* 1968), both at −80 °C. The media were supplemented with 150 ml glycerol/l prior to freezing. For propagating the strains, the respective media without glycerol were used and growth was monitored by measuring A_{650}.

For cheese trials, cultures were revived from frozen stocks, first by a transfer in reconstituted milk (100 g lait G/l; Standa Industrie, F-14050 Caen, France) at 2 ml inoculum/l until lactic acid reached 7·5 g/l as measured by Dornic acidity, followed by a second transfer on Phagex commercial medium (Standa Industrie) at 100 ml inoculum/l until lactic acid reached 9·5 g/l for *Lb. helveticus*, or by two consecutive transfers in yeast extract–lactate broth at 20 ml inoculum/l until A_{650} reached 1 for *Prop. freudenreichii*.

Preparation of crude cytoplasm

Cells were harvested by centrifugation (7000 ***g***, 20 min) at the beginning of the exponential growth phase and washed twice in cold sterile distilled water. The pellet was stored at −18 °C for 24 h before being resuspended in a volume of cold sterile distilled water 1/15th of the initial volume of the culture and then subjected to treatment in a French Press apparatus at 4 °C and 138 MPa for 5 min (one run). The suspension was centrifuged at 40000 ***g*** and 4 °C to eliminate unbroken cells and cell walls. The supernatant represented crude cytoplasm and was stored at −18 °C. Protein content was assayed by the micro-procedure of Bradford (Bio-Rad, F-94203 Ivry-sur-Seine, France) using bovine serum albumin (Sigma, F38297 Saint-Quentin Fallavier, France) as standard.

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Cheese manufacture

Small scale experimental Swiss cheeses were made from 10·3 kg thermized and microfiltered milk as described by Valence *et al.* (1998) with a starter comprising *Str. thermophilus* TA060, *Prop. freudenreichii* P23 and *Lb. helveticus* LH1 or LH77. Three cheeses were made in parallel from the same batch of milk, one with *Lb. helveticus* LH1 (LH1 cheese), one with LH77 (LH77 cheese) strain and one with LH77 but without brining. Samples were taken just after pressing (day 0), in the middle (day 16) and at the end (day 27) of the period in the cold room, at the middle (day 44) and the end (day 69) of warm room treatment and after 25 d cold storage (day 96).

Chemical analysis of cheeses

Total solids (International Dairy Federation, 1982), fat (Heiss, 1966) and NaCl content (International Dairy Federation, 1988) were determined in the ripened cheese on day 69. Lactate was monitored during ripening using a kit from Boehringer (D-68298 Mannheim 1, Germany).

Starter viability

A 10 g cheese sample was homogenized in 90 g trisodium citrate (20 g/l) with a Waring Blendor (Prolabo, F-94126 Fontenay-sous-Bois, France) once for 20 s at maximum speed then twice for 10 s at minimum speed. The viability of starter lactobacilli in cheese was measured by plating serial dilutions of samples on MRS agar (pH 5·4) and incubating at 37 °C for 48 h. *Prop. freudenreichii* cells were enumerated on a selective medium (Pal Propiobac; Standa Industrie) after incubation at 30 °C for 5 d and *Str. thermophilus* cells on M17 agar plate (Biokar 088, F-60000 Beauvais, France) after incubation at 43 °C for 16 h. Non-starter lactic acid bacteria were enumerated on FH agar plates (Isolini *et al.* 1990) after incubation at 37 °C for 3 d. Anaerobic growth conditions (Anaerocult A; Merck, F-94736 Nogent-sur-Marne, France) were used for all species and results were expressed as cfu/g cheese.

Aqueous cheese extracts

Aqueous cheese extracts were prepared as described by Valence *et al.* (1998). Each cheese sample (20 g) was homogenized with an Ultra-Turrax disperser (Bioblock, F-67403 Illkirch, France) for 2 min in 80 ml distilled water. The suspension was held at 38 °C for 20 min before centrifugation at 3000 ***g*** and 4 °C for 15 min. After removal of the upper solid fat layer the supernatant was filtered through Whatman no. 1 paper and then a 0·45 μm acetate filter (Sartorius, F-91127 Palaiseau, France) and stored at −18 °C until used.

