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Assessing the proteolytic and cheese ripening properties of single strains of *Lactococcus* in miniature cheeses

Shakeel-Ur-Rehman[a], Are H. Pripp[b], Paul L.H. McSweeney[a], Patrick F. Fox[a*]

[a] Department of Food Science and Technology, University College, Cork, Ireland
[b] Department of Food Science, Agricultural University of Norway, Aas, Norway

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Abstract — The performance of 11 single strains of *Lactococcus* (*Lc. lactis* ssp. *cremoris* 223, 227, 250, 255, 267, 303, AM1 and SK11 and *Lc. lactis* ssp. *lactis* UC317 [non-bitter strains], and *Lc. lactis* ssp. *cremoris* Wg2 and HP [bitter strains]) was studied in miniature Cheddar-type model cheeses. Different inocula were used to give equivalent rates of acidification during manufacture and the cheeses made differed in flavour development and proteolysis during ripening. Urea-polyacrylamide gel electrophoretograms (PAGE) of the water-insoluble fraction (WISF) of all cheeses were similar after 2 and 4 months of ripening, while the level of water-soluble nitrogen (WSN; as % of total N) showed only small differences between cheeses made with different starter strains. The cheese made with *Lc. lactis* ssp. *cremoris* Wg2 differed considerably from the other cheeses as indicated by urea-PAGE of the water-soluble fraction (WSF), reverse-phase high performance liquid chromatography (RP-HPLC) of the 70 % ethanol-soluble and -insoluble fractions of the WSF, concentration of amino acids and flavour characteristics; it had the highest pH (5.7) and the highest concentration of most of amino acids except Asp, Ile and Arg after 4 months of ripening. The cheese made with *Lc. lactis* ssp. *cremoris* SK11 received the highest score for flavour and that made with Wg2 received the lowest score. The results of this study indicate that the strain(s) of *Lactococcus* used as starter plays an important role in the biochemistry of cheese ripening and produces a characteristic profile of peptides and amino acids. © Inra/Elsevier, Paris.

single strain starters / miniature cheese / proteolysis

Résumé — Évaluation des propriétés protéolytiques de souches pures de *Lactococcus* pendant l'affinage de fromages miniatures. La performance de 11 souches pures de *Lactococcus* [*Lc. lactis* ssp. *cremoris* 223, 227, 250, 255, 267, 303, AM1 et SK11 et *Lc. lactis* ssp. *lactis* UC317 (souche

* Correspondence and reprints. pff@ucc.ie

non-amère), et *Lc. lactis* ssp. *cremoris* Wg2 et HP (souche amère)] est étudiée dans des fromages modèles miniatures de type cheddar. Différents inocula sont utilisés pour obtenir des taux équivalents d'acidification pendant la fabrication, et les fromages obtenus sont différents dans le développement de la flaveur et la protéolyse au cours de l'affinage. Les électrophorégrammes sur gel de polyacrylamide-urée (PAGE) de la fraction non-hydrosoluble (WISF) de tous les fromages sont similaires après 2 à 45 mois d'affinage, alors que le niveau d'azote hydrosoluble (en % de l'N total) montre seulement une petite différence entre les fromages fabriqués avec différents levains. PAGE de la fraction hydrosoluble (WSF), la CLHP phase inverse de fractions de la WSF insoluble et soluble dans 70 % d'éthanol, la concentration en acides aminés et les caractéristiques de la flaveur indiquent que le fromage fabriqué avec *Lc lactis* ssp. *cremoris* Wg2 est considérablement différent des autres fromages. Ce fromage après 4 mois d'affinage possède le plus haut pH (5,7) et la plus importante concentration en acides aminés excepté pour Asp, Ile et Arg, après 4 mois d' affinage. Le fromage fabriqué avec *Lc. lactis* ssp. *cremoris* SK11 a obtenu la note la plus élevée en flaveur et celui fabriqué avec Wg2 a obtenu la note la plus faible. Les résultats de cette étude indiquent que la (les) souche(s) de *Lactococcus* utilisée comme levains joue un rôle important dans la biochimie de l'affinage des fromages et produit un profil caractéristique de peptides et d'acides aminés.

souche pure / fromage miniature / protéolyse

1. INTRODUCTION

Lactic acid bacteria are added to cheese milk as starters during cheese manufacture primarily to produce lactic acid at an appropriate rate, but they also make an important contribution to proteolysis in cheese during ripening through the action of their cell envelope-associated proteinase (CEP) and intracellular peptidases [11, 12, 16, 19]. The significance of starter bacteria in proteolysis and flavour development in cheese during ripening is well documented [3, 7, 16, 17] and it is a widely held view that properly balanced flavour in Cheddar cheese cannot be produced without starter bacteria [22, 32].

The starter bacteria grow rapidly in the cheese milk and curd during the manufacture of Cheddar cheese, reaching 10^8–10^9 cfu·g^{-1} at salting, but subsequently decrease at a strain-dependent rate to approximately 1 % of maximum numbers within 1 month of ripening [23], probably due to low curd pH and the high salt concentration in the curd. The death and lysis of the starter cells are important, since intracellular peptidases are released into the cheese matrix where they degrade peptides, produced from casein by the coagulant or plasmin, to smaller peptides and amino acids [10]. Thomas and Pritchard [31] suggested that the main role of starter proteinases is the slow degradation of polypeptides produced from α_{s1}-casein by rennet; the peptide α_{s1}-CN(f1-23) is hydrolysed by the starter CEP [6, 14]. Lee et al. [21] showed that starter proteinases were required for the accumulation of small peptides and amino acids, which are involved in flavour development in Cheddar cheese. A positive relationship between proteolysis in cheese and the autolysis of starter has been established [4, 25, 35], suggesting that inter-strain differences in the level of autolysis/cell leakage is a major factor influencing the rate of secondary proteolysis in cheese. Muir et al. [24] found that differences in aroma, flavour and texture were associated with starter type. There have been few comparative studies using single strains of *Lactococcus* in cheese manufacture.

There have been numerous in vitro studies using the caseins or synthetic substrates to determine the types and specificities of proteinases and peptidases present in various starter bacteria [see 16, 19]. Such studies are useful in determining whether specific peptide bonds in the caseins can be hydrolysed. However, such studies provide little information on the usefulness of an organism in cheesemaking. Assessing the performance of different lactococcal strains during cheese ripening is expensive and time-consuming; it is therefore desirable to use model systems, provided that the conditions present in cheese can be replicated closely.

The aims of the present study were to determine the contributions of different individual strains of *Lactococcus* to cheese ripening in miniature Cheddar-type cheeses [29] with special reference to proteolysis and the formation of peptides and amino acids.

2. MATERIALS AND METHODS

2.1. Cheese manufacture

Lc. lactis ssp. *cremoris* strains 267 and 255 were obtained from Chr Hansen, Little Island, Cork, Ireland. The other strains used in this study (*Lc. lactis* ssp. *cremoris* 223, 227, 250, 303, AM1, SK11, HP, Wg2 and *Lc. lactis* ssp. *lactis* UC317) were obtained from the culture collection of the Department of Microbiology, University College, Cork. Individual strains were propagated in 10 % (w/w) sterile skim milk reconstituted from low-heat skim milk powder (RSM) (Irish Dairy Board, Dublin). The fully grown milk cultures of individual strains were inoculated at a level of 2 % into 10 mL of sterile RSM and incubated at 31 °C for 12 h (first propagation). The first propagation was used to inoculate 10 mL of sterile RSM at a level of 2 % which was incubated at 31 °C for 6–7 h (until a coagulum formed). The second propagation was used to inoculate RSM at 2 %, which was incubated at 22 °C for 12 h before adding to the cheese milk.

Preliminary trials were conducted to evaluate the growth rate of different strains of *Lactococcus* in sterile 10 % RSM and pasteurised whole milk. The starter inoculum for each strain required to produce acid at a comparable rate was determined by activity tests. Aliquots (10 mL) of 10 % RSM at 31 °C were inoculated with individual *Lactococcus* cultures at 1, 1.5, 2, 2.5 or 3 % (v/v) and subjected to the temperature profile used in Cheddar cheese manufacture. For cheesemaking, the level of inoculum for each strain was chosen based on the amount necessary to reduce the pH of milk to 5.2 in 5 h. Based on the results of activity tests, strains 223, AM1, 255, 227, 250 and HP were added to cheese milk at 2 %, while strains 303, SK11, UC317, 267, Wg2 were added at 2.8, 2.4, 2.6, 1.8 and 2.5 %, respectively.

Twelve miniature cheeses (one set) were manufactured in two batches of six cheeses in 1 day using the protocol developed by Shakeel-Ur-Rehman et al. [29]. The cheeses in batch 1 were made using strains 303, SK11, UC317, 223, AM1 and 267 and those in batch 2 using strains 255, 227, 250, Wg2, 223 and HP. *Lc. lactis* ssp. *lactis* UC223 was included as a 'control' strain in both batches within 1 day. Three sets of cheeses were made and the grouping of strains was the same in all sets. Milk was held at 30 °C for 30 min after addition of the starter. Rennet (Maxiren at 34 mL·100 L^{-1} milk) and $CaCl_2$ were then added and milk held at 30 °C until cutting (45–50 min). Temperature of the curds/whey mixture was then increased to 38 °C over 30 min and held at 38 °C until the pH reached 6.2 (about 30 min). The curds/whey mixture was then centrifuged at room temperature for 60 min (the temperature of the curd/whey mixture did not drop below 36 °C during centrifugation) and then the whey was drained. The curds in the centrifuge bottles were held in a water bath at 36 °C until pH decreased to 5.2–5.3 and then recentrifuged for 20 min. After further whey drainage, the cheeses were brine salted for 30 min at room temperature in the centrifuge bottles. After salting, the cheeses were removed from the bottles, wiped with tissue paper and vacuum packed in bags of MultiFlex PA/PE 20/70 C (Cryovac, Flersburg, Germany) with oxygen permeability of 45 $cm^3/m^2*d*bar$. The 24 cheeses of the first two sets were made from the same milk on 2 successive days. The 12 cheeses in the first set were ripened at 8 °C for 2 months, while the second set of 12 cheeses was ripened for 4 months. The third set of 12 cheeses was ripened at 8 °C for 4 months, but was not subjected to chemical analysis, but were retained for informal sensory analysis after 2 and 4 months for flavour and body on a 0–10 scale (0 = minimum score, 10 = maximum score) by two experienced commercial graders from the Irish Department of Agriculture, Food and Forestry. The three sets (36 cheeses) constituted the first trial and two trials were performed.

