Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Viability and contribution to proteolysis of an adjunct culture of *Lactobacillus plantarum* in two model cheese systems: Cheddar cheese-type and soft-cheese type

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Keywords
adjunct cultures, Cheddar cheese model, lactobacilli, proteolysis, ripening, soft-cheese model.

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2007/1535: received 19 September 2007, revised 19 February 2008 and accepted 20 February 2008

doi:10.1111/j.1365-2672.2008.03813.x

Abstract

Aims: The influence of the cheese-making process, ripening conditions and primary starter on the viability and proteolytic activity of an adjunct culture of *Lactobacillus plantarum* I91 was assessed in two miniature cheese models, representative of Cremoso Argentino and Cheddar cheeses.

Methods and Results: Cheeses with and without adjunct culture were made under controlled microbiological conditions and sampled during ripening for physicochemical and microbiological analyses. The addition of lactobacilli neither contributed to acid production nor caused changes to the composition of the cheeses. The strain studied exhibited good development and survival and showed a similar growth pattern in both cheese matrices. The adjunct culture caused changes to secondary proteolysis of both cheese types, which were evidenced by modification of peptide profiles and the increase in the levels of some individual amino acids as well as the total content of free amino acids. The changes observed were consistent with the acceleration of proteolysis in the two cheese models assayed.

Conclusion: *Lactobacillus plantarum* I91 has desirable and robust technological properties, which makes it a suitable adjunct culture for cheese-making.

Significance and Impact of the Study: Other cultures and environmental conditions prevailing in the food may affect the viability of adjunct cultures and its biochemical activities; this is the first report describing the successful performance of an adjunct culture of *Lact. plantarum* I91 in two different model cheese systems.

Introduction

Nonstarter lactic acid bacteria (NSLAB) are adventitious micro-organisms that develop in nearly all cheese varieties and consist mostly of facultative heterofermentative (mesophilic) lactobacilli; *Lactobacillus casei*, *Lact. paracasei*, *Lact. plantarum*, *Lact. rhamnosus* and *Lact. curvatus* are found most frequently in cheese (Fitzsimons *et al.* 1997).

The significance of NSLAB to cheese quality is controversial: while some authors have postulated that NSLAB are responsible for most quality defects in cheese (Khalid and Marth 1990; Galgano *et al.* 2001; Crow *et al.* 2002; Herreros *et al.* 2007; Rynne *et al.* 2007), other researchers reported that nonstarter lactobacilli did not alter flavour development in Cheddar cheese (Lloyd *et al.* 1980), or even that they positively influence secondary proteolysis and flavour (McSweeney *et al.* 1994; Hynes *et al.* 2003; Poveda *et al.* 2003; Di Cagno *et al.* 2006; Irigoyen *et al.* 2007). In this context, adjunct cultures of selected strains of NSLAB have been proposed to improve flavour intensity or to accelerate cheese ripening. In addition, adjunct of lactobacilli may suppress the growth of undesirable adventitious microbiota and thereby prevent the occurrence of defects (Banks and Williams 2004).

So far, many strains of cheese-isolated lactobacilli have been proposed as adjunct cultures, based on their contribution to secondary proteolysis and flavour formation in Cheddar (McSweeney *et al.* 1994), Danbo (Antonsson *et al.* 2003), Manchego (Poveda *et al.* 2003) and Armada cheeses (Herreros *et al.* 2007), among others, in which mesophilic starter cultures are mostly used. However, little information about the impact of adjunct lactobacilli on cheese ripening in combination with thermophilic starters is available. In addition, most adjunct cultures have been tested only in one cheese model, generally representative of the cheese from which they had been originally isolated (Broome *et al.* 1990; Herreros *et al.* 2007). Comparative studies on the behaviour of a given *Lactobacillus* strain in different cheese models are lacking; these studies can, however, provide interesting information, as cheese-making conditions (i.e. cheese technology, cooling rate, ripening temperature, etc.) and primary starter may favour or impair the growth of the adjunct culture and strongly influence its enzymatic activities (Wilkinson *et al.* 1994; Lane *et al.* 1997; Hynes *et al.* 2001). The finding of versatile cultures, with desirable technological properties robust enough to be expressed in different food environments and in the presence of different starter cultures, may be useful for the cheese industry and its suppliers.

Global production of Cheddar exceeds that of all cheese varieties and consequently Cheddar-type cheese is a common model for research (Banks 2002). On the other hand, production of Argentinean soft cheeses, among which Cremoso-type is the most important, is also significant as it accounted for 243 725 tn in 2006. Cremoso-type cheese constitutes a simple and reliable model for soft-cheese studies as it has no surface flora and its cheese-making procedure is easily miniaturized (Milesi *et al.* 2007).