Evidence for free active peptidases in aqueous cheese extracts

The aqueous cheese extracts obtained on day 16 were aseptically filtered through a 0·22 μm acetate filter (Sartorius) and incubated at 24 °C to simulate conditions prevailing during the ripening of Emmental in the warm room as described by Gagnaire *et al.* (1998). Total free NH_2 groups resulting from the action of free peptidases on casein peptides were assayed.

SDS-PAGE analysis of total protein content of the aqueous cheese extracts

SDS-PAGE was carried out as described by Valence & Lortal (1995) at 25 °C with an SDS polyacrylamide (140 g/l) separating gel and a constant voltage of 180 V for 1 h. Samples were mixed with an equal volume of Laemmli buffer (62·5 mM-Tris HCl,

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pH 6·8 containing (per l) 100 ml glycerol, 20 g SDS and 50 ml 2-mercaptoethanol) and boiled for 2 min (Laemmli, 1970); 20 μl of each sample was loaded on to the gel. Each sample was analysed at least twice.

Autolysis of starters in cheese

Autolysis was monitored during the ripening period by measuring the release of intracellular enzymes into the cheese matrix.

Lactate dehydrogenase. The lactate dehydrogenase (EC 1.1.1.27; LDH) activity was assayed as described by Thomas (1975). Activity was expressed as units of activity/ml aqueous cheese extract, where one unit of activity represented the amount of enzyme required for the reduction of 1 μmol pyruvate/min at 37 °C. Each assay was carried out in triplicate.

Dipeptidase. The 53·5 kDa dipeptidase (PepD) used as a species-specific lysis marker for *Lb. helveticus* by Valence *et al.* (1998) was detected by immunoblotting as described previously. Samples of aqueous cheese extracts were subjected to SDS-PAGE and then transblotted on to a nitrocellulose sheet (0·45 μm pore size; Millipore, F-78051 Saint-Quentin-en-Yvelines, France).

Extent of proteolysis in cheese

Determination of total free NH_2 groups. These were assayed according to the procedure of Church *et al.* (1985) with methionine (Sigma) as standard. Samples (75 μl) were incubated at 25 °C for 2 min with 1·5 ml *o*-phthaldialdehyde reagent (5 mM-sodium tetraborate–3·5 mM-SDS–6 mM-*o*-phthaldialdehyde containing 2 ml 2-mercaptoethanol/l). Absorbance was monitored at 340 nm and results expressed as mmol methionine equivalent/l. Each assay was performed in triplicate.

Analysis of free amino acids. Aqueous cheese extracts (100 μl) were first treated with 900 μl absolute ethanol at 20 °C at 1 h. The mixtures were centrifuged at 2400 ***g*** for 15 min and the supernatants (800 μl) dried in a Speedvac evaporator and subjected to derivatization with phenyl isothiocyanate (Pierce, Touzart & Matignon, F-94403 Vitry-sur-Seine, France). The amino acid derivatives were determined in triplicate using reversed-phase HPLC (Picotag C_{18} column; 150 × 3·9 mm i.d; Waters, F-78056 Saint-Quentin-en-Yvelines, France) according to Bidlingmeyer *et al.* (1984).

Chromatographic analysis. Filtered aqueous cheese extracts were diluted and their pH adjusted to 2 with trifluoroacetic acid (100 ml/l; Pierce, Touzart et Matignon). Portions (100 μl) were injected on to the analytical reversed-phase column (Lichrosper 100 RP C_{18} column; 124 × 4 mm i.d.; Merck) equilibrated at 40 °C at a flow rate of 1 ml/min with buffer A (HPLC grade trifluoroacetic acid (Millipore) in Milli-Q water, 1·06 ml/l). Elution was with a linear gradient from 50 to 680 ml/l of buffer B (1 ml trifluoroacetic acid/l in 800 ml acetonitrile/l Milli-Q water) over 62 min, followed by 680 ml B/l to pure B in 2 min. The absorbance was monitored at 214 nm with a u.v. detector (Spectra Physics SP 8490; Thermo Separation Products, F-78030 Les Ulis, France).