2.2. Analysis of cheeses

The cheeses were analysed in duplicate for moisture and protein [1], and salt using the method of Fox [9] and pH was determined as described by Shakeel-Ur-Rehman et al. [29]. Water-soluble (WSF) and -insoluble (WISF) fractions were prepared by a modification of the method of Kuchroo and Fox [15], as described by Shakeel-Ur-Rehman et al. [29]. The pH 4.6 soluble N was determined by macro-Kjeldahl. Proteolysis was studied as described by Lynch et al. [22]; WSFs and WISFs were analysed by alkaline urea-polyacrylamide gel electrophoresis (PAGE). Ethanol (70 %) -soluble and -insoluble subfractions of the WSF of 2- and 4-month-old cheeses were analysed by reverse-phase high performance liquid chromatography (RP-HPLC), as described by Lynch et al. [22]. Total free amino acids in the cheeses were determined by the Cd-ninhydrin method [8]. Individual amino acids in the 4-month-old cheeses were determined in the 2.0 % trichloroacetic acid (TCA)-soluble fraction of WSF by RP-HPLC according to the method of Cohen and Michaud [2]. Separation was performed using a Waters™ HPLC system consisting of a model 717 auto sampler, a model 626 pump and a Nova Pak™ C_{18} column (3.9 mm × 150 mm, 4 µm), certified for use with the AccQ Tag method (Millipore Corporation, Waters Chromatography Division, Milford, MA, USA) at a column temperature of 37 °C and a solvent flow rate of 1 mL·min^{-1}.

3. RESULTS AND DISCUSSION

In general, results presented are means from both trials. However, since HPLC, PAGE and sensory analysis were similar for both trials, results of only one are presented.

3.1. Composition

The composition of the cheeses made with different single strain starters were generally similar; the ranges for moisture, protein and salt were 39.72–41.12 %, 23.68–25.27 %, 1.61–1.67 %, respectively (*table I*) and were comparable to the composition of the minia-

Table I. Composition of 12 miniature cheeses made with different single strain *Lactococcus* starters. (Strain 223 was common to both batches of cheese made in a set.)

Tableau I. Composition de 12 fromages miniatures fabriqués avec différents levains de souche pure de *Lactococcus* (la souche 223 est commune aux deux lots de fromages fabriqués dans cette série).

Strain	Moisture[a]	Salt[a] (%)	Protein[a]	pH[c] 2 months	pH[c] 4 months
303	40.5 (1.17)[b]	1.66 (0.059)	25.2 (1.255)	5.10	5.10
SK11	39.8 (1.15)	1.61 (0.065)	25.1 (1.075)	5.04	5.03
317	41.1 (0.73)	1.62 (0.038)	24.6 (0.542)	5.08	5.10
223	41.0 (0.59)	1.61 (0.050)	24.6 (0.839)	5.16	5.17
AM1	39.9 (0.82)	1.65 (0.049)	24.8 (0.98)	5.07	5.07
267	40.4 (1.37)	1.67 (0.099)	24.1 (1.73)	4.92	4.96
255	40.5 (0.77)	1.65 (0.035)	23.6 (1.01)	5.05	5.01
227	40.8 (1.12)	1.66 (0.038)	23.9 (1.39)	5.03	5.03
250	39.8 (1.66)	1.66 (0.102)	24.6 (0.83)	5.20	5.10
Wg2	39.4 (1.75)	1.64 (0.108)	25.1 (1.12)	4.96	5.71
223	40.9 (1.44)	1.63 (0.026)	25.0 (1.17)	5.13	5.16
HP	39.7 (1.38)	1.61 (0.039)	24.8 (1.33)	5.04	5.05

[a] Mean of four cheeses, i.e. two from each of two trials; [b] figures in parentheses are standard deviations; [c] mean of two cheeses, one from each of two trials.

[a] Moyenne des quatre fromages, c'est-à-dire, deux de chacun des essais. [b] Les chiffres entre parenthèses correspondent à l'écart type. [c] Moyenne de deux fromages, un de chacun des deux essais.

ture Cheddar-type cheeses reported by Shakeel-Ur-Rehman et al. [29]. The cheeses made with *Lc. lactis* ssp. *cremoris* Wg2 had a higher pH after 4 months of ripening than the other cheeses, while cheeses made with strain 267 had the lowest pH, although all cheeses made had a similar pH (5.2–5.3) at day 1 (not shown).

3.2. Proteolysis

The pH 4.6-soluble nitrogen (SN) as a % of total N, in the 2-month-old cheeses ranged from 11 to 12 % and increased to 17 to 19 % in the 4-month-old cheeses (*table II*). Lynch et al. [22] found that the level of water-soluble nitrogen (WSN) was similar in cheeses made with or without starter, indicating that the starter organisms do not have a direct role in the production of WSN or pH 4.6-SN. Chymosin and plasmin are responsible for the production of most of the WSN [12]. All cheeses made using the different *Lactococcus* strains had similar urea-PAGE electrophoretograms, typical of Cheddar cheese, after 4 months of ripening (*figure 1*). This implies that *Lactococcus* strains either did not contribute or all strains contributed to a similar level to primary proteolysis in cheese. Studies on cheese with a controlled microflora have shown that the coagulant and, to a lesser extent plasmin, are responsible for the level of proteolysis detectable by gel-electrophoresis [13, 26, 33]. Urea-PAGE of WSFs of 4-month-old

Table II. pH 4.6-soluble nitrogen (N) (expressed as % of total N) of 12 miniature cheeses made with different single strain *Lactococcus* starters (mean of two cheeses, one from each of two trials). (Strain 223 was common to both batches of cheeses made in a set.)

Tableau II. Azote soluble N pH 4.6 (exprimé en % de l'azote total) de fromages miniatures fabriqués avec différents levains de souche pure de *Lactococcus* (moyenne de 2 fromages, un de chacun des deux essais). (La souche 223 est commune aux deux lots de fromages fabriqués dans cette série).

Strain	Age (months)	
	2	4
303	11.0	17.0
SK11	12.0	17.0
317	12.0	17.0
223	12.0	17.0
AM1	12.0	18.0
267	12.0	19.0
255	12.0	19.0
227	11.0	17.0
250	12.0	18.0
Wg2	11.0	18.0
223	12.0	17.0
HP	11.0	18.0

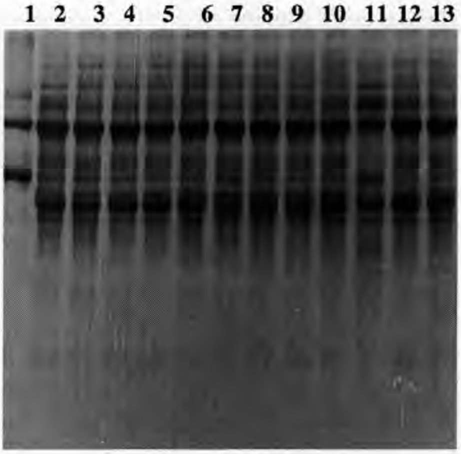

Figure 1. Urea-polyacrylamide gel electrophoretograms of water-insoluble fractions of 4-month-old miniature cheeses made with single strain *Lactococcus* cultures. Lane 1: sodium caseinate (control); lanes 2–13: cheeses made using strains 303, SK11, 317, 223, AM1, 267, 255, 227, 250, Wg2, 223 or HP, respectively.

Figure 1. Électrophorégrammes en gel de polyacrylamide-urée des fractions non hydrosolubles de fromages miniatures affinés 4 mois, fabriqués avec des cultures de souches pures de *Lactococcus*. Série 1 : caséinate de sodium (contrôle), séries 2–13 : fromages fabriqués avec les souches 303, SK11, 317, 223, AM1, 267, 255, 227, Wg2, 223 ou HP, respectivement.

cheeses made with the different *Lactococcus* strains showed differences in the peptide profiles (*figure 2*), indicating different contributions of individual *Lactococcus* starter contribute to the formation of intermediate-sized peptides.

Differences in peptide profiles were apparent in the RP-HPLC of ethanol-soluble and – insoluble fractions of WSFs of 4-month-old cheeses made with different *Lactococcus* strains (*figures 3a, b, 4a, b*). The RP-HPLC chromatograms of the ethanol-soluble and -insoluble fractions of WSF of 4-month-old cheeses showed that the cheeses could be classified into three groups: I, that made with Wg2; II, those made with SK11 or AM1; and III, those made with 223, 227, 250, 255, 267, 303, UC317 or HP (*figures 3a, b, 4a, b*). This grouping has been confirmed by Pripp et al. [27] by performing a detailed multi-variate component statistical analysis (MVCA) of RP-HPLC chromatograms of ethanol-soluble and -insoluble fractions of WSFs of the cheeses in this study. RP-HPLC is a useful technique for studying the peptidase activities of starters [30]. Farkye et al. [7] also found differences in the RP- HPLC profiles of WSFs of slurries made using *Lc. lactis* ssp. *lactis* 650 or *Lc. lactis* ssp. *cremoris* 62M, indicating differences in proteolytic activity among strains.

