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Protocol for the manufacture of miniature cheeses

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Abstract — A protocol has been developed for the manufacture of model miniature cheeses. Pasteurised milk (63 °C × 30 min) at 31 °C was placed in each of 6 wide-mouth centrifuge bottles (200 mL). The standard protocol for the manufacture of Cheddar cheese was then followed up to completion of cooking. When the pH of the curd reached 6.2, curds and whey in the bottles were centrifuged for 60 min at 1700 *g*. The supernatant whey was drained and the matted curd was held in the bottles in a water bath at 36 °C and turned every 15 min until pH of the curd reached 5.2–5.3. The acidified curd, inverted with respect to the first centrifugation, was recentrifuged at 1 700 *g* for 20 min. After whey drainage, the miniature cheeses (20–22 g) were immersed in brine (20 % NaCl and 0.05 % $CaCl_2$) for 30 min and then vacuum packed. Cheeses were analysed for moisture, salt and pH; statistical analysis showed no significant ($P > 0.05$) variation within a batch, between batches made on the same day or on different days. Miniature cheeses were compared to a control Cheddar cheese (made in a pilot plant from 20 L milk) after 2 or 4 months of ripening at 8 °C. The miniature cheeses were similar to the control in terms of gross composition, proteolysis and sensory scores. The model system developed in this study will be useful for screening the cheesemaking properties of starters, adjuncts or enzymes or for small-scale work involving expensive reagents. © Inra/Elsevier, Paris.

miniature cheeses / Cheddar cheese / model cheese system

Résumé — **Protocole de fabrication de mini-fromages**. Un protocole de fabrication de fromages modèles miniatures a été développé. Du lait pasteurisé (63 °C × 30 min) était réparti à 31 °C dans 6 flacons pour centrifugation à ouverture large (200 mL). Le protocole standard de fabrication du Cheddar était suivi jusqu'en fin de cuisson. Lorsque le pH du caillé atteignait 6,2, le caillé et le sérum contenus dans les flacons étaient centrifugés 60 min à 1 700 *g*. Le surnageant était éliminé et le caillé soudé était conservé dans les flacons dans un bain-marie à 36 °C et retourné toutes les 15 min jusqu'à ce que le pH du caillé atteigne 5,2–5,3. Le caillé acidifié était à nouveau centrifugé 20 min à 1700 *g* avec les flacons retournés par rapport à la première centrifugation. Après égouttage du sérum, les fromages miniatures (20–22 g) étaient immergés en saumure (20 % NaCl et 0,05 % $CaCl_2$) pendant 30 min puis emballés sous vide. L'extrait sec, la teneur en sel et le pH des fromages ont été déterminés ; l'analyse statistique n'a montré aucune variation significative ($P > 0,05$) au sein d'un lot de fabrication, entre lots réalisés le même jour ou à des jours différents. Les mini-fromages ont été comparés à un Cheddar témoin (réalisé en usine pilote à partir de 20 L de lait) après 2 et 4 mois

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d'affinage à 8 °C. Les mini-fromages étaient semblables au témoin quant à leur composition grossière, la protéolyse et l'analyse sensorielle. Le système modèle mis au point dans cette étude sera utile pour des criblages des propriétés et de l'aptitude fromagère de levains, bactéries auxiliaires, enzymes ou encore pour des travaux à petite échelle mettant en œuvre de réactifs onéreux. © Inra/Elsevier, Paris.

fromage miniature / Cheddar / système modèle

1. INTRODUCTION

Assessment of the performance of enzymes, starters or adjuncts during cheese ripening is expensive and time-consuming. Thus, a model system in which their role can be assessed rapidly appears desirable, provided that the conditions in cheese can be replicated closely. Many model systems have been developed, including cheese slurries, cheese pastes, cheese- like products or mixing of dairy ingredients to give a product with a chemical composition similar to that of cheese. Farkye et al. [1] blended unsalted curd, heat-shocked lactic acid bacteria, NaCl and sterile distilled water to give a slurry containing 57–70 % moisture, 18–23 % protein, 3.34 % NaCl in moisture and a pH of 4.85–5.32. Although the composition of the dry matter of this system was similar to cheese and it ripened quickly, it had a far higher moisture content than hard cheeses. Salles et al. [7] mixed milk fat, milk powder, NaCl, water, native calcium phosphocaseinate (prepared by microfiltration of skim milk) and glucono-δ-lactone to prepare a base with a chemical composition similar to that of cheese; the mixture was heated at 33 °C for 2.5 h, 0.15 mL rennet extract were added and the mixture homogenised at 33 °C for 1 h. The resulting cheese-like product contained 60–61.5 % moisture and 23.4–23.8 % protein. This model could be prepared easily, but its body, texture and water activity were different from normal cheese. Smit et al. [8] prepared a cheese paste by homogenising grated Gouda cheese (6 weeks old or a mixture of older and younger cheeses) and adding water to a final level of 50–55 %. The slurry was heated at 80 °C for 3 min and cooled to 30 °C to give a cheese paste with a solid consistency when cold. It was claimed that the product prepared by this method could be liquified by heating to 55 °C, without separation of fat, thus allowing incorporation of non-starter lactic bacteria or enzymes; the product regained its original structure on cooling. This system had a composition and texture similar to natural cheese, but had the disadvantage that the heat-treatment given to the curd would change its chemical and physical characteristics and inactivate enzymes from the starter, rennet and other sources. The consistency of this model cheese was different from real cheese. Youssef [9] made a cheese-like product (synthetic cheese model) by blending calcium paracaseinate – calcium phosphate complex (freeze-dried powder in which plasmin was inactivated by a high heat-treatment before freeze-drying), lactic acid and numerous buffers to simulate the aqueous phase of cheese; the enzyme system(s) to be studied was then added. This system had an aqueous phase similar to that of cheese, but its composition differed in several respects from that of real cheese, e.g., by addition of buffers and caseins. de Jong and de Groot-Mostert [3] found that proteolysis in simple model substrates, e.g., solutions of casein, paracasein or synthetic peptides or milk, does not reflect the actual progress of proteolytic processes in cheese. The texture of all of the above models was also different from real cheese and their ripening characteristics have not been compared to real cheese.