RESULTS

Chemical analysis of the cheeses

These results were similar to those obtained for small scale experimental Swiss cheese by Valence *et al.* (1998) and were in agreement with previous values obtained for Swiss cheese (Buisson *et al.* 1987; Steffen *et al.* 1993). Total solids, moisture in non-fat substance and pH at moulding were (means ± SD) 585 ± 2 g/kg, 579 ± 2 g/kg and 6·38 ± 0·02 respectively. The pH of the cheese fell to 5·20 ± 0·02 within the first 24 h.

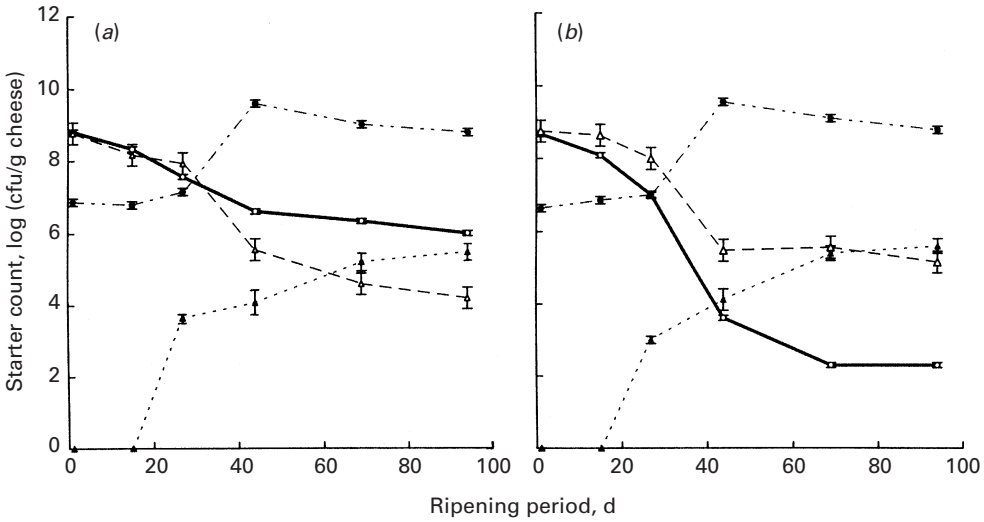

Fig. 1. Counts of starter strains during ripening in experimental cheeses made with (*a*) *Lactobacillus helveticus* ITGLH1 and (*b*) *Lb. helveticus* ITGLH77 in addition to *Streptococcus thermophilus* TA060 and *Propionibacterium freudenreichii* P23. ○, *Lb. helveticus*; ●, *Propionibacterium freudenreichii*; △, *Streptococcus thermophilus*; ▲, non-starter lactic acid bacteria. Values are means from three trials with SD indicated by vertical bars.

Both *Lb. helveticus* strains produced similar acidification (results not shown) and lactate contents ($13{\cdot}0 \pm 0{\cdot}1$ g/kg on day 1). The ripened cheeses had $623{\cdot}5 \pm 2{\cdot}5$ g total solids/kg, 530 ± 2 g moisture/kg non-fat substance, $464{\cdot}5 \pm 1{\cdot}5$ g fat/kg dry matter and $9{\cdot}3 \pm 0{\cdot}2$ g salt/kg moisture.

Viability of starter and non-starter bacteria during ripening

The maximal growth of *Lb. helveticus* and *Str. thermophilus* (5×10^8 cfu/g cheese) was followed by a dramatic decrease in viability during ripening in the cold room: 96 and 98% for LH1 and LH77 respectively (Fig. 1). At the end of ripening the viable counts of *Lb. helveticus* were 10^6 and 2×10^2 cfu/g cheese for LH1 and LH77 respectively. The viability of *Str. thermophilus* also fell sharply, at a similar rate for all cheeses. The maximal growth of *Prop. freudenreichii* was reached in the warm room, followed by a decrease in viability of 85% (Fig. 1). The non-starter lactic acid bacteria, not detectable at the beginning of the ripening, did not exceed 10^6 cfu/g cheese at any time (Fig. 1). Omitting the brining stage had no effect on the changes in viability for any starter strain (results not shown).