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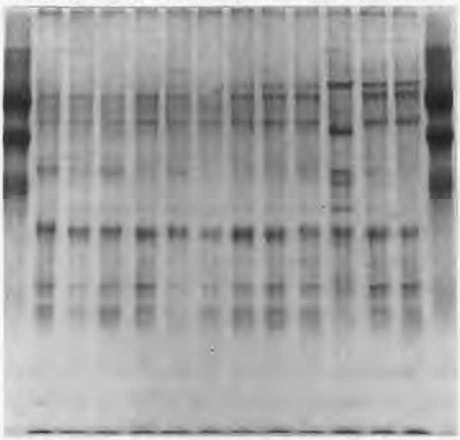
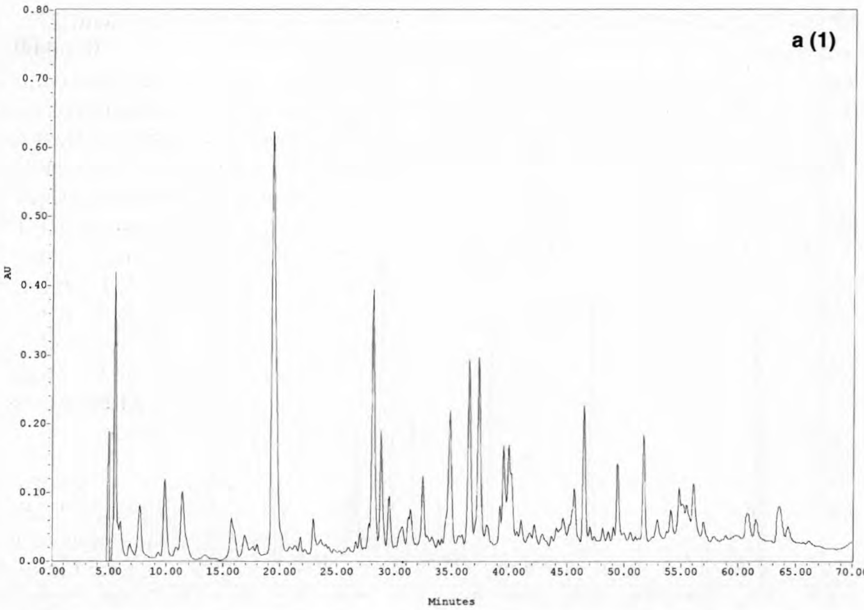


Figure 2. Urea-polyacrylamide gel electrophoretograms of the water-soluble fractions of 4-month-old miniature cheeses made with single strain *Lactococcus* cultures. Lanes 1 and 14: sodium caseinate (control); lanes 2–13: cheeses made using strains 303, SK11, 317, 223, AM1, 267, 255, 227, 250, Wg2, 223 or HP, respectively.

Figure 2. Électrophorégrammes en gel de polyacrylamide-urée de la fraction hydrosoluble des fromages miniatures affinés 4 mois, fabriqués avec des cultures de souches pures de *Lactococcus*. Série 1 et 14 : caséinate de sodium (contrôle), séries 2–13 : fromages fabriqués avec les souches 303, SK11, 317, 223, AM1, 267, 255, 227, Wg2, 223 ou HP, respectivement.

The CEPs of *Lactococcus* strains have been classified into three groups: P_I, P_{III} and mixed $P_{I/III}$ type [34]. The P_I type acts primarily on β-casein and is found in *Lc. lactis* ssp. *cremoris* Wg2. The P_{III} type which acts on α_{s1}-, κ- and β-caseins, is found in *Lc. lactis* ssp. *cremoris* SK11 and AM1. The CEP of *Lc. lactis* ssp. *cremoris* HP and *Lc. lactis* ssp. *lactis* UC317 is of the P_I/P_{III} type and degrades both α_{s1}- and β-caseins in a manner that distinguishes them from P_I or P_{III} proteinases [6, 20]. The specificity of the CEP of other strains used in the study has not been determined. Differences in the specificity of CEP of the different lactococcal strains may explain the differences in the RP-HPLC peptide profiles observed in this study.

The cheeses made with strain Wg2 had the highest total concentration of free amino acids after 2 and 4 months of ripening; only small differences were observed in the levels of total free amino acids in the cheeses made with other strains (*table III*). The level of total free amino acids found in the cheeses made using *Lc. lactis* ssp. *cremoris* 223 or *Lc. lactis* ssp. *lactis* UC317 was similar to those found by Lane and Fox [17, 18] in Cheddar cheeses made using these strains. O'Donovan et al. [25] reported that the concentration of free amino acids was highest in cheeses made with AM2 and lowest in cheeses made with HP. However, in this



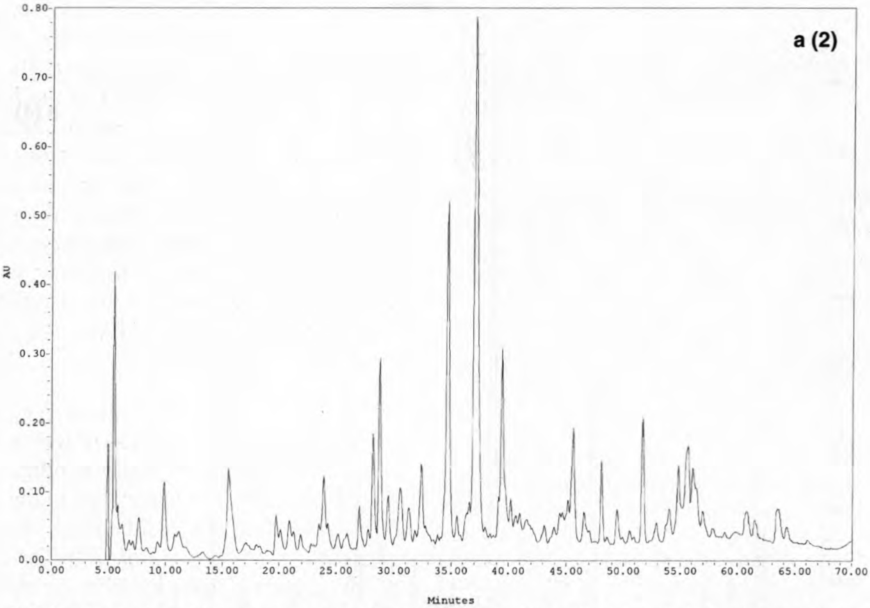
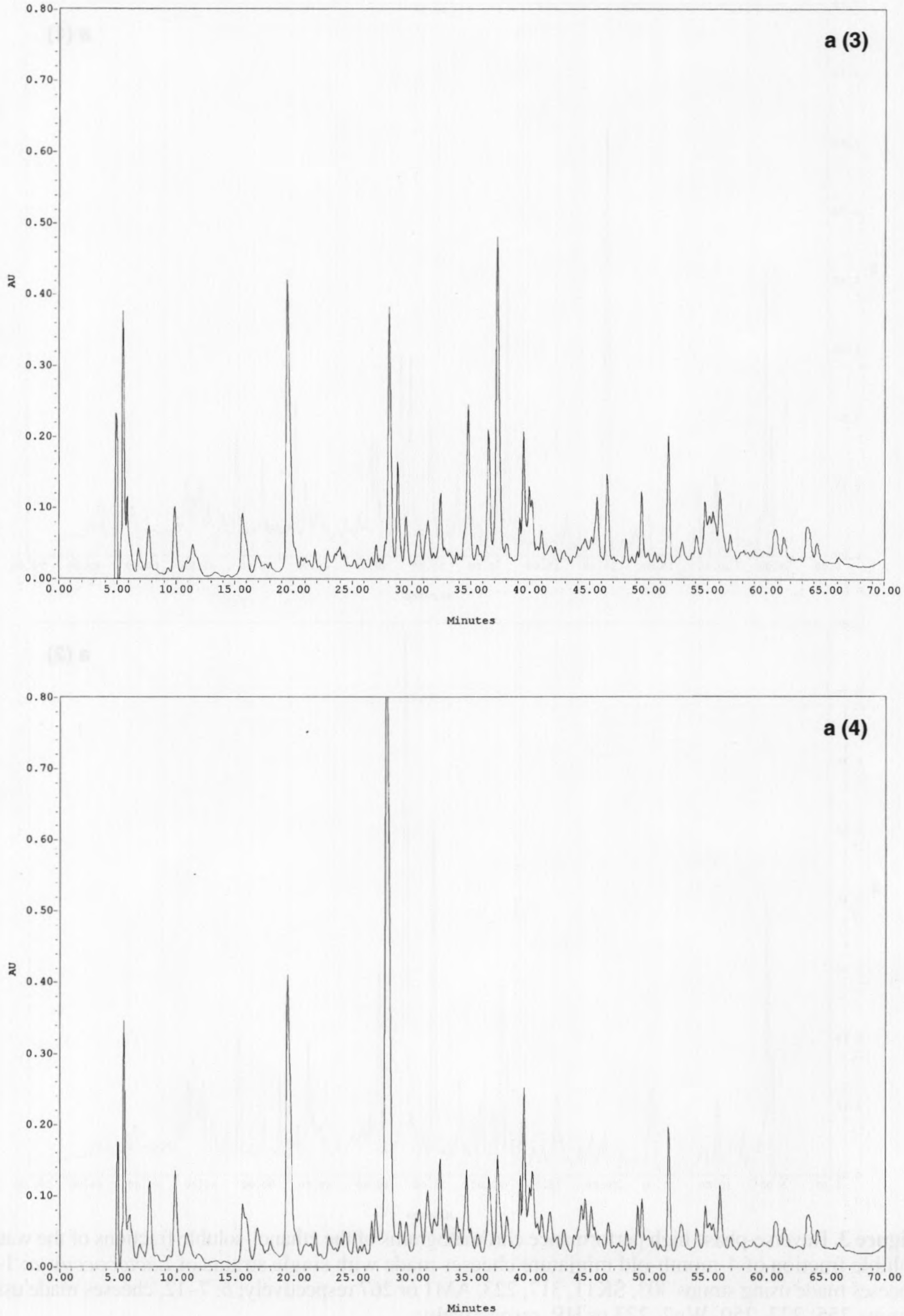


Figure 3. Reverse-phase high performance chromatograms of the ethanol-soluble fractions of the water-soluble fraction of 4-month-old miniature cheeses made with single strains of *Lactococcus. a*: 1–6, cheeses made using strains 303, SK11, 317, 223, AM1 or 267 respectively; *b*: 7–12, cheeses made using strains 255, 227, 250, Wg2, 223 or HP, respectively.