The objective of this study was to assess the impact of an adjunct culture of *Lact. plantarum* I91 on the proteolysis in miniature Cremoso and Cheddar-type cheeses made under controlled microbiological conditions. In addition, we evaluated the effect of the cheese-making process, ripening conditions and starter composition on the growth, survival and biochemical activity of the selected strain.

Materials and methods

Starter and adjunct cultures

Lyophilized *Streptococcus thermophilus* (Diagramma S.A., Santa Fe, Argentina) was used as a starter culture in the manufacture of miniature Cremoso cheese, while frozen concentrated *Lactococcus lactis* subsp. *lactis* (Christian Hansen, Horsholm, Denmark) was selected for miniature Cheddar cheese-making. *Strepococcus thermophilus* was reactivated in sterile reconstituted skim milk powder at 43°C for 45 min before adding into the cheese-milk, while *L. lactis* was inoculated directly. Both starter cultures were added to the milk at a level of 10^6 CFU ml^{-1}.

Adjunct culture consisted of *Lact. plantarum* I91, isolated from a good quality 2-month-old Tybo Argentino cheese. The culture was obtained from the collection of the Instituto de Lactología Industrial (INLAIN, CONICET, Facultad de Ingeniería Química, Universidad Nacional del Litoral, Santa Fe, Argentina). This strain was previously identified and characterized by means of *in vitro* assays. Technological properties as well as resistance to biological barriers and probiotic characteristics were evaluated and suggested that the culture had potential for use as an adjunct in cheese-making (Bude-Ugarte *et al.* 2006). Stock culture was stored frozen at −80°C in MRS broth (Britania, Buenos Aires, Argentina) supplemented with 15% (v/v) glycerol. Before being used, the strain was cultivated twice in MRS broth at 34°C overnight. Cells from an aliquot of this culture were harvested by centrifugation (8000 ***g*** for 20 min at 4°C), washed twice with 0·1 mol l^{-1} sodium phosphate buffer (pH 7), and re-suspended in the same buffer. The adjunct culture was added into the vats containing cheese-milk of experimental cheeses at a level of 10^6 CFU ml^{-1}.

Cheese models

Two miniature cheese models were studied, i.e. miniature Cremoso and Cheddar-type cheeses. Cremoso cheese-making and analyses were performed at the INLAIN (CONICET, Universidad Nacional del Litoral, Santa Fe, Argentina), while for Cheddar-type cheeses both the manufacture and analyses were carried out at the Department of Food and Nutritional Sciences (University College, Cork, Ireland).

Miniature Cheddar cheese model

Miniature Cheddar cheeses were manufactured from 200 ml of batch-pasteurized (65°C for 30 min) milk by the procedure of Shakeel-Ur-Rehman *et al.* (1998) adapted to aseptic conditions. The bulk raw milk used for manufacture was obtained from a local farmer and standardized to a casein-to-fat ratio of 0·7:1·0. Four miniature Cheddar cheeses were prepared on each cheese-making day: two control cheeses (Cch) containing only starter culture and two experimental (Ech) cheeses with the addition of both starter and adjunct culture. Cheese manufacture was carried out in a laminar air-flow unit using sterile utensils. Cheeses were vacuum packed and ripened at 8°C for up to 60 days.

Gross composition and the pH of the cheeses were determined in triplicate at 30 days of ripening. Moisture (oven drying at 102 ± 1°C) and protein content (Kjeldahl method) were analysed according to International Dairy Federation standard methods (International Standard, 4A 1982 and International Standard, 20B 1993, respectively). Salt content was determined by the potentiometric method of Fox (1963). The pH was measured (Orion pH meter, model 720; Orion Research Inc., Boston, MA, USA) during the cheese-making and in the final products by placing the electrode of the pH meter in contact with the curd or grated cheese, respectively.

Microbiological counts were performed in duplicate after 1, 15, 30, 45 and 60 days of ripening. Starter population was determined on M17 agar (Merck, Darmstadt, Germany) after incubation at 30°C for 3 days. The adjunct culture and NSLAB in experimental and control cheeses, respectively, were enumerated on Rogosa agar (Merck) after incubation at 30°C for 5 days under aerobic conditions.

Proteolysis was monitored on 1-, 30-, and 60-day-old cheeses by urea-PAGE, RP-HPLC of peptide profiles and for levels of individual free amino acids (FAA). The pH 4·6-soluble and -insoluble fractions of the cheeses were obtained by a slight modification of the method of Kuchroo and Fox (1982) as described by Sousa and McSweeney (2001).

Urea-PAGE (12·5% T, 4% C, pH 8·9) of the freeze-dried pH 4·6-insoluble fraction of the cheeses was performed by using a Protean II XI vertical slab-gel unit (Bio-Rad Laboratories Ltd, Watford, UK) according to the method of Andrews (1983), as modified by Shalabi and Fox (1987). The gels were stained directly with Coomassie Brillant Blue G250, as described by Blakesley and Boezi (1977), and destained in a few changes of distilled water.