Bacterial lysis in cheese

The LDH activity of aqueous extracts of LH1 and LH77 cheeses increased during ripening in the cold and warm rooms (Fig. 2), with maximal activity being reached at the end of ripening (day 69). For LH1 cheese, LDH activity was already detectable on day 0; it then increased faster and was always 5–7-fold the level found in LH77 cheese. The absence of brining had no effect on the LDH activity (Fig. 2). The intracellular LDH activities for *Lb. helveticus* LH1 and LH77 and *Str. thermophilus* TA060 were (units/mg cytoplasmic proteins, means $\pm$ SD) $1{\cdot}2 \pm 0{\cdot}2$, $3{\cdot}0 \pm 0{\cdot}2$ and $2{\cdot}9 \pm 0{\cdot}1$ respectively. *Prop. freudenreichii* exhibited no NAD-dependent LDH activity. As the cheeses were made from microfiltered thermized milk, the LDH detected could only have come from the thermophilic lactic starters, i.e. *Lb. helveticus* or *Str. thermophilus*.

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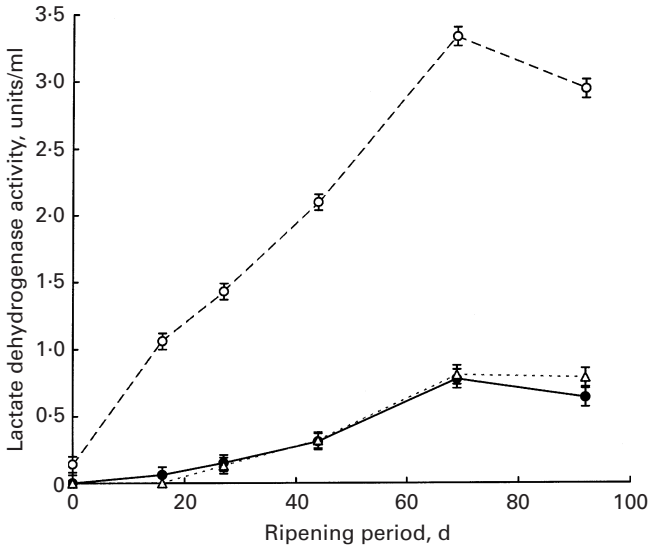

Fig. 2. Lactate dehydrogenase activity in the aqueous extracts from experimental cheeses at different times of ripening. Cheeses were made with (in addition to *Streptococcus thermophilus* TA060 and *Propionibacterium freudenreichii* P23) ○, *Lactobacillus helveticus* ITGLH1; ●, △, *Lb. helveticus* ITGLH77; ●, brined; △, non-brined. Values are means from three trials with SD indicated by vertical bars. (One unit of activity corresponds to the amount of enzyme required for the reduction of 1 μmol pyruvate/min at 37 °C.)

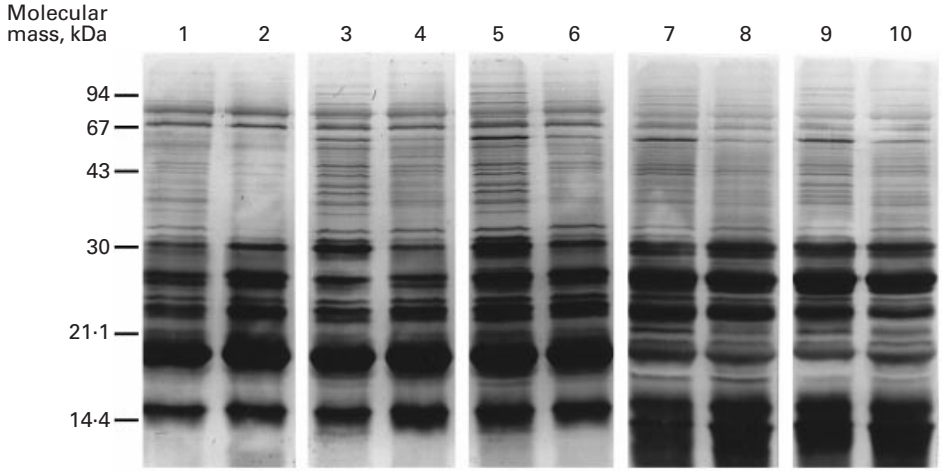

Fig. 3. SDS-PAGE of aqueous extracts of experimental cheeses at different times of ripening showing differences in the protein profiles depending on the *Lactobacillus helveticus* starter used. The cheeses were made with *Lactobacillus helveticus* ITGLH1 (lanes 1, 3, 5, 7 and 9) or *Lb. helveticus* ITGLH77 (lanes 2, 4, 6, 8 and 10) in addition to *Streptococcus thermophilus* TA060 and *Propionibacterium freudenreichii* P23. Lanes 1 and 2, day 1; lanes 3 and 4, day 16; lanes 5 and 6, day 27; lanes 7 and 8, day 44; lanes 9 and 10, day 69. Molecular masses of standard proteins are indicated on the left.