Figure 3. Chromatogramme RP-HPLC de la fraction soluble dans l'éthanol issu de la fraction hydrosoluble des fromages miniatures affinés 4 mois fabriqués avec des souches pures de *Lactococcus. a* : 1–6 : fromages fabriqués avec les souches 303, SK11, 317, 223, AM1 ou 267 respectivement ; *b* : 7–12 : fromages fabriqués avec les souches 255, 227, 250, Wg2, 223 ou HP, respectivement.



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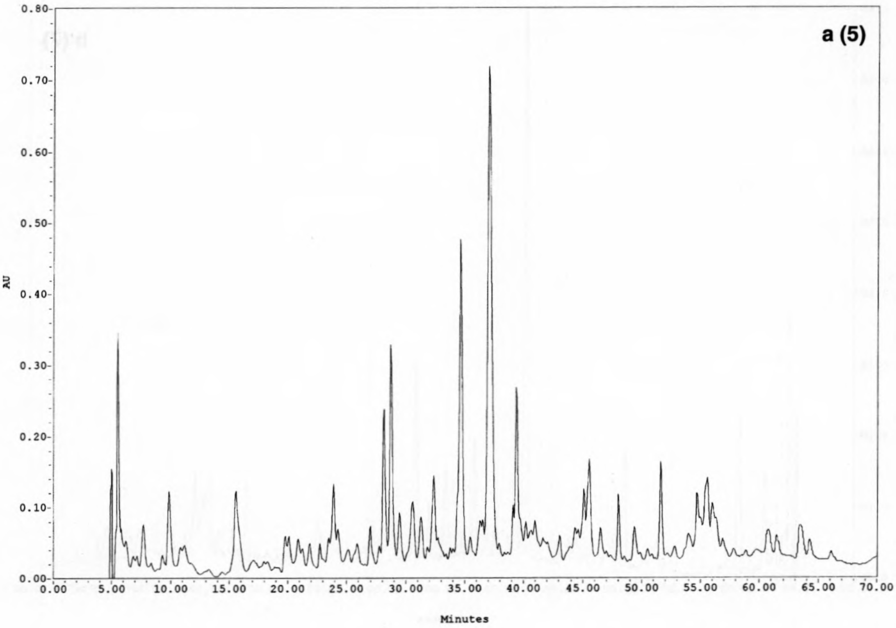

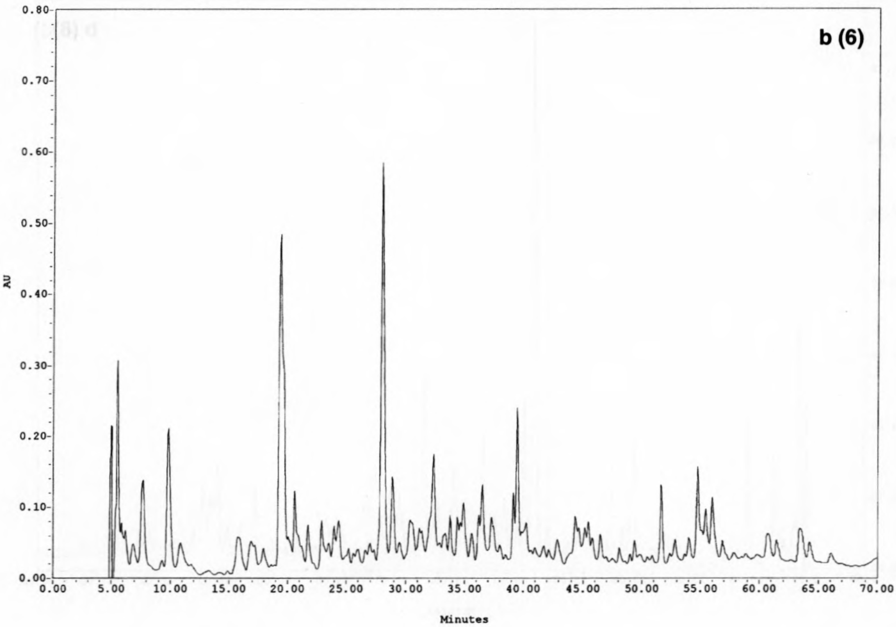

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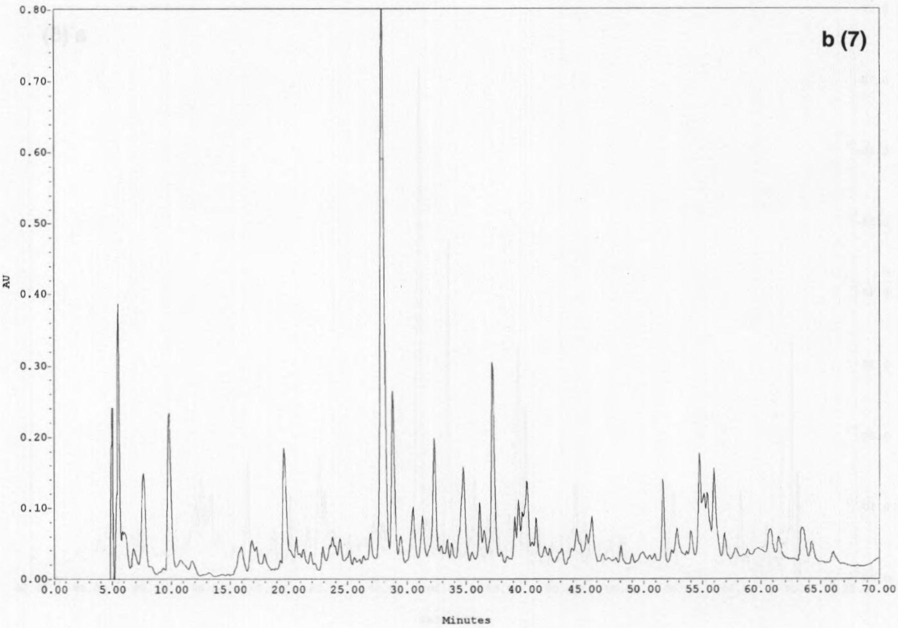