Peptide profiles of the pH 4·6-soluble fraction were determined by RP-HPLC using the equipment and the assay conditions as described by Poveda *et al.* (2003). The pH 4·6-soluble extracts were freeze-dried, dissolved in mobile phase A (10 mg ml^{-1}) and centrifuged at 15 000 ***g*** for 10 min. The samples were then filtered through a 0·45 μm filter and an aliquot (40 μl) of the filtrate was injected onto the column.

Levels of individual FAA in the freeze-dried pH 4·6-soluble fraction of the cheeses on one and 60 days of ripening were determined using the AccQ.Tag method (Waters Corporation, Milford, MA, USA). A given mass of the lyophilized pH 4·6-soluble fraction (10–20 mg depending on the ripening time and type of cheese) was dissolved with an appropriate volume of distilled water. Internal standard solution (20 μl of 2·5 mmol l^{-1} α-aminobutyric acid in 0·1 mol l^{-1} HCl; Sigma-Aldrich, St Louis, MO, USA) was added to 980 μl of the reconstituted pH 4·6-soluble fraction and filtered through a 0·45 μm filter (Millex; Millipore, São Paulo, Brazil). Derivatization was performed by mixing 20 μl of diluted pH 4·6-soluble fraction with 60 μl of borate buffer (pH 8·8) and 20 μl of the derivatizing solution (10 mmol l^{-1} 6-aminoquinolil-*N*-hydroxysuccinylcarbamate). The reaction mixture was kept at room temperature for 1 min and then incubated in a water bath at 55°C for 10 min, after which 20 μl of the derivatized sample was injected into the HPLC column. Concentrations of each FAA were determined by RP-HPLC using a quaternary pump, an on-line degasser and UV/VIS detector, all Series 200 (Perkin Elmer, Norwalk, CT, USA), fitted with a Nova-Pak™ C18 (150 × 3·9 mm, 4 μm particle size) analytical (Waters Corporation) and a C18 (15 × 3·2 mm, 7 μm particle size) guard columns (Perkin Elmer). Data were recorded with an interface module connected to a computer using the Turbochrom® software (Perkin Elmer). UV detection was performed at 248 nm. Chromatographic separation was performed at a column temperature of 37°C and at a flow rate of 1 ml min^{-1} using two solvents as mobile phase. Solvent A was prepared by diluting (2:20, v/v) a solution provided by the AccQ.Tag kit (trihydrate sodium acetate 19%, phosphoric acid 6–7%, triethylamine 1–2%, water 72–73%) with double distilled water, and solvent B was acetonitrile–double distilled water 60:40 (v/v). Separation was achieved according to the gradient described by Bergamini (2007).

Miniature soft-cheese model

Four replicate cheese-makings of miniature Cremoso cheese, an Argentinean soft-cheese variety, were conducted on different days with different milks under controlled microbiological conditions, according to the procedure described by Milesi *et al.* (2007). The bulk raw milk used for manufacture was obtained from a nearby dairy plant (Milkaut S.A., Franck, Santa Fe, Argentina) and batch-pasteurized at 65°C for 30 min. On each day of manufacture, four miniature Cremoso cheeses were prepared from 2000 ml of milk, two control (Ccr) and two experimental cheeses (Ecr). As in the previous model, control and experimental cheeses only differed in the presence of the adjunct culture of *Lact. plantarum* I91. Cheeses were vacuum packed and ripened at 5°C up to 60 days.

Chemical composition of 3-day-old cheeses was assessed in triplicate as described for miniature Cheddar-type cheeses. Streptococci were enumerated on duplicate plates of Skimmed Milk Agar (Britania) by incubation at 37°C for 48 h after 0 (fresh curd), 3, 15, 30, 45 and 60 days of ripening. The same samples were screened in

duplicate for adventitious and adjunct lactobacilli, in control and experimental cheeses, respectively, by plating on MRS agar (Britania) and incubating at 34°C for 48 h under anaerobic conditions. Urea-PAGE and RP-HPLC were performed after 3, 30 and 60 days of ripening using the instruments and the methods described by Milesi *et al.* (2007).

Individual FAA content was determined in the water-soluble fraction of 3- and 60-day-old cheeses. Cheese samples (5 g) were homogenized with 15 ml of distilled water by using mortar and pestle and incubated in a water bath at 40°C for 1 h. The suspension was centrifuged at 3000 ***g*** for 30 min. The supernatant was then filtered through fast flow filter paper and the volume was made up to 25 ml with distilled water. The water-soluble fraction of the cheeses was diluted with the internal standard solution and derivatized as described before, then injected into the chromatograph. FAA were analysed by RP-HPLC using the method described above.