The objective of this study was to develop a model cheese system, with a gross composition, texture and consistency very similar to those of Cheddar cheese, but for which the time and cost of manufacture and ripening are greatly reduced.

2. MATERIALS AND METHODS

Preliminary trials were first conducted to develop a protocol for small-scale manufacture of cheese with a composition close to that of Cheddar cheese. The final protocol developed enabled the manufacture of 6 miniature Cheddar-type cheeses in one batch.

2.1. Protocol for the manufacture of miniature cheese

Raw milk was obtained from CMP Dairy, Cork, Ireland, and pasteurised in beakers at 63 °C for 30 min. Pasteurised milk was transferred to 6 wide-mouth plastic centrifuge bottles (200 mL), cooled to 31 °C and maintained at this temperature in a water bath. Starter (2 %, v/v, *Lactococcus lactis* subsp. *lactis* UC 223, obtained from the Microbiology Department, University College, Cork) was added to the milk which was ripened for 30 min. $CaCl_2$ (132 µL of a 1 mol·L^{-1} solution / 200 mL milk) was then added. Rennet (Maxiren-15, Gist-Brocades, Seclin, France) was added (43.5 µL /200 mL milk) and the milk held for 40–50 min until a firm coagulum formed. The coagulum in the bottles was cut manually by cutters made of wires stretched 1 cm apart across a frame. The cut curd particles in the whey were healed for 2 min and then stirred slowly for 10 min using glass rods. The temperature was then increased to 38 °C over 30 min and the curds /whey mixture stirred continuously at 38 °C until the pH reached 6.2. Curds and whey, in the centrifuge bottles, were then centrifuged at room temperature for 60 min at 1700 *g* in an MSE 'MAJOR' centrifuge (MSE Scientific Instruments, Crawley, Sussex, England). These centrifugation conditions were chosen to achieve a moisture level of 38–40 % in the miniature cheeses. The whey was drained and the curds held in the centrifuge bottles in a water bath at 36 °C. Cheeses were inverted in the bottles every 15 min until the pH decreased to 5.2–5.3 and were then recentrifuged at 1 700 *g* for 20 min, with the top of the cheeses during the first centrifugation now on the bottom (to give a smooth surface on both sides). After further whey drainage, the cheeses were brine salted (20 % NaCl, 0.05 % $CaCl_2.2H_2O$) for 30 min at room temperature in the centrifuge bottles. After salting, the cheeses were removed from the bottles, wiped with tissue paper, vacuum packed and ripened at 8 °C.

The above procedure required about 6 h to produce a batch of 6 cheeses; it was possible to manufacture 2 batches (i.e., 12 cheeses) in an 8 h day.

2.2. Experimental design

The experiment was performed in two parts. In the first part, 24 miniature cheeses were manufactured from same milk in four batches (I, II, III, IV) over two days and were analysed for moisture, salt and pH (see below). The data were analyzed statistically to establish the repeatability of the method.

In the second part of the experiment, 18 miniature cheeses in three batches (V, VI, VII) and a control Cheddar cheese (2 kg) were made from same milk pasteurised at 63 °C for 30 min. The control cheese (from 20 L milk) was made in the pilot plant of the Department of Food Technology, University College, Cork, Ireland, by a standard protocol [4]. The control cheese and 12 miniature cheeses (batches V and VI) were made on the same day and were ripened at 8 °C for 2 (batch V) or 4 (batch VI) months and compared with the control cheese of same age for composition and proteolysis (see below). The third batch (VII) of six miniature cheeses was made on the next day, ripened at 8 °C and graded (see below) after 2 and 4 months, together with the control Cheddar cheese.

2.3. Analysis of cheeses

Moisture, protein and salt were determined by standard methods, as described by Lynch et al. [6]. pH was determined by placing the electrodes of the pH meter in contact with the curd or grated cheese. Water-soluble extracts (WSE) were prepared by a modification of the method of Kuchroo and Fox [5]. Grated cheese (15 *g*) and distilled water (30 mL) were homogenised in plastic stomacher bags and the pH adjusted to 4.6 using 1 mol·L^{-1} HCl; the mixture was incu-

bated at 40 °C for 1 h and centrifuged at 3000 *g* for 30 min at 4 °C. Proteolysis was studied as described by Lynch et al. [6]. Water-soluble (WSF) and -insoluble fractions (WISF) were prepared and analysed by alkaline urea-polyacrylamide gel electrophoresis (PAGE). Total free amino acids were determined by the Cd-ninhydrin method [2]. Ethanol (70 %) – soluble and – insoluble sub-fractions of the WSE of 4 month-old cheeses were analysed by HPLC, as described by Lynch et al. [6].

The miniature cheeses in batch IV were graded informally for flavour and texture on a 0–10 scale by two graders from the Irish Department of Agriculture, Food and Forestry.

Data from the first part of the study were subjected to two-way-analysis of variance (ANOVA) using Stat Work stastistical software (Release 1.2; Cricket Software Inc, Philadelphia, USA) for mean squares and probability. The same software was used to determine means and standard errors for the data from second part of the experiment.

3. RESULTS AND DISCUSSION

The levels of moisture, salt and pH of the miniature cheeses were within the ranges 37.87–40.06 %, 1.44–1.50 % and 5.19–5.23, respectively (*table I*). Statistical analysis by two-way ANOVA showed no significant differences ($P > 0.05$) within a batch, between batches made on the same day or between batches made on different days (*table I*, batches I, II, III, IV), thus establishing the reliability of the method.

Table I. Composition (means of duplicates) of miniature cheeses (20 g) made in four batches from the same milk over two days.

Tableau I. Composition (moyennes de deux répétitions) des mini-fromage (20 g) réalisés avec le même lait au cours de quatre fabrications sur deux jours.