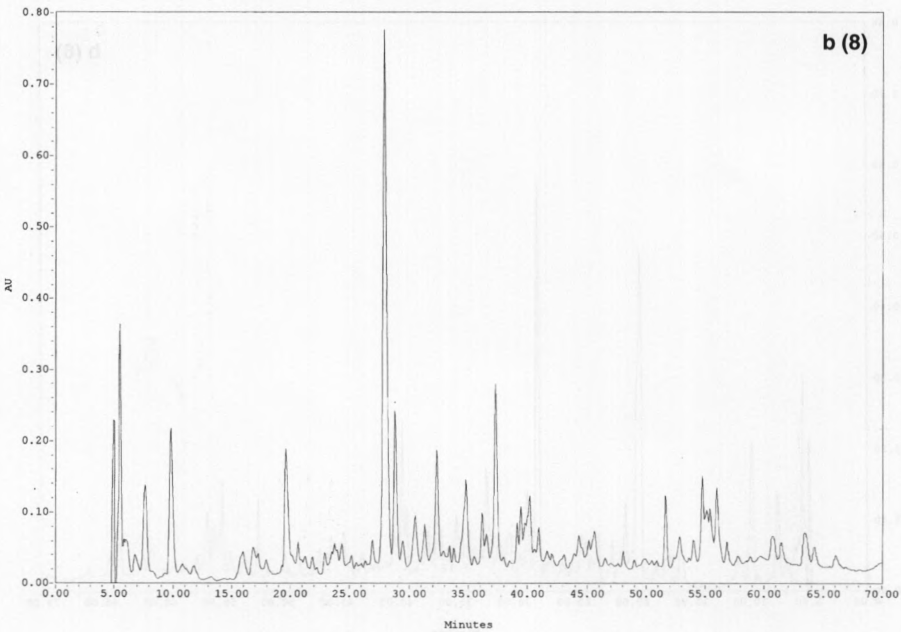

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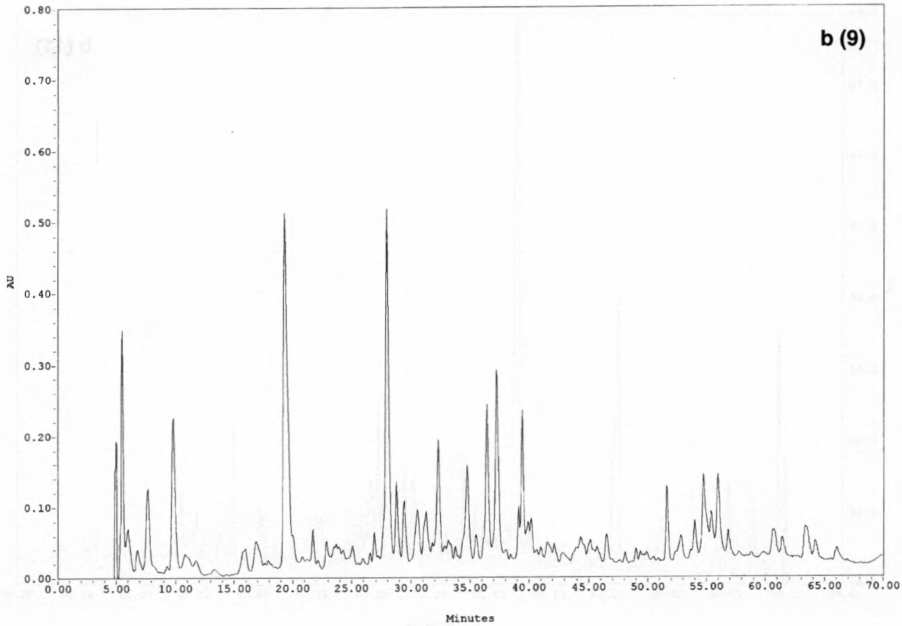

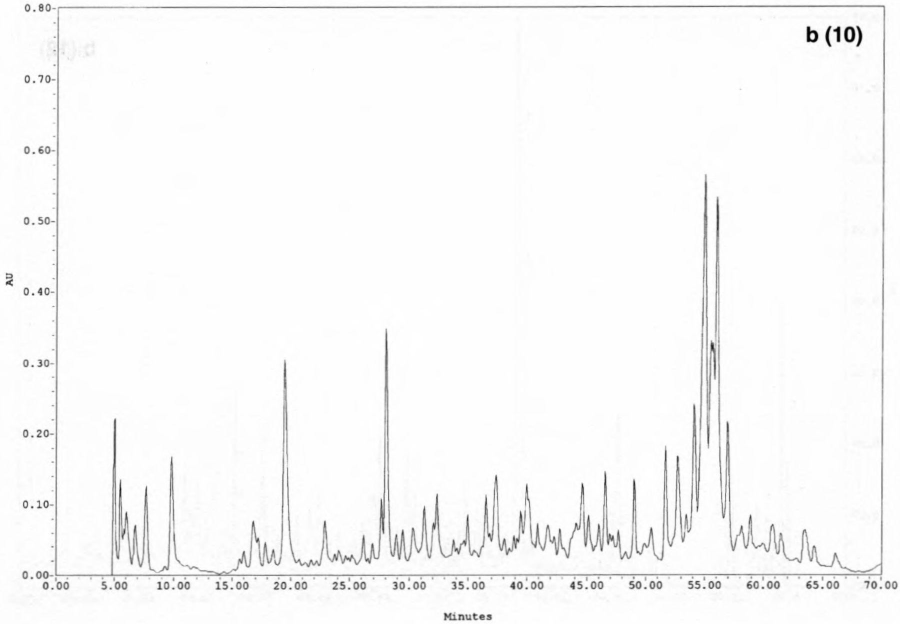

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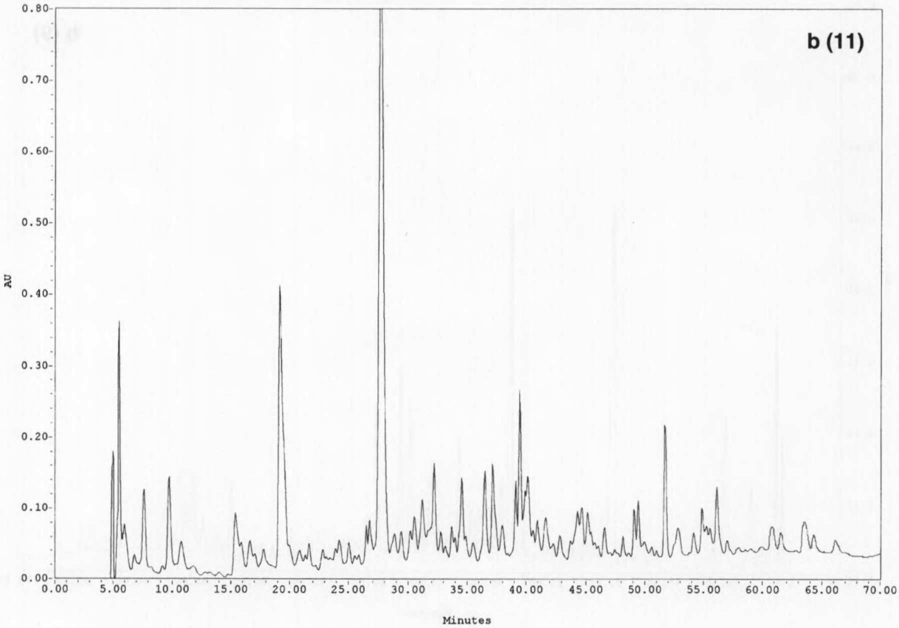

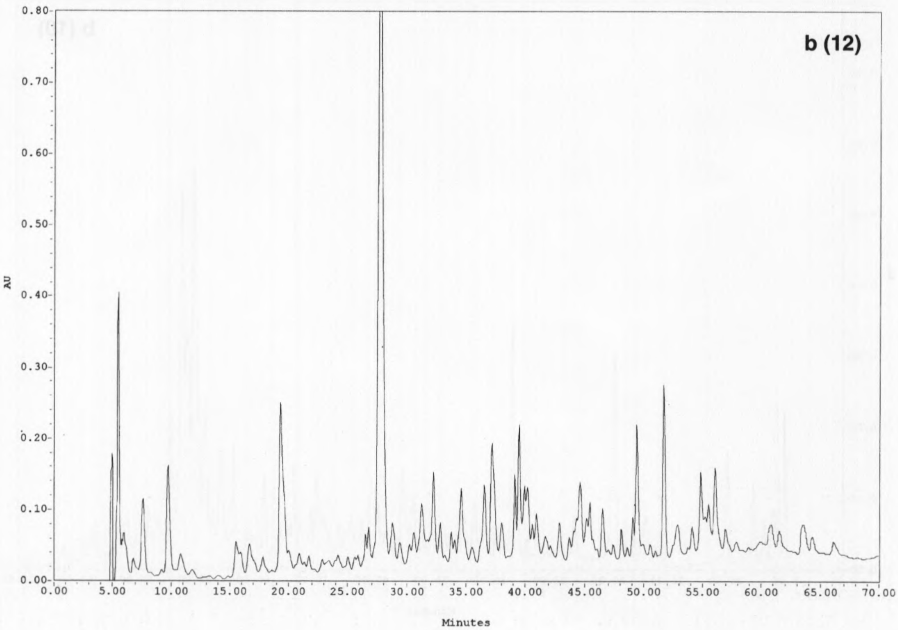

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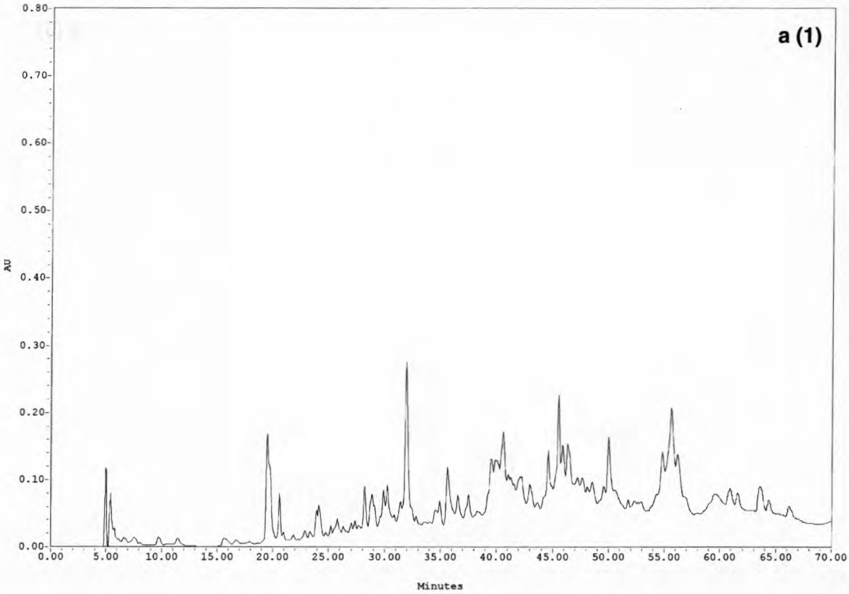