Statistical analyses

Data from chemical composition, microbiological counts and individual content of FAA of miniature Cheddar and Cremoso cheeses made with and without added lactobacilli were analysed by one-way ANOVA using SPSS 10·0 (SPSS Inc., Chicago, IL, USA). The significance level was set at $P < 0{\cdot}05$. Multivariate statistical analysis of peptide profiles was used to evaluate the contribution of the adjunct culture tested to proteolysis in both cheese models. Chromatographic data were subjected to principal component analysis (PCA) and nonhierarchical cluster analysis (CA) (*K*-means) using the covariance matrix with SPSS 10·0 to reduce the dimensionality of the data, evaluate the distribution of variables and samples and detect any eventual grouping (Bergamini *et al.* 2006).

Results

Cheese composition and primary proteolysis

In both model cheeses, no significant differences ($P > 0{\cdot}05$) were found between control and experimental cheeses for pH, moisture, salt-in-moisture and protein content. For Cheddar-type cheeses, these parameters ranged between 5·21 and 5·22, 37·28 and 37·64 (% w/w), 4·69 and 4·96 (% w/w), 24·61 and 24·81 (% w/w), respectively, and for Cremoso cheeses, they ranged between 5·17 and 5·21, 50·54 and 51·75 (% w/w), 2·24 and 2·39 (% w/w), 19·23 and 19·39 (% w/w), respectively (not shown). Composition was similar to that of previously reported for conventional and miniature Cheddar and Cremoso cheeses (Shakeel-Ur-Rehman *et al.* 1998; Milesi *et al.* 2007). The evolution of pH during cheese-making followed the same pattern in all cheeses of each type, regardless of the addition of the adjunct culture. Similarly, the final pH recorded after the incubation or cheddaring steps was similar in both control and adjunct-treated cheeses.

The presence of the adjunct culture did not affect the breakdown of intact casein in both model cheeses at any ripening time, which was evidenced by very similar electrophoretic patterns, characterized by an extensive and progressive breakdown of α_{s1}-casein – earlier and more extensive in Cremoso cheeses than in Cheddar-type cheeses – and little or no degradation of β-casein (results not shown).

Evolution of starter cultures and lactobacilli

The numbers of starter, adventitious and adjunct lactobacilli in the miniature Cheddar- and Cremoso-type cheeses at different ripening periods are shown in Figs 1 and 2. In miniature Cheddar-type cheeses, lactococci counts decreased almost one log cycle (from approx. 9·5 to 8·3 $\log_{10}$ CFU g^{-1} cheese) over the course of ripening in the cheeses made with and without adjunct culture. On the other hand, initial counts of adjunct lactobacilli in the experimental cheeses were around 8 $\log_{10}$ CFU g^{-1} cheese, then increased slightly during the first 30 days and remained constant at high levels (approx. 8·4 $\log_{10}$ CFU g^{-1}) until the end of ripening. In miniature Cremoso-type cheeses, starter population (*Strep. thermophilus*) was over 10^9 CFU g^{-1} during the whole ripening period in all cheeses regardless of the addition of adjunct culture, and numbers of adjunct lactobacilli in experimental cheeses increased rapidly up to 10^8 CFU g^{-1} and then remained

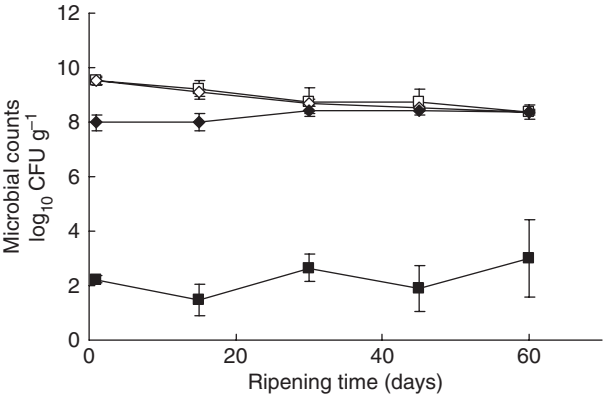

Figure 1 Evolution of starter bacteria, NSLAB and *Lactobacillus plantarum* I91 during ripening of miniature control and adjunct-treated Cheddar-type cheeses made under controlled microbiological conditions. Lactococci from starter culture and lactobacilli in control (□, ■) and experimental (◇, ◆) cheeses.