	Moisture (%) Batch				Salt (%) Batch				pH Batch			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
Cheese												
1	39.42	37.87	38.32	38.80	1.50	1.39	1.44	1.51	5.21	5.23	5.24	5.19
2	38.68	39.14	38.95	39.64	1.47	1.44	1.44	1.50	5.18	5.20	5.20	5.20
3	38.67	39.42	39.02	39.37	1.50	1.50	1.50	1.50	5.20	5.22	5.23	5.20
4	39.47	38.48	39.72	38.07	1.43	1.48	1.47	1.40	5.23	5.20	5.25	5.23
5	39.70	39.05	38.95	38.53	1.44	1.45	1.43	1.47	5.20	5.20	5.20	5.21
6	40.06	39.49	39.19	39.19	1.47	1.45	1.41	1.50	5.23	5.20	5.23	5.20

		Mean squares		
Source of variation	df[a]	Moisture	Salt	pH
Between days	1	0.0002	0.0001	0.0004
Between batches	1	0.1463	0.0008	0.0014
Interaction	1	0.4163	0.0068	0.0012
Total	47			
		Probability > F[b]		
Between days	1	0.985	0.869	0.342
Between batches	1	0.571	0.532	0.072
Interaction	1	0.341	0.072	0.089
Total	47			

[a] degrees of freedom, [b] F-ratio
[a] degré de liberté, [b] ratio F.

In the second part of the experiment, miniature cheeses were compared with a pilot-scale Cheddar cheese manufactured from 20 L of milk. The levels of salt, moisture, protein and pH in 6 miniature cheeses after 2 (batch V) and 4 (batch VI) months of ripening were close to those in the pilot-scale control Cheddar cheese of similar ages (*table II*). These results indicate that the miniature cheeses made on a laboratory scale were comparable to Cheddar cheese made on a pilot scale with respect to moisture, salt, protein, pH and proteolysis. Levels of water-soluble nitrogen (WSN), as % of total N, in the six miniature cheeses of batch V were similar to that for the control after 2 months of ripening (mean ± standard error = 15.74 % ± 0.1518). The WSN levels in the six 4 month miniature cheeses in batch VI varied from 17.01 to 19.66 % compared with 17.89 % ± 0.5518 for the pilot-scale Cheddar cheese (*table III*).

Urea-PAGE of WISF and WSF of six miniature cheeses (batch VI) after 4 months of ripening were similar to the control cheese of the same age (*figure 1*). HPLC chromatograms of ethanol-soluble and -insoluble sub-fractions of the WSF of the 4 month-old miniature cheeses (batch VI) were similar and comparable to the control cheese of the same age (*figures 2* and *3*). The total concentration of free amino acid in miniature cheeses after 2 (batch V) or 4 (batch VI) months of ripening were similar to the levels in control cheese of same age and had means of 5.31 mg leucine / g cheese ± 0.0396 for batch V and 7.17 mg leucine / g cheese ± 0.1395 for batch VI (*table III*).

The control cheese received higher scores for flavour than the miniature cheeses (batch VII) after 2 months of ripening, but the scores for body were similar for all cheeses; after 4 months, miniature cheeses received,

Table II. Comparison of composition (means of duplicates) and pH of two batches (V, VI) of miniature (M) cheeses made on the same day from the same milk. Batch V was compared with the control (20 L scale) after 2 months and Batch VI after 4 months of ripening.

Tableau II. Comparaison de la composition (moyennes de deux répétitions) et du pH de deux lots de fabrication (V et VI) de mini-fromages (M) réalisés avec le même lait le même jour. Le lot V est comparé au Cheddar témoin (réalisé à partir de 20 L de lait) après 2 mois d'affinage et le lot VI après 4 mois.

Cheese	Age (months)							
	2	4	2	4	2	4	2	4
	Batch							
	V	VI	V	VI	V	VI	V	VI
	Moisture (%)		Salt (%)		Protein (%)		pH	
1	39.30	40.19	1.72	1.61	23.43	24.56	5.23	5.26
2	39.31	39.17	1.73	1.62	23.60	24.31	5.22	5.30
3	39.70	38.50	1.70	1.64	23.58	24.51	5.12	5.28
4	39.68	40.55	1.70	1.67	23.25	24.56	5.11	5.27
5	39.68	38.15	1.76	1.68	23.25	24.56	5.13	5.36
6	39.65	39.05	1.74	1.67	23.58	24.56	5.13	5.28
Mean	39.55	39.26	1.72	1.64	23.44	24.50	5.15	5.29
Standard error	0.078	0.382	0.096	0.011	0.067	0.040	0.021	0.014
Control	38.04	38.34	1.79	1.76	23.15	24.56	5.11	5.18

Table III. Water-soluble nitrogen (WSN, expressed as % total N), and total free amino acids (AA, mg leucine/g cheese) in two batches (V, VI) of miniature cheeses and in a control Cheddar cheese (20 L scale) made on same day after 2 and 4 months of ripening.

Tableau III. Azote soluble dans l'eau (WSN, exprimée en pourcentage de l'azote total), et somme des acides aminés libres (AA, mg leucine/g cheese) dans deux lots de fabrication (V, VI) de mini-fromages et dans le Cheddar témoin (réalisé à partir de 20 L de lait) produit le même jour, après 2 et 4 mois d'affinage.