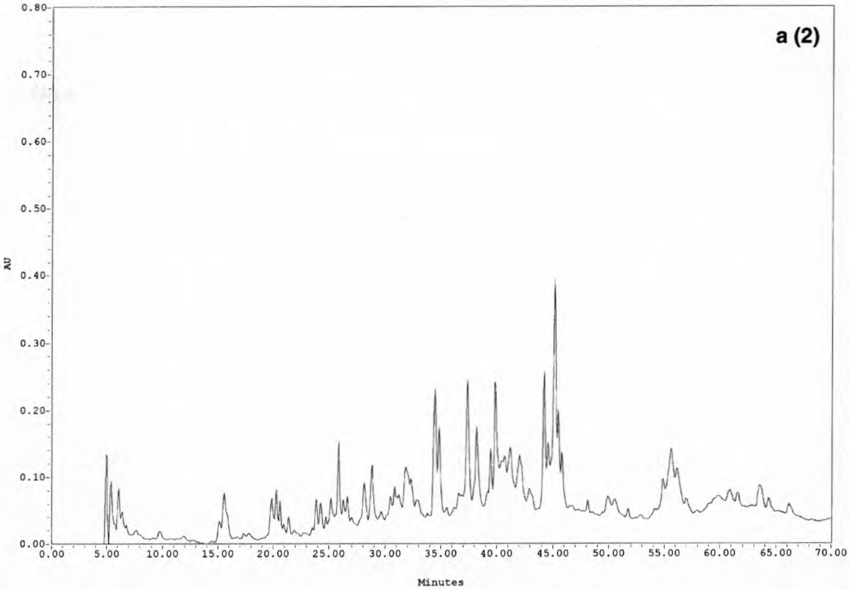

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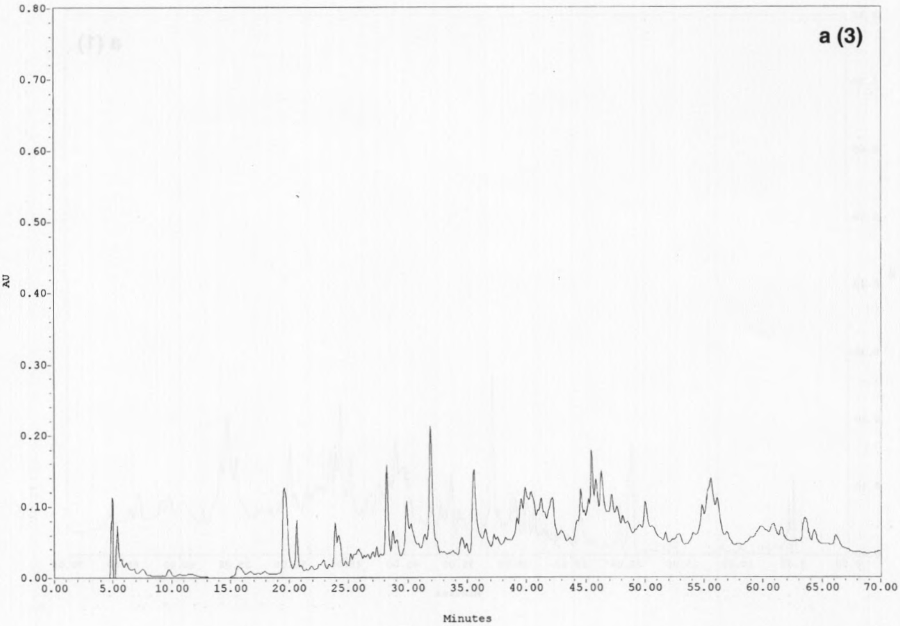
Figure 4. Chromatogramme RP-HPLC de la fraction insoluble dans l'éthanol issu de la fraction hydrosoluble des fromages miniatures affinés 4 mois fabriqués avec des souches pures de *Lactococcus. a* : 1–6 : fromages fabriqués avec les souches 303, SK11, 317, 223, AM1 ou 267 respectivement ; *b* : 7–12 : fromages fabriqués avec les souches 255, 227, 250, Wg2, 223 ou HP, respectivement.



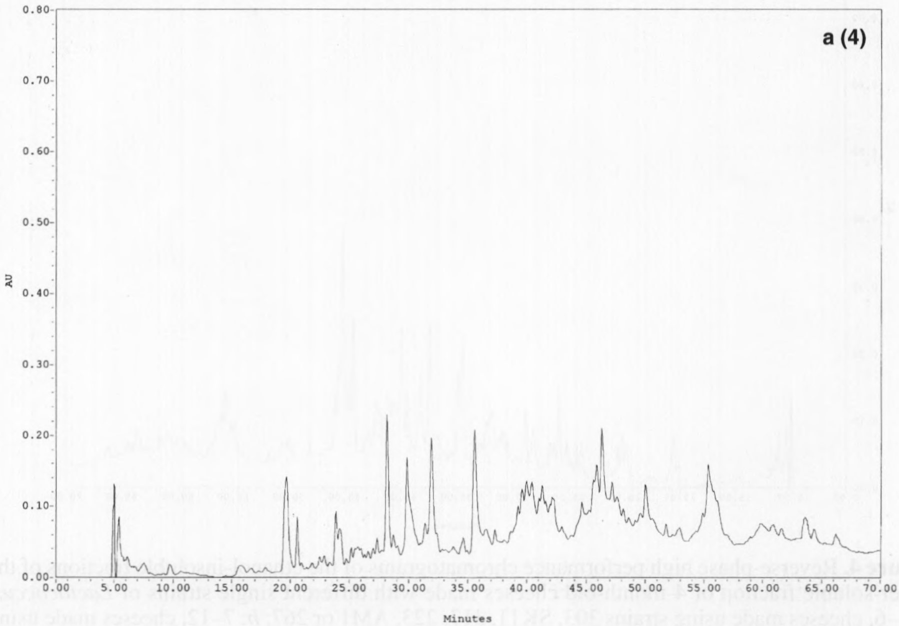
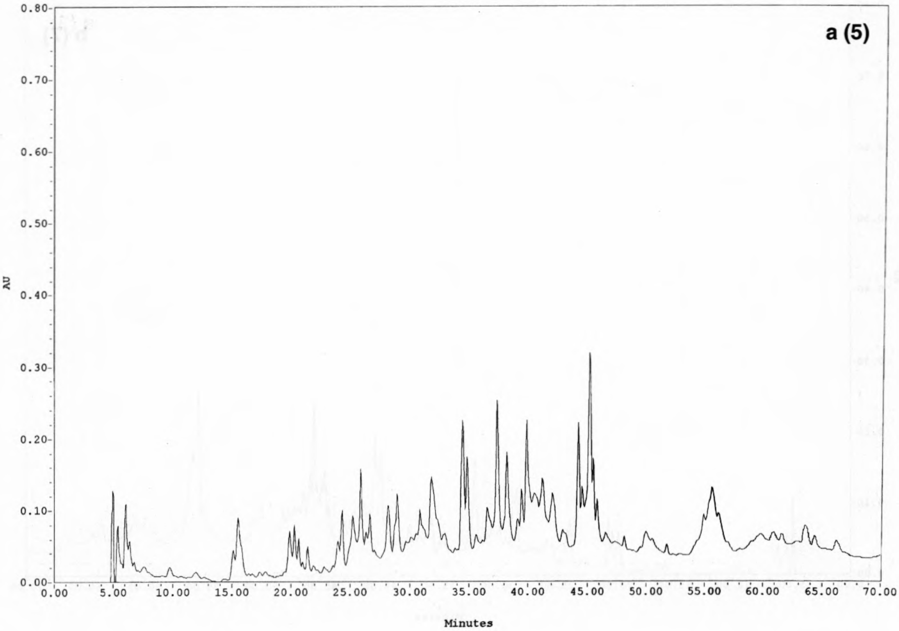
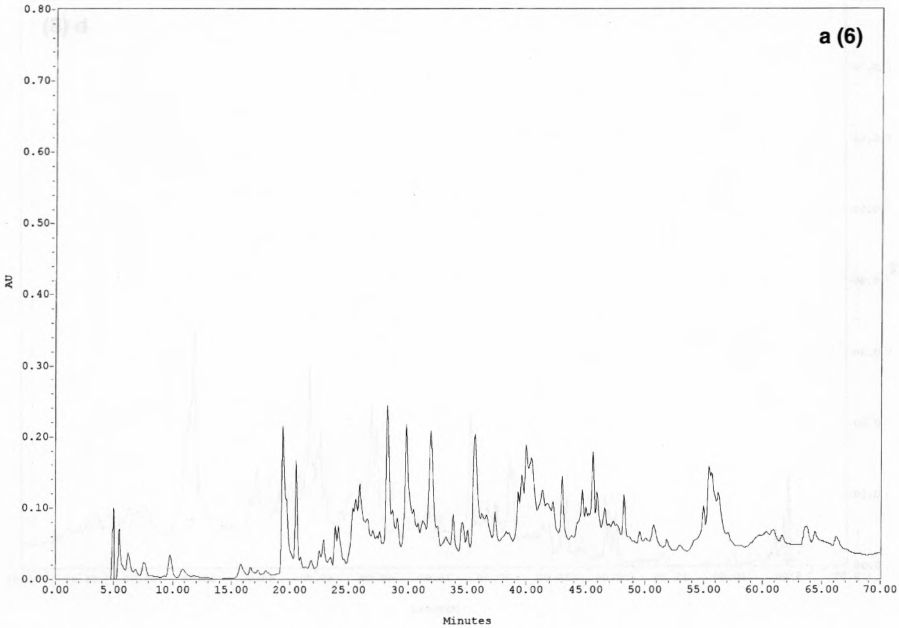