As the SDS-PAGE profile of the milk did not contain proteins in the range 30–60 kDa, any detected of this size were assumed to come from bacterial starters (Valence *et al.* 1998). SDS-PAGE of aqueous extracts of LH1 and LH77 cheeses during ripening revealed, for an equal volume loaded per lane, an increase in the intensity and number of protein bands > 30 kDa between days 0 and 16 (Fig. 3). At day 16, the intensity for these proteins for LH1 cheese was 8–10-fold that for LH77

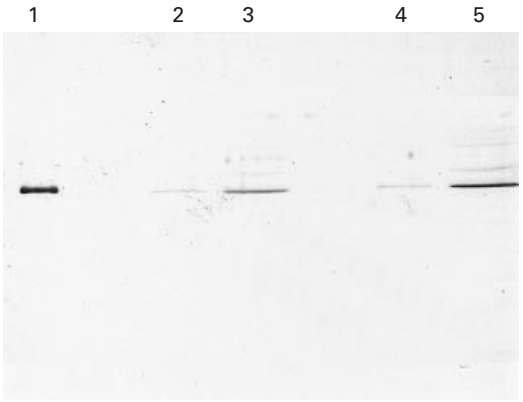

Fig. 4. Immunoblotting with anti-peptidase serum showing the autolysis of *Lactobacillus helveticus* strains in experimental cheeses at different ripening times. Lane 1, purified PepD (3 ng); cheeses made with (in addition to *Streptococcus thermophilus* TA060 and *Propionibacterium freudenreichii* P23) lanes 2 and 3, *Lb. helveticus* ITGLH77; lanes 4 and 5, *Lb. helveticus* ITGLH1. Lanes 2 and 4, day 1; lanes 3 and 5, day 27.

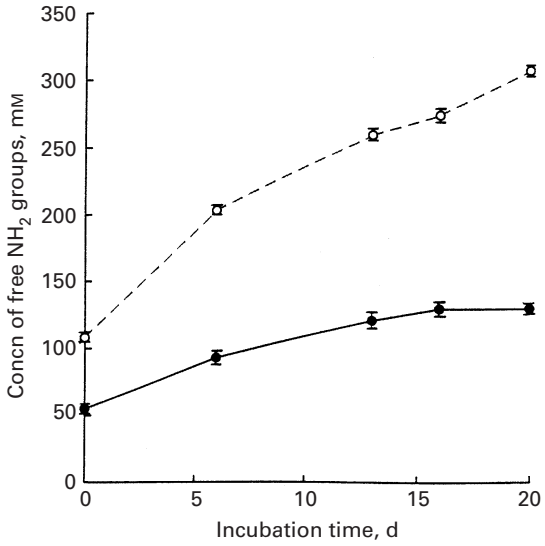

Fig. 5. Release of free amino groups in aqueous extracts of experimental cheeses obtained after 16 d ripening, sterilized by filtration and incubated statically at 24 °C for 20 d. Extracts were from cheeses made with (in addition to *Streptococcus thermophilus* TA060 and *Propionibacterium freudenreichii* P23) ○, *Lactobacillus helveticus* ITGLH1; ●, *Lb. helveticus* ITGLH77. Values are means from three trials with SD indicated by vertical bars.

cheese. (This was determined by loading increasing quantities of aqueous extracts of LH77 cheese and densitometric analysis; results not shown). The intensity then rose steadily between days 16 and 44.

A weak band of PepD was detected as early as day 0; in the cold room this increased until day 27 (Fig. 4), after which no variation could be detected to the end of ripening (results not shown). The intensity varied with the strain of *Lb. helveticus*: on day 27 that for LH1 cheese was 3-fold that for LH77 cheese (results not shown). However, the value for LH1 cheese may have been underestimated owing to saturation of the Western blotting (Valence *et al.* 1998).