Cheese	Age (months)			
	2		4	
	V WSN	Batch VI AA	V WSN	Batch VI AA
1	16.42	5.120	19.55	6.740
2	15.39	5.340	17.01	7.230
3	15.85	5.361	16.83	7.681
4	15.67	5.338	19.66	6.848
5	15.67	5.382	16.80	7.232
6	15.45	5.363	17.49	7.323
Mean	15.74	5.31	17.89	7.17
Standard Error	0.1518	0.0396	0.5518	0.1395
Control	15.20	5.440	17.50	6.940

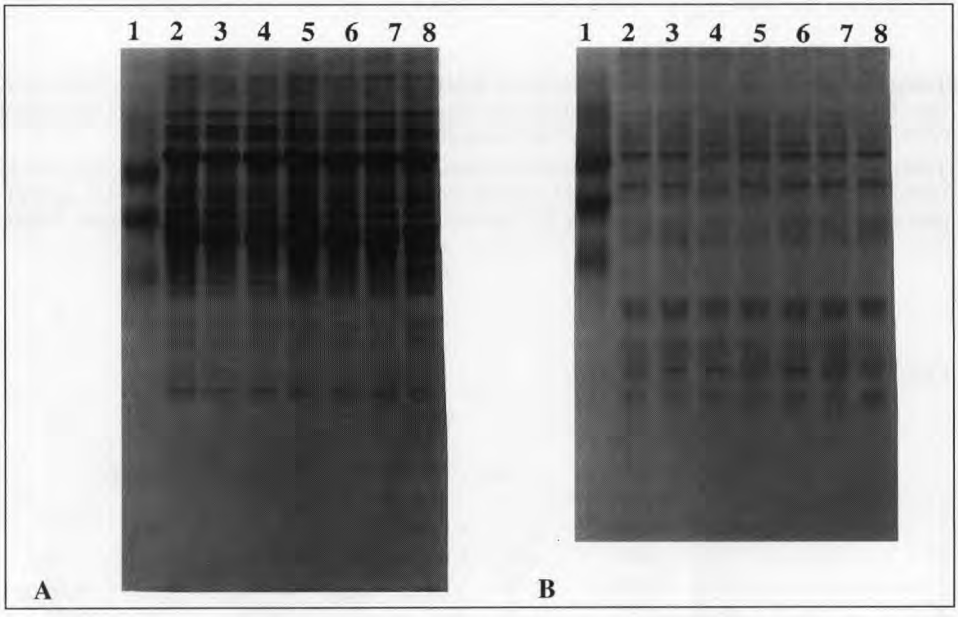


Figure 1. Urea-polyacrylamide gel electrophoretograms of water-insoluble (**A**) and -soluble (**B**) fractions of 4 month- old cheeses. Lane 1: sodium caseinate, lanes 2–7: 6 miniature cheeses made in a single batch, lane 8: control Cheddar cheese (2 kg).

Figure 1. Électrophorétogrammes obtenus en gel urée-polyacrylamide des fractions insolubles (**A**) et solubles (**B**) dans l'eau d'un fromage de 4 mois. Ligne 1 : caséinate de sodium, lignes 2 à 7 : les 6 mini-fromages réalisés au cours d'une même fabrication ; ligne 8 : Cheddar témoin (2 kg).