Figure 4. Continued.
Figure 4. Suite





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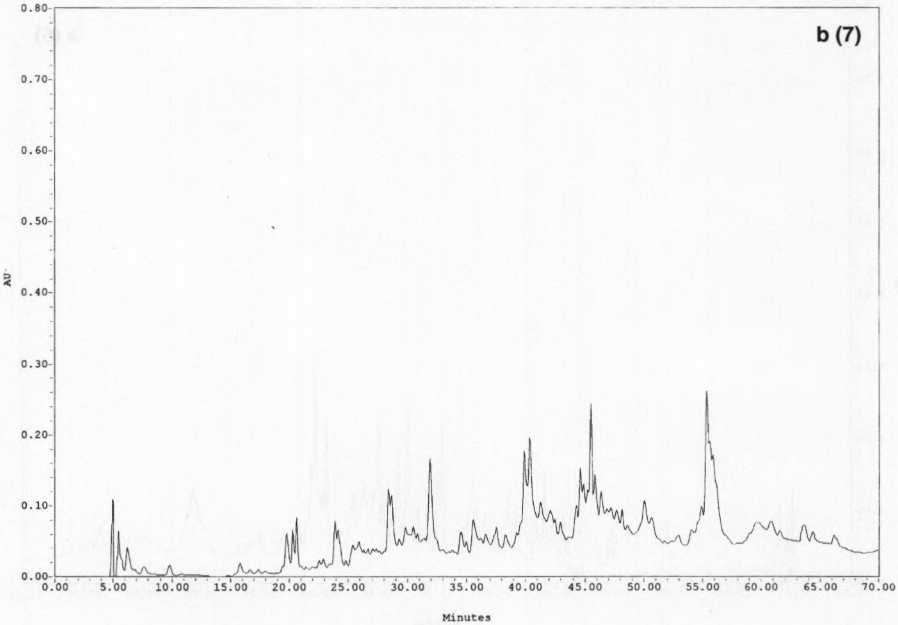

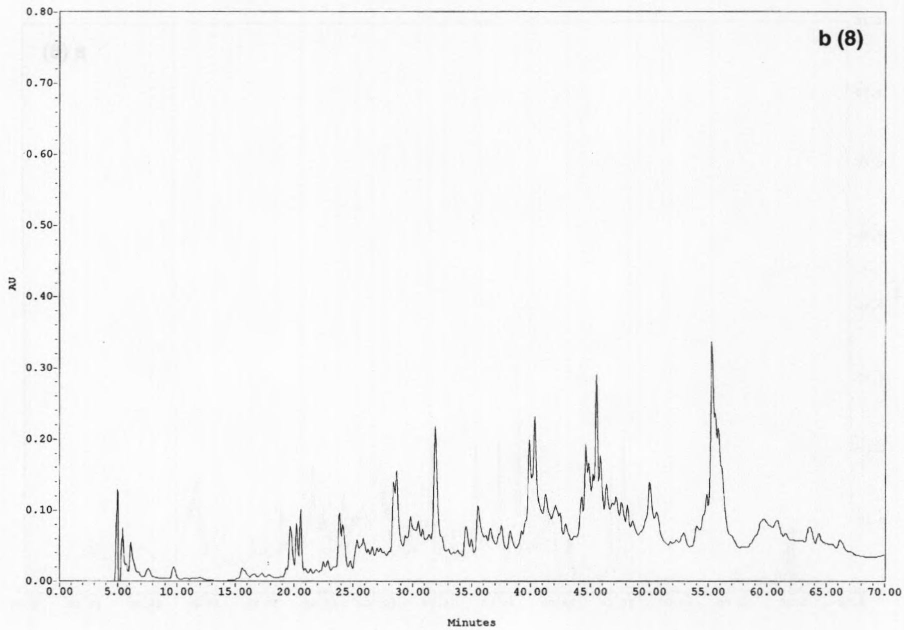

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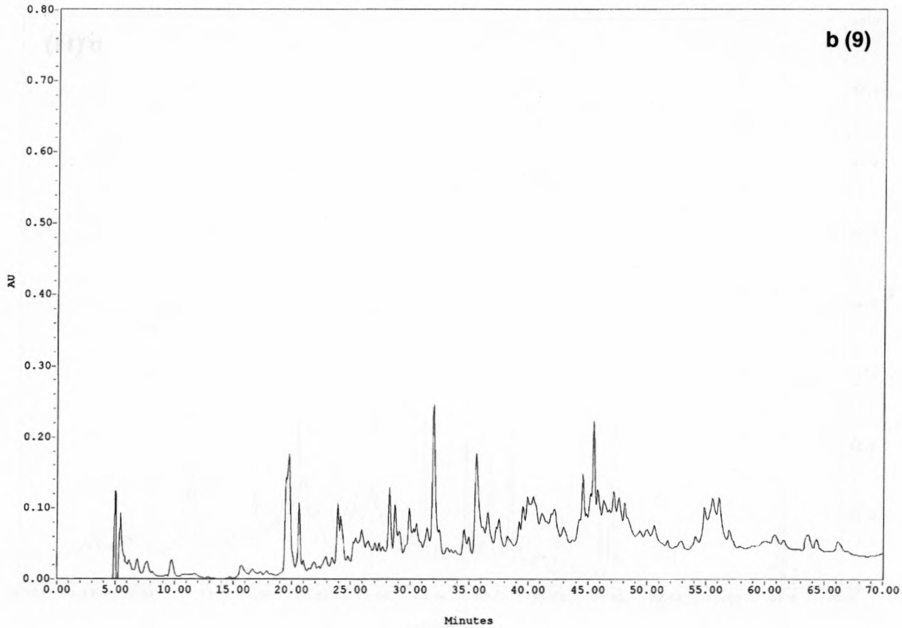

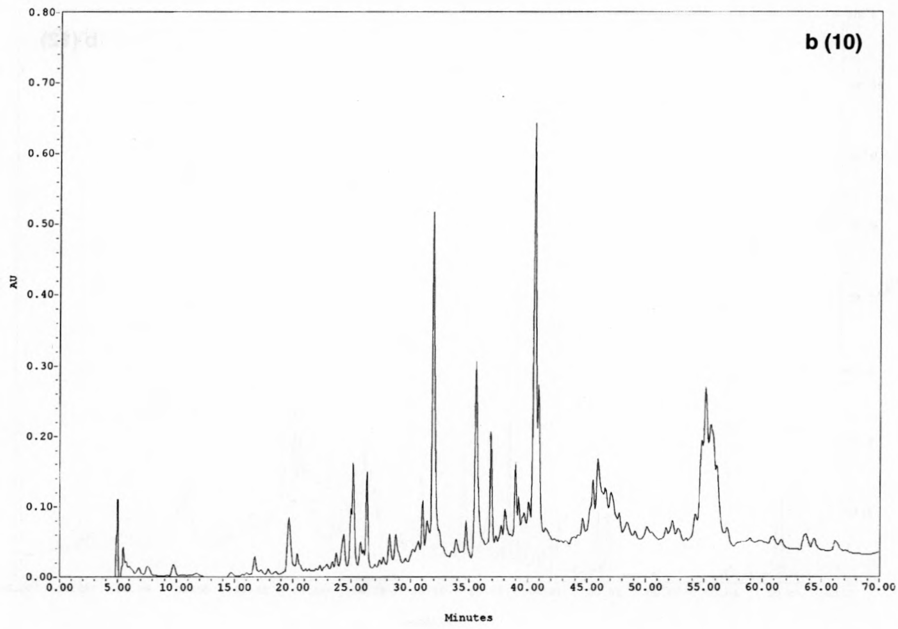

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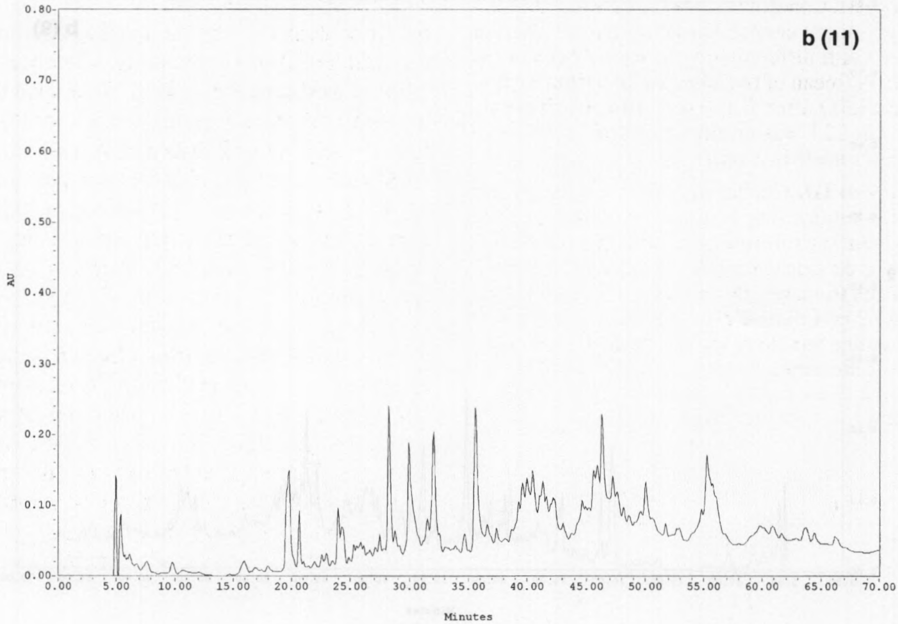

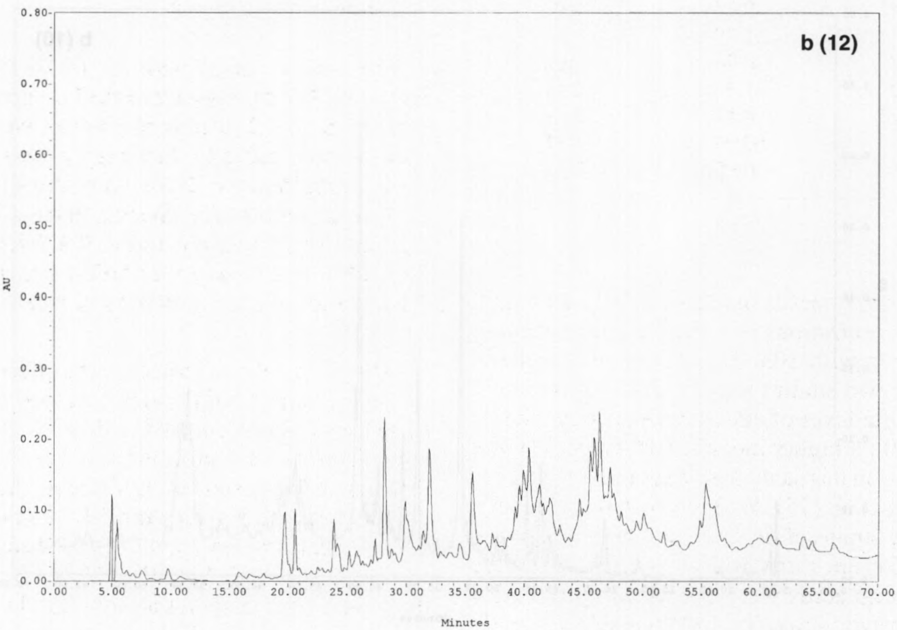

Figure 4. Continued.
Figure 4. Suite

Table III. Concentration of total free amino acids (mg Leu·g^{-1} cheese) in 12 miniature cheeses made with different single strain *Lactococcus* starters (mean of two cheeses, one from each of two trials) after 2 and 4 months of ripening. (Strain 223 was common to both batches of cheeses made in a set.)