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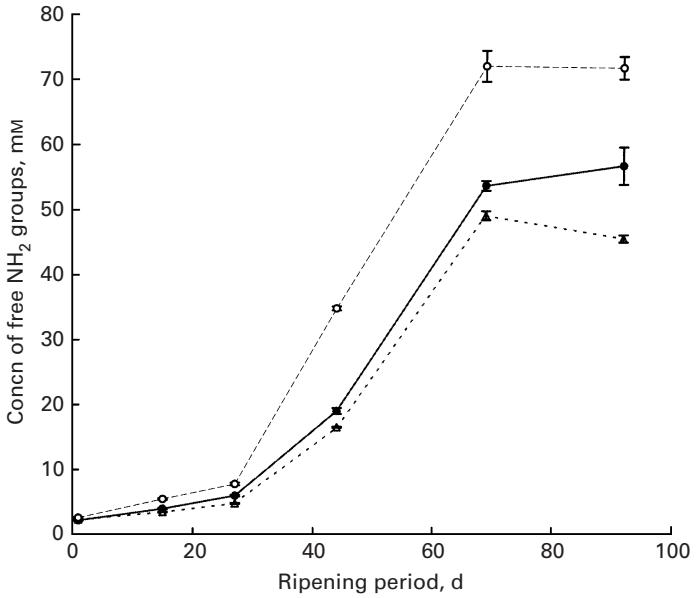

Fig. 6. Formation of free amino groups during the ripening of experimental cheeses made with (in addition to *Streptococcus thermophilus* TA060 and *Propionibacterium freudenreichii* P23) ○, *Lactobacillus helveticus* ITGLH1; ●, △, *Lb. helveticus* ITGLH77; ● brined; △, non-brined. Values are means from three trials with SD indicated by vertical bars.

Evidence of free active peptidases

The NH_2 groups increased sharply in the sterile aqueous extracts of cheese obtained at day 16 and incubated at 24 °C (Fig. 5), showing the presence of active peptidases released through lysis of the starters for both LH1 and LH77 cheeses.

Proteolysis

The concentration of NH_2 groups remained steady during cold room ripening, then increased sharply in the warm room and remained constant during subsequent cold storage (Fig. 6). At day 69 their concentration was higher (+35%) in LH1 cheese than in LH77 cheese and was slightly higher (+10%) for brined than non-brined LH77 cheeses. The peptide profiles of aqueous cheese extracts, analysed by reversed-phase HPLC, revealed differences during ripening (results not shown). Between days 0 and 27, no changes in profiles could be detected and they were similar for both strains. At day 44, particular peaks appeared and/or varied strongly during warm room ripening. Peaks corresponding to small hydrophilic peptides and free amino acids (retention time < 15 min), already detectable by day 27, increased until the end of the ripening, sharply for LH1 cheese and moderately for LH77 cheese. Peaks corresponding to large hydrophobic peptides (retention time > 40 min) were present from the beginning of ripening and increased between days 27 and 44 for both types of cheeses. Thereafter these decreased until the end of the ripening in LH1 cheese but remained almost constant for LH77 cheese.

Free amino acids were present in cheeses at very low concentrations at the end of cold room ripening and increased mainly in the warm room. Total amino acid concentrations in aqueous extract of cheeses increased during warm room ripening from 18 to 380 mM for LH77 and from 35 to 660 mM for LH1 cheeses. Qualitatively the profiles were similar for both strains, the predominant amino acids being Pro,

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Leu, Lys, Val, Gly and Glx (results not shown), in agreement with previous observations for full size Swiss cheeses (Salvat-Brunaud *et al.* 1995).

No significant differences could be detected between brined and non-brined cheeses either in free amino acids or chromatographic profiles (results not shown). The difference of +10% in free NH_2 groups for brined LH77 cheese compared with non-brined cheese could be attributed to activation of plasmin by salt (Fox & Stepaniak, 1993).