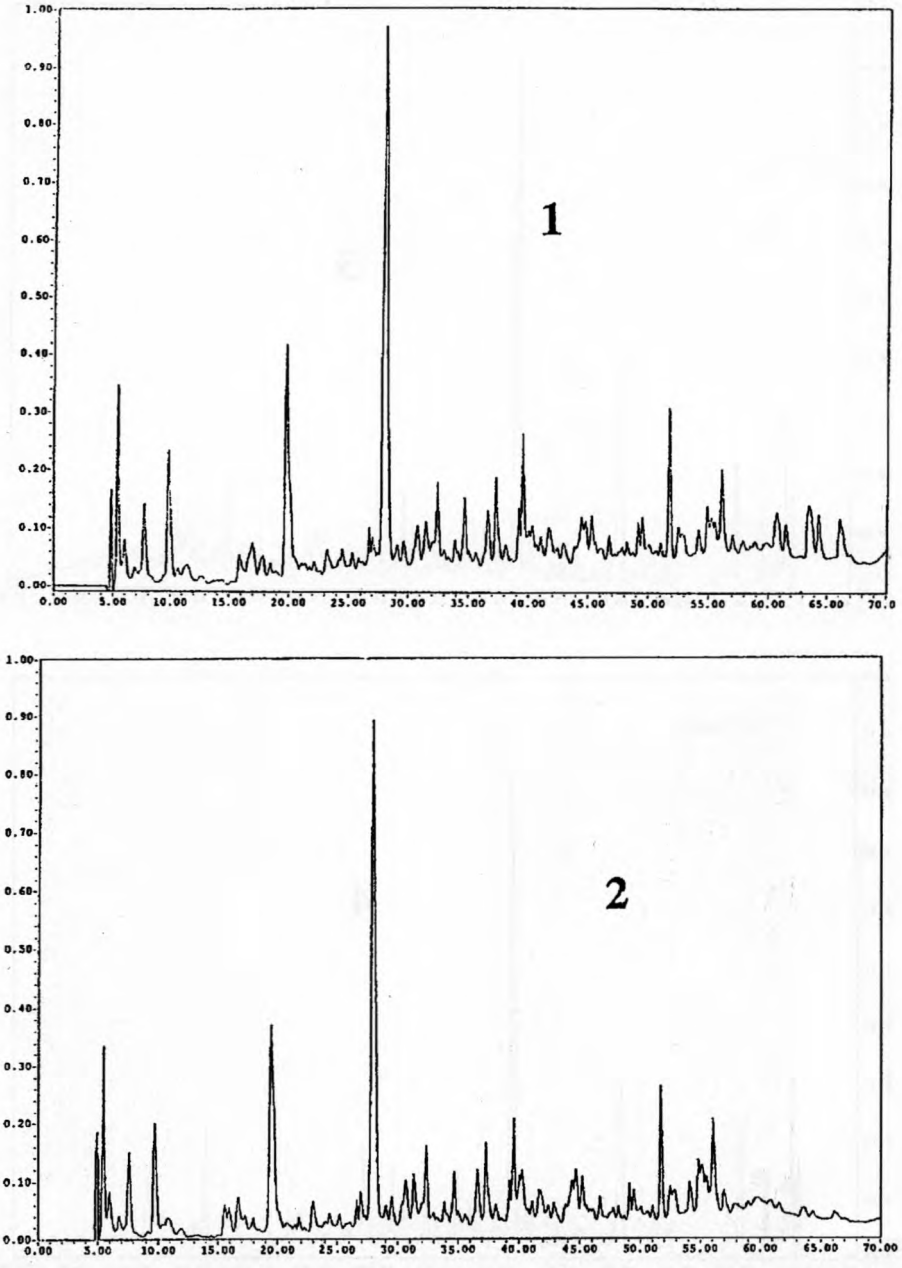
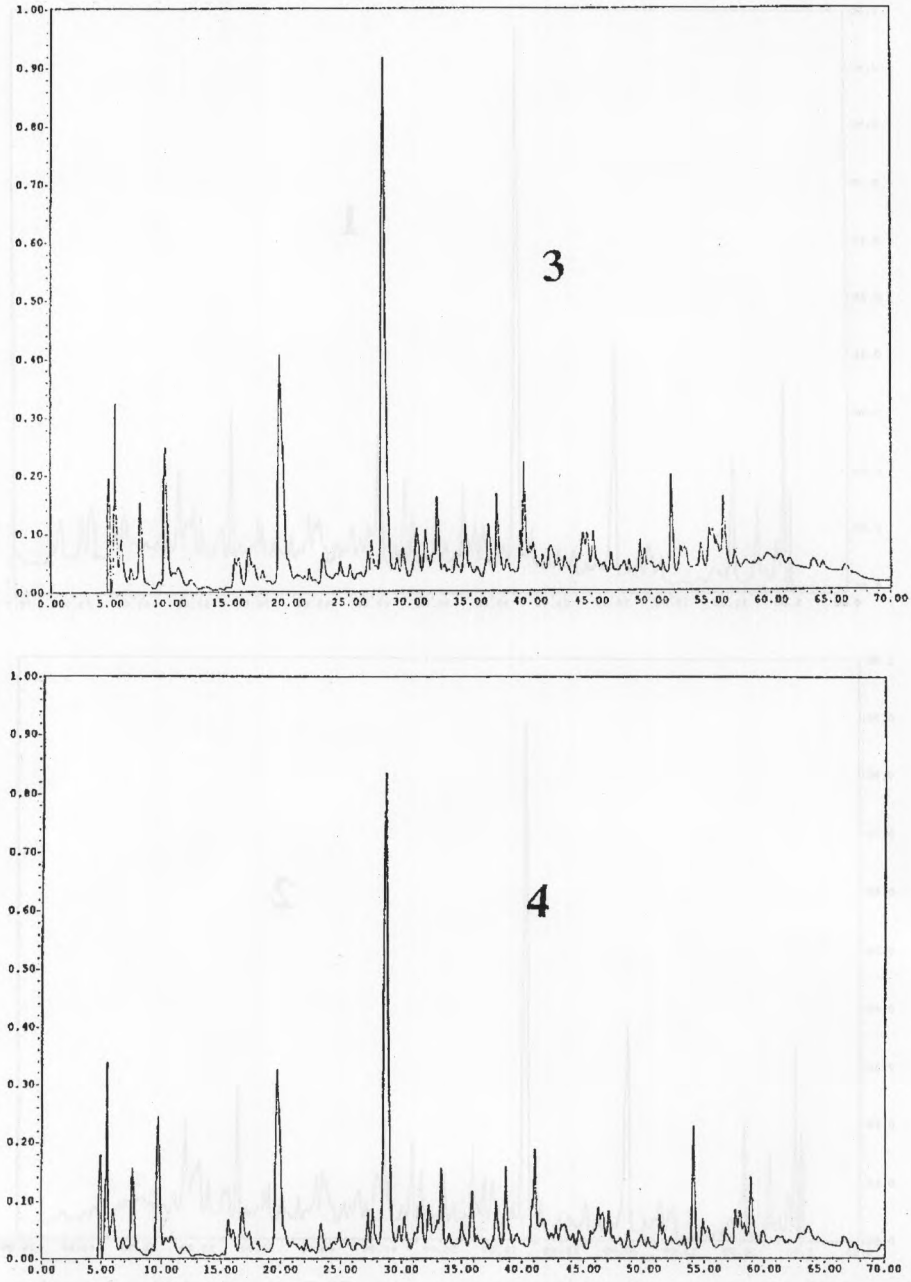


Figure 2. High performance liquid chromatograms of the 70 % ethanol-soluble sub-fractions of water-soluble fraction of six (1–6), 4 month-old miniature cheeses, made in a single batch and a control Cheddar cheese (2 kg) (7).

Figure 2. Chromatogrammes HPLC de la sous-fraction soluble dans l'éthanol à 70 % contenue dans la fraction soluble dans l'eau de six (1 à 6) mini-fromages de 4 mois réalisés au cours d'une même fabrication et du Cheddar témoin (2 kg) (7).