Tableau III. Concentration en acides aminés libres totaux (mg Leu·g^{-1} de fromage) dans 12 fromages miniatures fabriqués avec des levains de souche pure de *Lactococcus* (moyenne de deux fromages, un de chacun des deux essais) après 2 et 4 mois d'affinage (la souche 223 est commune aux deux lots de fromages fabriqués dans cette série).

Strain	Age (months)	
	2	4
	mg Leu·g^{-1} cheese	
303	0.93	1.70
SK11	0.98	2.19
317	1.05	1.74
223	1.03	2.11
AM1	1.65	2.09
267	0.95	1.97
255	1.83	3.86
227	0.96	2.08
250	1.47	2.44
Wg2	4.16	6.61
223	0.94	1.81
HP	0.90	2.35

study, cheeses made with HP had higher concentrations of amino acids than those made with 303. The differences between the two studies may be due to differences in the level of starter added to the cheese milk; a higher inoculum of strain 303 was used in this study than was used by O'Donovan et al. [25]. Wilkinson et al. [35] found that strains of *Lc. lactis* ssp. *cremoris* lyse at different rates and higher levels of free amino acids were produced by the most autolytic strain (i.e., AM2) and lower levels by the least autolytic strain (i.e., HP).

The cheeses made with *Lc. lactis* ssp. *cremoris* Wg2 contained the highest levels of nearly all amino acids measured (*table IV*). The cheeses made with SK11 or 267 as starter contained the highest and lowest concentration of Asp, respectively. The cheeses made with strains HP or Wg2 developed the highest and lowest concentration of Arg, respectively. The cheeses made with strain AM1 developed the highest concentration of Ile. O'Donovan et al. [25] noted that Glu, Leu, Lys were the principal amino acids in cheeses made with HP, AM2 or 303. According to Wilkinson et al. [35], Glu, Leu, Arg, Lys, Phe, Ser are the principal amino acids in Cheddar cheese, while Engels et al. [5] reported that Glu, Leu, Phe, Val, Pro and Lys were the principal amino acids in Cheddar, Gouda, Edam and Gruyere. The concentrations of the free amino acids found in this study were analysed by MVCA by Pripp et al. [27], who confirmed that cheeses made with Wg2 were different to all others.

3.3. Sensory analysis

The cheeses made with *Lc. lactis* ssp. *lactis* UC 317 were awarded the best score for flavour after 2 months of ripening, while cheeses made using *Lc. lactis* ssp. *cremoris* strains 250, Wg2 or HP received the lowest flavour scores. The cheeses made with *Lc. lactis* ssp. *cremoris* strains 303, SK11, 223, 255 or 227 were awarded similar flavour scores after 2 months of ripening (*table V*).

After 4 months of ripening, the cheeses made with strain SK11 were awarded the best flavour score, followed by 303. SK11 is considered to be a non-bitter strain [21]. Roberts and Wijesundera [28] found that a cheese slurry made with strain SK11 developed a clean, cheesy flavour. The cheeses made with strain Wg2, AM1 or 255 received the lowest flavour scores after 4-months of ripening. The body of cheeses made with strain Wg2 were very poor after 4 months. Cheese made with strains UC 317, UC 223, UC227 or 250 received similar flavour scores (*table IV*).

Table IV. Sensory scores awarded by two graders on a 0–10 point scale (0 = minimum score, 10 = maximum score) to 12 miniature cheeses made with different single strain *Lactococcus* starters after 2 and 4 months of ripening. (Strain 223 was common in both batches of cheeses made in a set.)

Tableau IV. Note sensorielle décernée par un jury de deux personnes dans une gamme de note de 0–10 (0 = note minimale, 10 = note maximale) pour 12 fromages miniatures fabriqués avec différents levains de souche pure de *Lactococcus* après 2 et 4 mois d'affinage (la souche 223 est commune aux deux lots de fromages fabriqués dans cette série).

Strain	Age (months)			
	2		4	
	Flavour	Body	Flavour	Body
303	7	7	8	7
SK11	7	6	9	6
317	8	7	7	7
223	7	7	9	7
AM1	7	7	5	5
267	6	7	6	5
255	7	7	5	5
227	7	6	7	7
250	5	5	7	7
Wg2	5	5	5	1
223	7	7	7	7
HP	5	5	7	7

4. CONCLUSION

The miniature Cheddar-type cheese model system developed by Shakeel-Ur-Rehman et al. [29] was found to be very useful for screening *Lactococcus* strains for cheesemaking. The miniature cheeses closely resemble Cheddar cheese [29]; therefore, it was possible to identify the proteolytic characteristics of different individual strains of lactococci in these miniature cheeses. The miniature cheeses represent a better model system than milk as proteolysis in milk does not accurately reflect the actual proteolytic pathways in cheese. The miniature cheeses have been compared to pilot scale Cheddar cheese [29] but not to commercial Cheddar cheese. The strains of *Lactococcus* influenced cheese ripening by producing a variety of small peptides, different total concentrations of amino acids and different individual free amino acid profiles. Phe, Leu, Lys, Glu, Pro, Tyr, Val, Ser and Ile were the principal amino acids in cheeses produced by most strains. The cheeses made with different strains showed marked differences in flavour. Strain Wg2 was found to differ considerably from the other strains studied and had a negative influence on cheese quality. Pripp et al. [27] analysed cheeses of this study by performing a detailed MVCA of the peak areas of the peptides of the RP-HPLC chromatograms of ethanol-soluble and -insoluble fractions of WSF and classified the single strains of *Lactococcus* into three groups: I, Wg2; II, AM1 and SK11; and III, 223, 227, 250, 255, 267, 303, UC317 and HP.

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Table V. Concentrations of individual amino acids (µmol·g^{-1} cheese) in 12 miniature cheeses made with different single strain *Lactococcus* starters (mean of two cheeses, one from each of two trials) after 4 months of ripening. (Strain 223 was common to both batches of cheeses made in set.)

Tableau V. Concentration de chaque acide aminé (µmol·g^{-1} fromage) dans 12 fromages miniatures fabriqués avec des levains de souche *Lactococcus* (moyenne de deux fromages, un de chacun des deux essais) après 4 mois d'affinage (la souche 223 est commune aux deux lots de fromages fabriqués dans cette série).

Strain	Asp	Ser	Glu	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Met	Lys	Ile	Leu	Phe
303	1.10	7.99	3.50	1.69	4.22	1.45	2.37	3.90	19.60	5.35	7.43	2.98	19.68	2.96	18.54	15.39
SkII	3.67	2.23	7.12	2.37	2.93	4.53	1.51	1.90	2.55	2.55	4.39	1.77	12.38	3.71	12.50	13.04
317	1.22	8.10	7.44	1.05	3.31	1.90	1.80	2.40	12.82	4.15	6.83	2.43	20.76	5.35	13.15	10.56
223	1.23	7.41	9.74	1.07	5.73	9.53.	4.62	2.55	7.90	8.25	12.09	3.39	28.38	2.33	17.25	26.05
AMI	2.36	10.08	12.85	1.98	4.31	8.43	2.29	3.10	3.45	3.75	7.92	4.34	21.4	12.69	13.88	15.90
267	0.97	11.47	8.45	2.50	6.34	5.35	2.29	2.90	6.30	10.03	12.31	4.88	23.45	6.06	21.30	13.73
255	1.55	21.32	16.83	3.26	7.01	7.83	4.76	4.40	5.30	8.90	13.63	5.09	16.56	3.91	28.56	25.23
227	0.82	8.75	16.49	2.52	5.08	4.31	3.65	2.95	6.30	8.15	11.51	4.06	30.76	3.94	18.39	21.91
250	1.31	11.15	9.35	3.12	7.59	4.61	4.30	4.30	10.40	5.00	11.67	3.47	24.20	4.16	20.95	32.17
Wg2	1.78	15.91	20.60	5.65	13.82	1.80	13.39	9.25	34.90	17.10	21.46	13.39	45.12	10.84	30.05	39.63
223	1.14	9.01	15.30	1.87	5.71	9.91	4.46	3.25	8.40	8.05	12.26	5.17	32.34	5.74	22.26	26.89
HP	1.63	8.08	13.5	2.54	5.37	10.50	4.63	2.85	7.05	11.30	11.03	4.33	19.77	3.96	17.68	23.07

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