DISCUSSION

Autolysis of *Lb. helveticus* during the ripening of Swiss type cheese has previously been demonstrated for strain LH77; it occurred early and coincided with a drastic reduction in lactobacilli counts and the release of intracellular PepD (Valence *et al.* 1998). In the present study autolysis of *Lb. helveticus* was confirmed for this strain and compared with another industrial strain, LH1. All the results demonstrated a more extensive autolysis of LH1 compared with LH77. Indeed, the PepD signal, which is specific for lysis of *Lb. helveticus*, was higher for the LH1 strain. Moreover, since the LDH released must have come from *Lb. helveticus* or *Str. thermophilus* and, assuming similar behaviour of the *Str. thermophilus* in the two cheeses, the difference of the LDH released would reflect the greater autolysis of LH1 (5–7-fold that of LH77). This was further confirmed by comparison of the protein profiles of the aqueous extracts of the cheeses and by the release of PepD. It should be noted that the decrease in viability in the cold room (96 and 98%) was almost identical, showing that, in the case of LH77, a significant part of the population was dead or could not be grown, but was not lysed. Hence we suggest that decrease in viability of *Lb. helveticus* in cheese cannot be regarded as a reliable indicator of lysis. We also found that for both strains brining had no effect on starter viability or the extent of lysis of *Lb. helveticus*, at least for small scale Swiss cheeses.

Autolysis can have an effect on proteolysis only if the peptidases released remain active in the cheese matrix. Our results demonstrated the presence of free active peptidases in cheese, in agreement with results for lactococci in Cheddar (Law *et al.* 1974; Wilkinson *et al.* 1994*b*) and thermophilic starters in Emmental (Gagnaire *et al.* 1998) cheese. The proteolysis was shown to take place mainly in the warm room, as with full size Emmental cheeses (Thierry *et al.* 1998). The various indices used showed greater proteolysis in LH1 cheese. The overall intracellular peptidase activity towards β-casein peptides was shown to be slightly higher for LH1 than for LH77 (+15%; our unpublished work). Thus the greater degree of proteolysis for LH1 may be due to a more extensive lysis coupled with a slightly higher peptidase activity. However, it should be pointed out that although lysis for LH1 was 5–7-fold that of LH77, proteolysis (as measured by free amino acid content) was only 1·8-fold that of LH77. This suggests that there are limiting factors in Swiss cheese such as limited diffusion of the peptidases into the cheese matrix after their release and/or substrate limitation around the colonies. Moreover, even non-lysed LH77 cells could participate in the proteolysis, as suggested recently for permeating lactococci by Niven & Mulholland (1998).

The strain dependence of autolysis has been established *in vitro* for lactococci and *Lb. helveticus* (Langsrud *et al.* 1987; Lortal *et al.* 1997), and was confirmed in cheese for lactococci (Boutrou *et al.* 1998; Chapot-Chartier *et al.* 1994; Wilkinson *et al.* 1994*a*, *b*) and for *Lb. helveticus* in the present study. There are several hypotheses to explain this strain dependence. There may be an association between the presence of a prophage and the ready lysis of a strain in cheese, and this hypothesis recently

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received experimental support for *Lactococcus lactis* (Lepeuple *et al.* 1997). Among *Lb. helveticus* strains, lysogeny appears widespread: 47% of the strains are lysogenous, harbouring complete or defective prophages (Séchaud, 1990; Carminati *et al.* 1997). When induced by mitomycin C, the growth of LH1 and LH77 was not altered at all (Valence, 1998), suggesting that they are non-lysogenous, but this would have to be confirmed by further work. Minor variations in bacterial cell wall composition, such as the degree of peptidoglycan reticulation or *O*-acetylation, or the content of secondary polymers, may also have major effects on lysis (Logardt & Neujahr, 1975; Niskasaari *et al.* 1989). Finally, differences in zymograms suggest variations in the effectiveness of autolysins between one strain and another in the same species (Valence & Lortal, 1995). Incidentally, comparison of LH1 and LH77 autolysis in buffered solutions indicated greater autolysis for LH77, the reverse of the results obtained in cheeses. Selection of strains on the basis of their autolysis in buffered solutions may reflect only differences in autolysin efficiency or cell wall composition and not the presence of prophages, whose expression requires a growing culture. This highlights the need to develop predictive tests for lysis in *Lb. helveticus* species which are also able to respond to the lysogenic state of a strain.

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