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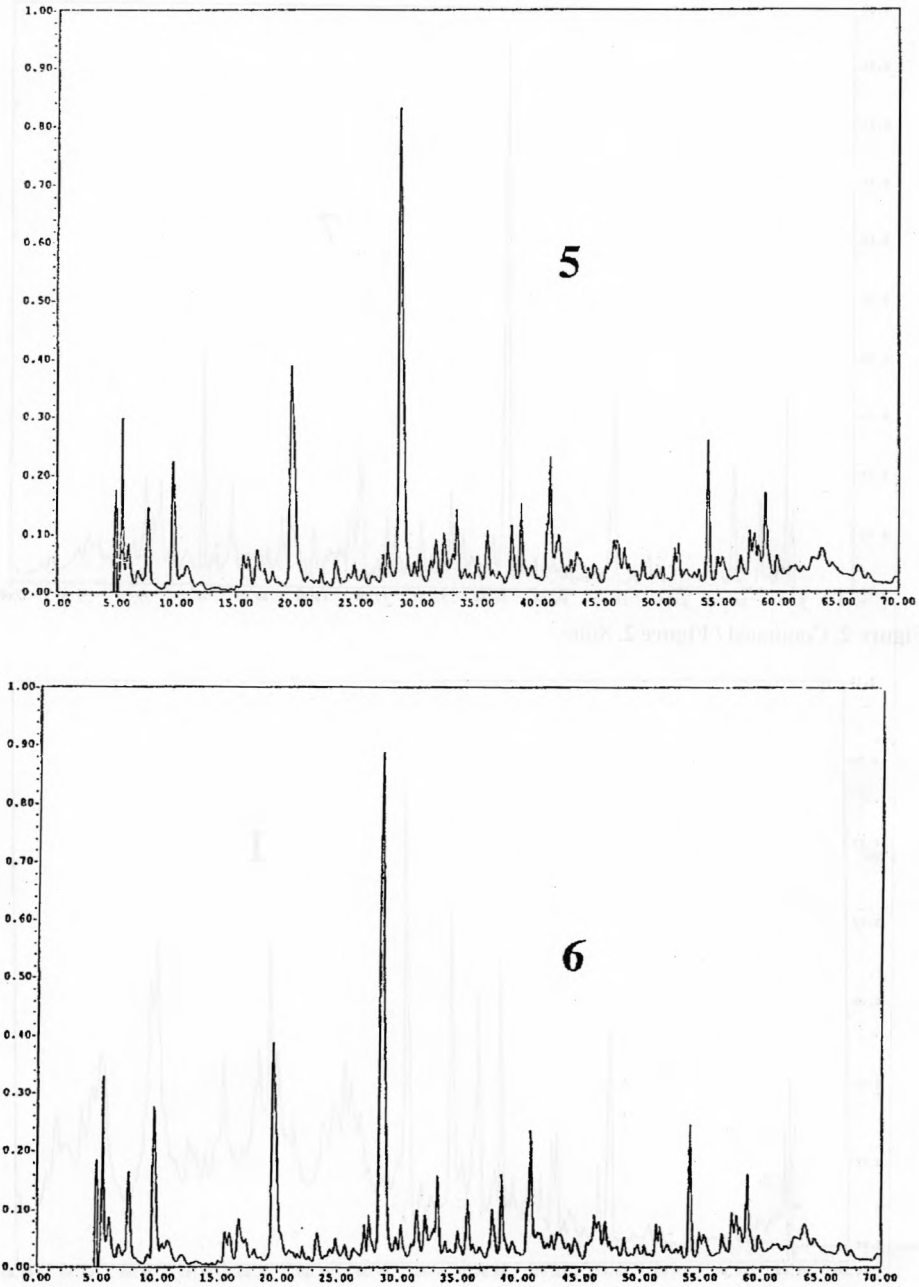

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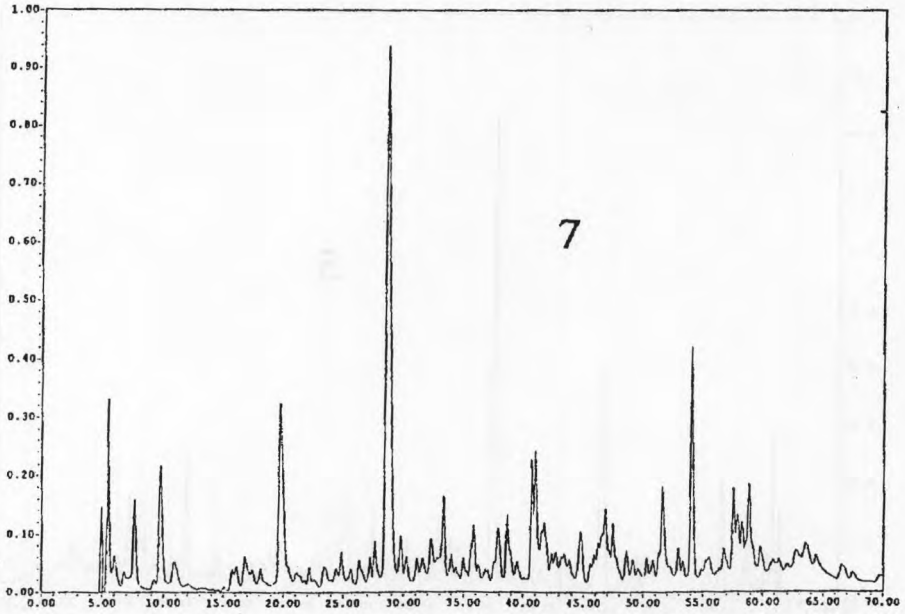