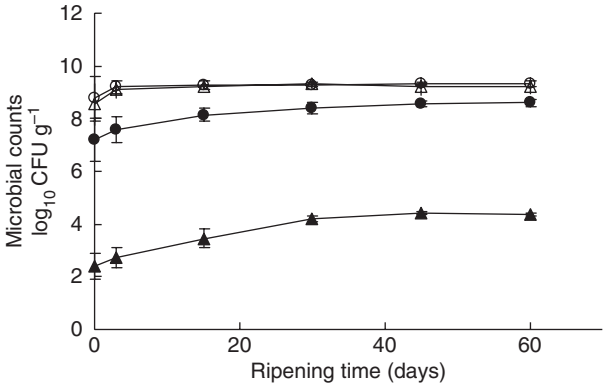

Figure 2 Evolution of starter bacteria, NSLAB and *Lactobacillus plantarum* I91 during ripening of miniature control and adjunct-treated Cremoso cheeses, made under controlled microbiological conditions. Streptococci from starter culture and lactobacilli in control (△, ▲) and experimental (○, ●) miniature Cremoso cheeses.

constant during ripening. Adventitious lactobacilli in the Cheddar-type control cheeses remained at very low numbers ($<10^3$ CFU g^{-1} cheese), while in Cremoso-type control cheeses were somewhat higher ($<10^4$ CFU g^{-1}).

Peptide profiles and free amino acids

Peptide profiles of miniature Cheddar- and Cremoso-type cheeses made with or without adjunct culture showed some quantitative differences, which were mainly evident at the end of the ripening period (not shown). For Cheddar-type cheeses, 10 peaks were selected and used as variables for PCA after visual comparison of the chromatograms. These peaks, identified as '*a*'–'*j*', showed the greatest variation between cheese samples according to both cheese type and ripening time. The first two PCs explained 98·1% of the total variation of the data. As can be observed in the loading plot (Fig. 3a), all selected variables exhibited high impact on PC1, while only peaks *a*, *f* and *g* showed an influence on PC2. In the score plot (Fig. 3b), samples were grouped according to ripening time on PC1, while they tended to gather by type of cheese on PC2. Peaks *f*, *i* and *g*, located on the negative side of PC2, were present at higher levels in 60-day-old control cheeses, while peaks *a* and *c*, and to lesser extent *b* and *d*, located on the positive side of PC2, dominated the chromatograms of the 60-day-old adjunct-treated cheeses. The samples were classified into three different groups by applying CA (Fig. 3b). Cluster one contained all 1-day-old cheeses, either with or without added adjunct lactobacilli. Cluster two was composed of both 30-day-old control and adjunct-treated cheeses as well as of 60-day-old control cheeses. Cluster three consisted of adjunct-treated cheeses at 60 days of ripening.

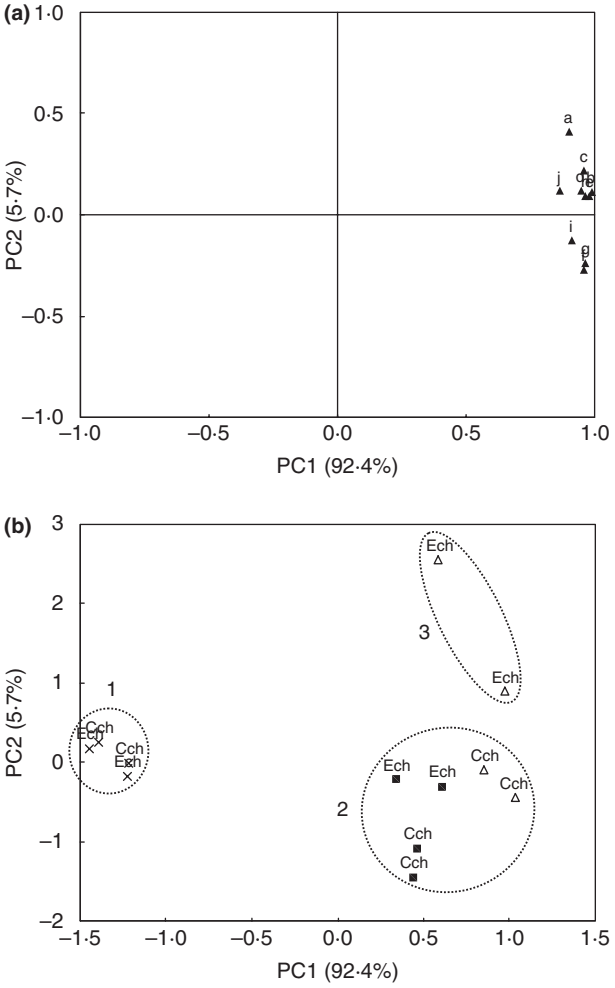

Figure 3 Principal component (PC) analysis of data from RP-HPLC chromatograms of miniature control and adjunct-treated Cheddar-type cheeses, made under controlled microbiological conditions. (a) Loading plot of independent variables on PC1 and PC2. (b) Score plot on PC1 and PC2 of 1-(x), 30-(■) and 60-(△) day old cheeses. Cch and Ech, control and adjunct-treated cheeses, respectively. Ellipses enclose cheese samples in the same cluster as defined by cluster analysis (*K*-means).

For Cremoso-type cheeses also, 10 peaks were selected, labelled '*a*'–'*j*' and were used as independent variables for PCA. These peaks were similarly chosen because of their high variability between cheeses, but were not necessarily equivalent to those used in profiles of Cheddar-type cheeses. The first two PCs accounted for 98·3% of the data variation. A plot of the independent variables on PC1 and PC2 showed that all selected peaks had a strong influence on PC1 while only peaks *f*, *i*, *j*, *a*, and to lesser extent, *c* and *g* impacted on PC2 (Fig. 4a). Figure 4(b) shows the score plot of the samples on PC1 and PC2 from miniature control (Ccr) and experimental (Ecr) Cremoso cheeses after 3, 30 and 60 days of ripening.

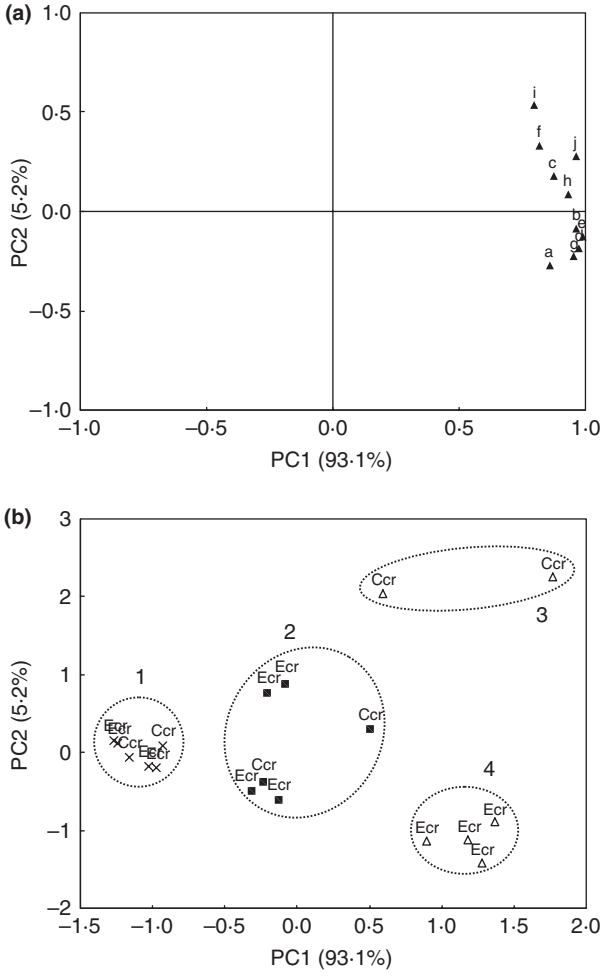

Figure 4 Principal component analysis of data from RP-HPLC chromatograms of miniature control and adjunct-treated Cremoso cheeses, made under controlled microbiological conditions. (a) Loading plot of independent variables on PC1 and PC2. (b) Score plot on PC1 and PC2 of 3-(x), 30-(■) and 60-(△) day old cheeses. Ccr and Ecr, control and adjunct-treated cheeses, respectively. Ellipses enclose cheese samples in the same cluster as defined by cluster analysis (*K*-means).

While samples were grouped according to ripening time on PC1, they were distributed by cheese type on PC2. Peaks *c*, *f*, *h*, *i*, *j*, located on the positive side of PC2 dominated chromatograms of 60-day-old control cheeses, while the rest, located on the negative side of PC2, were present at higher levels in 60-day-old adjunct-treated cheeses. The samples were classified into four different groups by applying CA, which are indicated on Fig. 4(b) by ellipses. All 3-day-old cheeses were grouped in the same cluster (cluster one) regardless of the type of cheese. Similarly, cluster two enclosed both 30-day-old control and adjunct-treated cheeses. Cluster three and four consisted of 60-day-old control and adjunct-treated cheeses, respectively.

Concentrations of individual FAA at the beginning and at the end of the ripening period for miniature control and adjunct-treated Cheddar- and Cremoso- type cheeses are shown in Figs 5 and 6. Total FAA content of all cheeses increased as ripening progressed, but significant differences were found in the levels of total FAA between control and adjunct-treated cheeses. In both cheese models, the level of total FAA in the cheeses with adjunct lactobacilli was twofold higher ($P < 0{\cdot}05$) than in their respective control cheeses after 60 days of ripening.

In Cheddar-type cheeses, major individual FAA in both control and adjunct-treated cheeses at the end of ripening were serine, glutamate, proline, lysine, leucine and phenylalanine which accounted for about 70% of the total FAA. The concentration of these amino acids as well as the levels of glycine, histidine, threonine, alanine, tyrosine and valine at 60 days of ripening was higher in the adjunct-treated cheeses compared with those in their controls; however, only significant differences ($P < 0{\cdot}05$) in the content of proline and tyrosine were detected when data were subjected to ANOVA.