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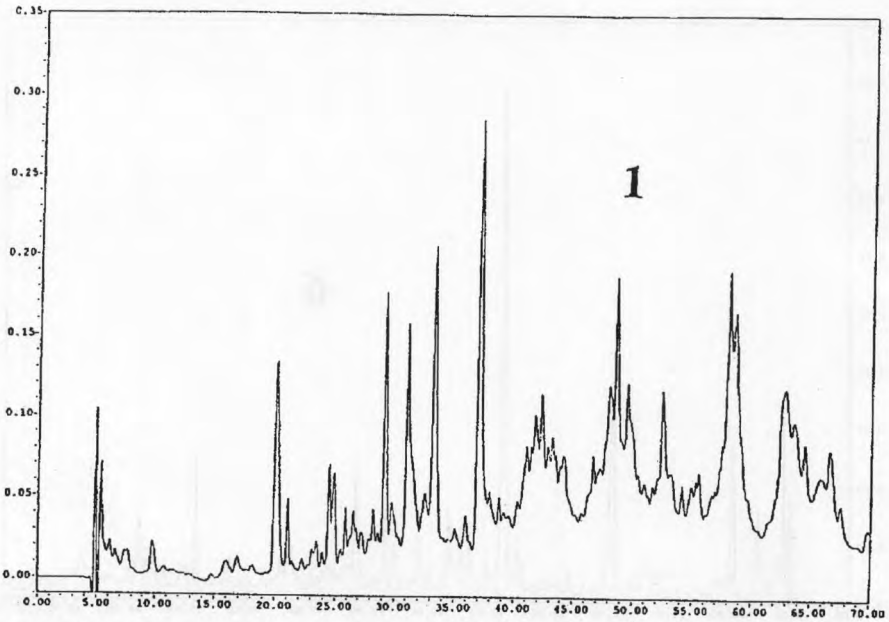
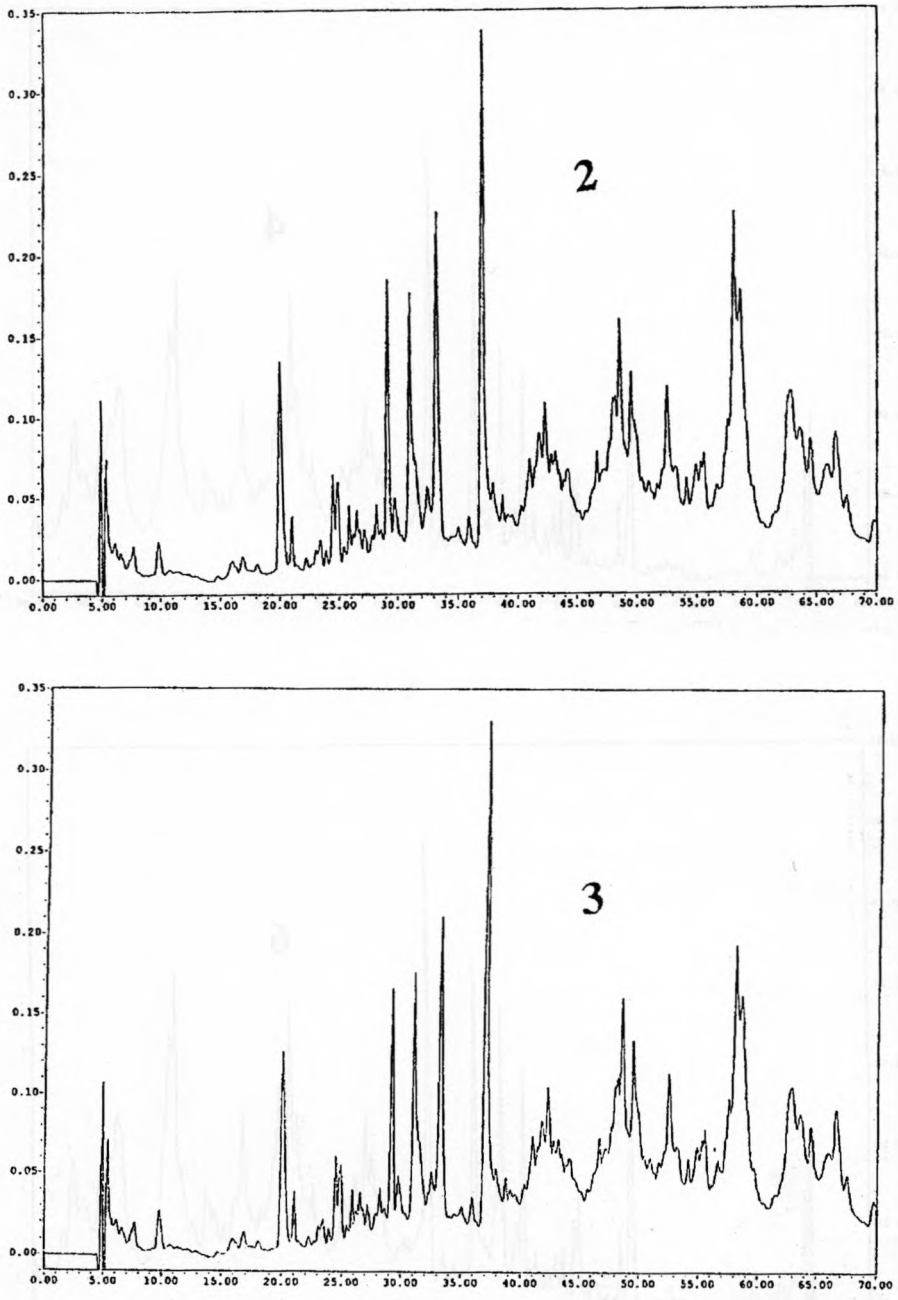


Figure 3. High performance liquid chromatograms of 70 % ethanol-insoluble sub-fractions of water-soluble fraction of six (1–6), 4 month-old miniature cheeses, made in a single batch and a control Cheddar cheese (2 kg) (7).

Figure 3. Chromatogrammes HPLC de la sous-fraction insoluble dans l'éthanol à 70 % contenue dans la fraction soluble dans l'eau de six (1 à 6) mini-fromages de 4 mois réalisés au cours d'une même fabrication et du Cheddar témoin (2 kg) (7).