In Cremoso-type cheeses, the most abundant FAA in 60-day-old control and adjunct-treated cheeses were arginine, alanine, proline, lysine, leucine and phenylalanine, which accounted for about 80% of the total FAA. Adjunct-treated cheeses contained significantly higher concentrations ($P < 0{\cdot}05$) of serine, histidine, arginine, threonine, proline, tyrosine, leucine and phenylalanine than the control cheeses after 60 days of ripening.

Discussion

Lactobacillus plantarum I91 neither contributed to acid production during cheese manufacture and ripening nor caused changes to the gross composition of the cheeses, which is an important technological feature to prevent deviations from standard cheese-making and overall quality of the product (Crow *et al.* 2002).

The decrease in mesophilic starter cell populations during ripening of miniature Cheddar-type cheeses was consistent to that of other studies on conventional Cheddar cheese (Broome *et al.* 1990; McSweeney *et al.* 1994), while the different behaviour of thermophilic starter, which remained at high numbers during ripening of Cremoso-type cheeses, was also typical of this variety (Bude-Ugarte *et al.* 2006). In both model cheeses, after 60 days of ripening, NSLAB were present at a much lower level (at least four log cycles) than in commercial or pilot-plant cheeses, manufactured either in open vats or under controlled microbiological conditions (Broome *et al.* 1990; McSweeney *et al.* 1994; Bude-Ugarte *et al.* 2006). This result demonstrates the effectiveness of the cheese-making processes and the measures applied to control the growth

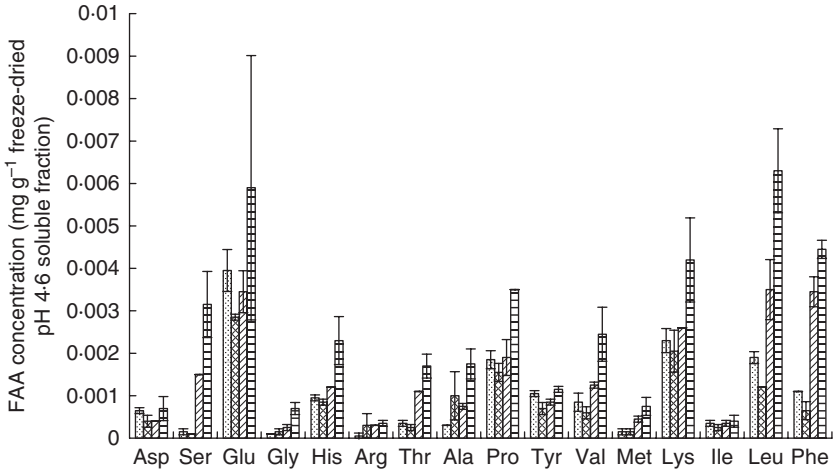

Figure 5 Individual free amino acid profiles of miniature control and adjunct-treated Cheddar-type cheeses, made under controlled microbiological conditions, after 1 and 60 days of ripening. Cch and Ech, control and adjunct-treated cheeses, respectively. ⊡, Cch-1 day; ⊠, Ech-1 day; ▨, Cch-60 days; ▤, Ech-60 days.

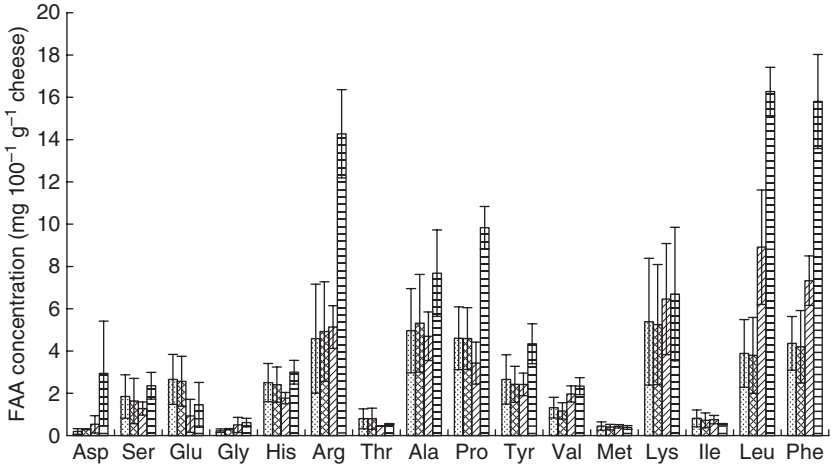

Figure 6 Individual free amino acid profiles of miniature control and adjunct-treated Cremoso cheeses, made under controlled microbiological conditions, after 3 and 60 days of ripening. Ccr and Ecr, control and adjunct-treated cheeses, respectively. ⊡, Ccr-3 days; ⊠, Ecr-3 days; ▨ , Ccr-60 days; ▤, Ecr-60 days.

of undesirable adventitious micro-organisms, which in turn is important to prevent the enzymatic activities of the adjunct culture from being masked by the biochemical activities of non-controlled biota.

In both cheese models, the starter population grew to similar numbers in control and experimental cheeses throughout ripening, suggesting that the addition of *Lact. plantarum* I91 did not influence the growth and survival of the starter bacteria. On the other hand, the counts of adjunct lactobacilli in the cheese models studied showed that the selected strain grew rapidly in the curd during manufacture and adapted well to both cheese technologies. The differences in cheese environment defined by cheese-making and ripening conditions as well as the level and species of the starter culture did not interfere with the development and survival of *Lact. plantarum* I91. On the contrary, slight differences were found in the counts of adventitious lactobacilli between the cheese models, as they were approximately one log order lower in the miniature Cheddar-type cheeses compared with that in the miniature Cremoso cheeses. Even though miniature Cheddar-type cheeses were ripened at a higher temperature, which facilitates the growth of NSLAB (Shakeel-Ur-Rehman *et al.* 2000), the raw milk used for their manufacture had better microbiological quality (result not shown), which could explain the lower NSLAB numbers found in this cheese type.

Proteolysis in all cheeses significantly increased with ripening time, as evidenced by electrophoretic and peptide profiles and levels of FAA. In agreement with previous reports (McSweeney *et al.* 1994; Hynes *et al.* 2003; Poveda *et al.* 2003; Di Cagno *et al.* 2006), no differences in primary proteolysis were observed in the electrophoretic patterns of cheeses made with or without adjunct lactobacilli in both model cheeses. Overall, mesophilic lactobacilli are weakly proteolytic and do not contribute considerably to hydrolysis of intact casein during cheese ripening (Di Cagno *et al.* 2006).

On the other hand, *Lact. plantarum* I91 caused noticeable modifications in the peptide profiles in both model cheeses. In the miniature Cremoso cheese model, CA enclosed control and adjunct-treated cheeses in the same group both at 3 and at 30 days of ripening, but they were differentiated from each other at the end of the ripening stage. As for miniature Cheddar-type cheese model, the tested strain not only introduced changes in the peptide profile but also accelerated ripening as peptide profiles of 30-day-old experimental cheeses were similar to those of the control cheeses at the end of ripening (60 days), and 60-day-old experimental cheeses were more proteolysed than the controls. The fact that *Lact. plantarum* I91 is able to increase secondary proteolysis in both model cheeses is encouraging with regard to its impact on cheese quality via formation of background flavour and supplying FAA, which are the main precursors of most volatile aroma compounds (Yvon 2006). Other nonstarter lactobacilli cultures have shown a very slight peptidolytic activity in cheese (McSweeney *et al.* 1994; Hynes *et al.* 2001; Michaelidou *et al.* 2003).

The addition of *Lact. plantarum* I91 also resulted in a noticeable increase in levels of total FAA in the two model cheeses and caused modifications to their FAA profiles. The content of several amino acids was significantly higher in the adjunct-treated Cremoso cheeses compared with that of their respective control cheeses at the end of the ripening, while in the adjunct-treated Cheddar-type cheeses, significant differences were only found in levels of a few amino acids. This was not surprising as variability among cheese samples was high (frequently the case in cheese-making experiments). Increased concentrations of total and individual FAA in cheeses with added lactobacilli were also reported for other cheese varieties, although it depends on the cultures (starter and adjunct) and cheese composition (McSweeney *et al.* 1994; Hynes *et al.* 2001; Poveda *et al.* 2003; Di Cagno *et al.* 2006; Irigoyen *et al.* 2007; Morea *et al.* 2007).

Lactobacillus plantarum I91 was found to be a versatile and suitable adjunct culture for the manufacture of Cheddar-type and Cremoso cheeses, which may be considered as representative of uncooked hard and soft cheeses, respectively. The culture did not contribute to acid production, exhibited good development and survival in cheeses and enhanced secondary proteolysis, all desirable technological properties whose robustness was evidenced by the similar trends found in the two model cheese systems. The results of this study suggest that the use of *Lact. plantarum* I91 as adjunct culture for the manufacture of the two cheese varieties studied may contribute to cheese quality enhancement. However, further studies are needed to evaluate the contribution of the strain studied on the flavour formation in cheese.

Acknowledgements

This work was partly funded by a grant from the Programme Alβan, a European Union Program of High Level Scholarships for Latin America (Scholarship No. E05D050374AR). The authors wish to acknowledge the Agencia Nacional de Promoción Científica y Tecnológica (Proyecto PICTO 09-13227) for the financial support provided for this research.

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