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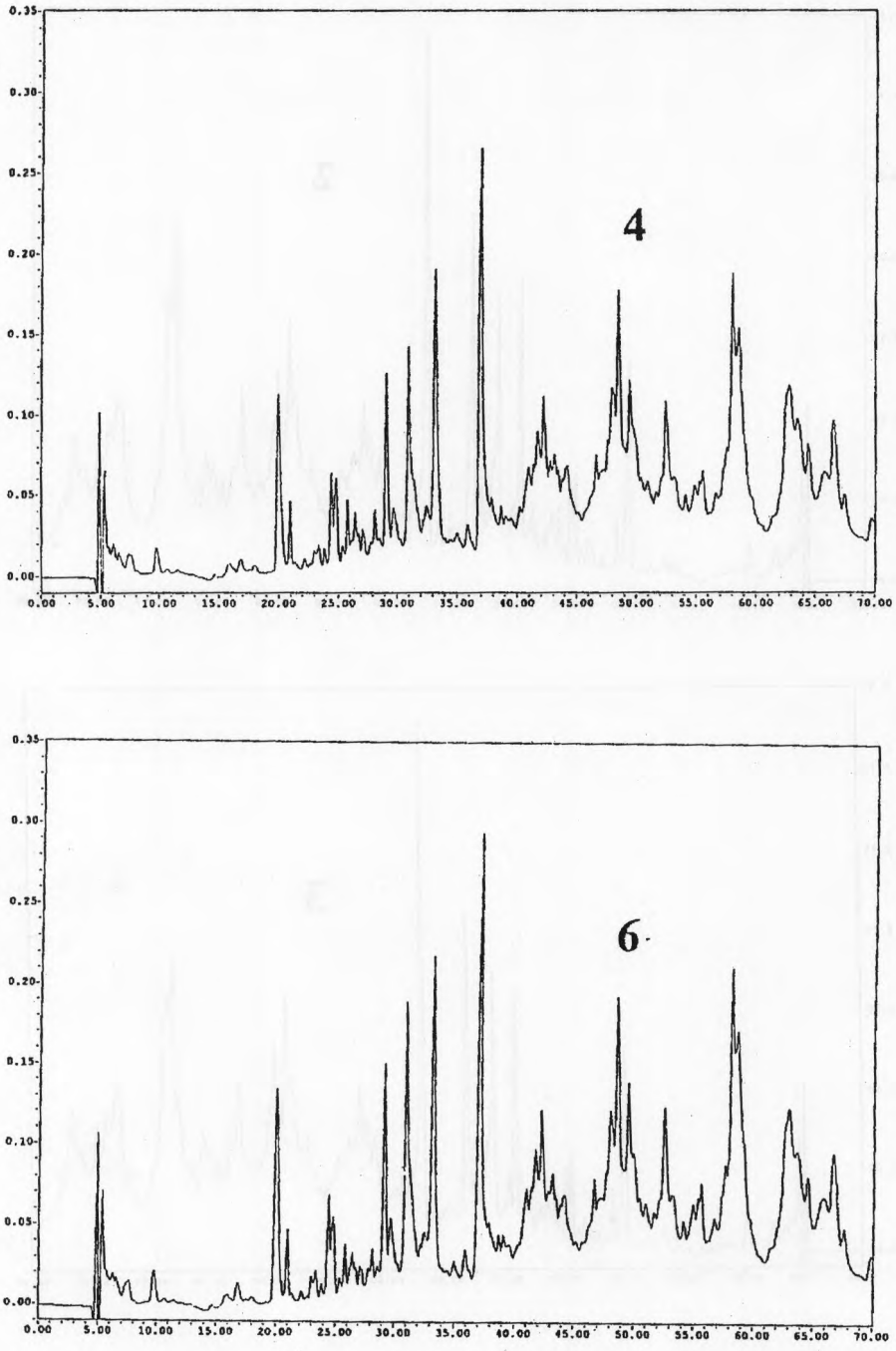

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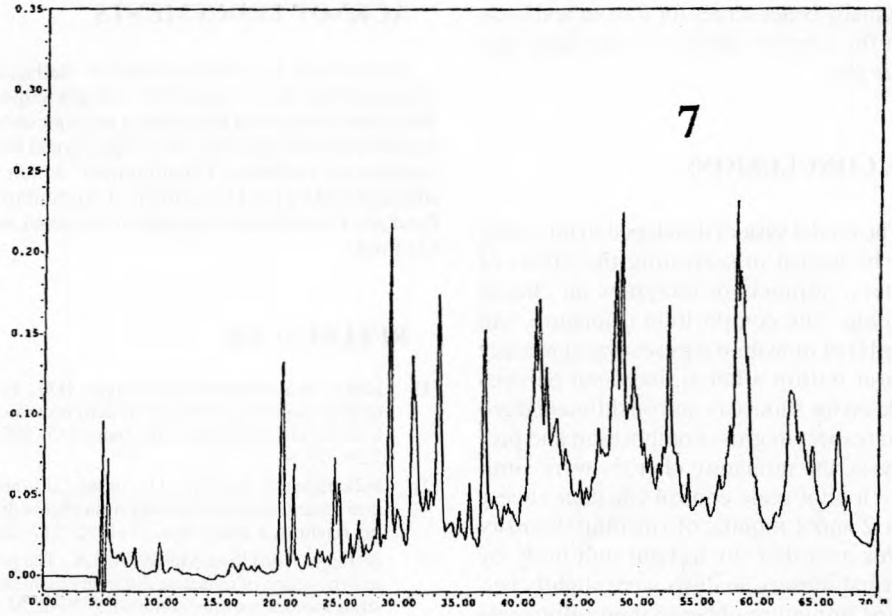

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Table IV. Sensory scores awarded by two graders from Irish Department of Agriculture, Food and Forestry to 6 miniature cheeses (Batch VII) and a control Cheddar (20 L scale) after 2 and 4 months of ripening.

Tableau IV. Cotations sensorielles attribuées par deux juges de l'Irish Department of Agriculture, Food and Forestry, à 6 mini-fromages (lot VII) et au Cheddar témoin (réalisé à partir de 20 L de lait) après 2 à 4 mois d'affinage.

Cheese	Age (months)			
	2		4	
	Flavour	Body	Flavour	Body
1	7	5	7	7
2	7	5	7	7
3	7	5	7	7
4	7	5	7	7
5	7	5	7	7
6	7	5	7	7
Control	9	5	6	6

marginally better scores for flavour and body than the control cheese of the same age (*table IV*).

4. CONCLUSIONS

The model system developed in this study will be useful in screening the effect of starters, adjuncts or enzymes on cheese ripening. The composition (moisture, salt and pH) of miniature cheeses was stastically similar within a batch, between batches made on the same day and on different days. With respect to gross composition and proteolysis, the miniature cheeses were similar to a pilot scale control Cheddar cheese after 2 and 4 months of ripening. Sensory scores awarded for flavour and body by informal sensory analysis were slightly better for miniature cheeses than pilot-scale control Cheddar cheeses after 4 months of ripening. There were some differences in gross composition within batches of miniature cheeses, and between miniature cheeses and pilot-scale control Cheddar, but these were slight, and comparable to variations obtained between Cheddar cheeses made on a pilot-scale.

The model cheese system developed in this study is rapid to prepare, cheap and much closer in terms of gross composition and proteolysis to cheese than any other model system proposed to date. It will be useful for small scale work involving expensive reagents or for screening large numbers of cultures. However, as with all models, one must eventually scale up to pilot-scale or commercial cheese manufacture to confirm results.

ACKNOWLEDGEMENTS

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