

Effects of commercial enzymes on proteolysis and ripening in Cheddar cheese

MG Wilkinson¹, TP Guinee¹, DM O'Callaghan¹, PF Fox²

¹ National Dairy Products Research Centre, Moorepark, Fermoy, Co Cork;

² University College, Department of Food Chemistry, Cork, Ireland

(Received 20 February 1992; accepted 3 July 1992)

Summary — The effects of exogenous enzyme preparations, *ie* FlavourAge-FR or DCA 50, on proteolysis flavour textural development in high (38%) or low (35%) moisture Cheddar cheeses ripened at 4 or 10 °C for 180 days were investigated. Proteolysis, as measured by nitrogen soluble in water (WSN), 75% ethanol (ALC-N) or 5% PTA (PTA-N), was highest in cheeses with added FlavourAge-FR, intermediate in DCA 50-treated cheeses and lowest in control cheeses at both 4 or 10 °C. The WSN was chromatographed by fast protein liquid chromatography (FPLC) and free amino acid analysis. The concentrations of low molecular weight (< 10 000) peptides and free amino acids were highest in FlavourAge-FR-treated cheeses while DCA 50-treated cheeses had intermediate levels compared to control cheeses at 4 or 10 °C. Polyacrylamide gel electrophoresis of the cheeses during ripening showed that FlavourAge-FR caused significant proteolysis of both α_{s1} - and β -caseins, while DCA 50 caused no significant degradation of β -casein. Cheese texture, as measured by yield value, was affected most by FlavourAge-FR treatment. Taste panel analysis of the cheeses indicated little acceleration of flavour development by any of the treatments, and in some cases enzyme treatment led to off-flavours and textural defects.

maturation / protéinase / protéolyse / accélération / flavour

Résumé — Effets d'enzymes commerciales sur la protéolyse et l'affinage de fromages de type Cheddar. Les effets de préparations d'enzymes exogènes, «FlavourAge-FR» ou «DCA 50», sur la protéolyse, le goût et le développement de la texture de fromages de type Cheddar à haute (38%) ou basse (35%) humidité affinés à 4 ou 10 °C pendant 180 j ont été étudiés. La protéolyse, mesurée par la quantité d'azote soluble dans l'eau (WSN), dans 75% d'éthanol (ALC-N) ou dans 5% PTA (PTA-N) était la plus importante dans les fromages fabriqués avec addition de FlavourAge-FR, moyenne dans les fromages traités avec DCA 50, la protéolyse étant la plus basse pour les fromages de contrôle à 4 ou 10 °C. L'azote soluble dans l'eau était chromatographié par chromatographie liquide rapide sur gel et analyse des acides aminés libres. Les concentrations en peptides de faible poids moléculaire (< 10 000) et en acides aminés libres étaient supérieures dans les fromages traités avec la FlavourAge-FR, alors que les fromages traités avec DCA 50 avaient des niveaux moyens, comparés aux fromages de contrôle à 4 ou 10 °C. Des électrophorèses sur gel polyacrylamide des fromages en cours d'affinage ont montré que la FlavourAge-FR provoquait une protéolyse significative des caséines α_{s1} - et β DCA 50 n'entraînait pas de dégradation significative de la caséine β . La texture du fromage, mesurée par la résistance à l'écrasement, était surtout affectée par le traitement avec l'enzyme FlavourAge-FR. L'évaluation sensorielle des fromages a montré que, quel que soit le traitement, le développement du goût était légèrement accéléré, et dans quelques cas, le traitement par ces enzymes menait à des défauts de goût et de texture.

affinage / protéolyse / protéase / arôme / fromage

INTRODUCTION

Ripening of cheese is a complex process involving the gradual breakdown of carbohydrate, fat and protein into organic acids, free fatty acids, peptides and free amino acids, respectively; these changes result in the conversion of curd to a cheese having the desired texture and flavour characteristics of the intended variety (Fox, 1989a, b). Ripening itself is a costly and time-consuming process so that any acceleration would obviously be of considerable benefit to the producer, provided that the final product has the same flavour, rheological and sensory attributes as the untreated cheese (Fox, 1989b). Proteolysis is generally regarded as a primary requisite for good flavour development in Cheddar cheese (Fox, 1989a); most methods used to accelerate ripening have, therefore, involved acceleration of casein breakdown by proteinases and/or peptidases.

The major methods used to accelerate ripening include the elevation of ripening temperature (Aston *et al*, 1983, 1985), addition of exogenous proteinases/peptidases (Law and Wigmore, 1982, 1983; Hayashi *et al*, 1990), inclusion of starter bacteria which have been heat freeze-shocked and/or use of mutant starter bacteria, *eg* lactose-negative (*lac*⁻) and/or proteinase negative (*prt*⁻) strains as an additional source of proteinases and/or peptidases (Grieve and Dulley, 1983; Bartels *et al*, 1987a, b).

In recent years the availability of commercial enzyme preparations has led to a choice of available options to accelerate the ripening of Cheddar cheese. The aims of this study were to evaluate the effect of various enzyme preparations on proteolysis in, and on the rheology and sensory attributes of high and low moisture Cheddar cheeses.

MATERIALS AND METHODS

Culture preparation

L. lactis subsp. *cremoris* C25 and *L. lactis* subsp. *cremoris* G11, were obtained in mixed culture as freeze-dried pellets from Chr Hansen Ltd (Little Island, Cork, Ireland). The cultures were propagated twice in 10% reconstituted skim milk (RSM) at 21 °C for 18 h prior to use.

Manufacture of Cheddar cheese

Cheddar cheese was manufactured using a 1.5% inoculum of the mixed-strain starter culture. Curds were cooked at 38.5 °C, pitched at pH 6.1 and milled twice at pH 5.2, to ensure chips of a uniform size and to improve salt and enzyme absorption. Salt was added at a rate of 2.7% (w/w). The curds were moulded and pressed overnight at 4.2 kgf/cm², and stored at 4 to 10 °C. Samples were removed at various stages throughout the 180-d ripening period for assessment of proteolysis and rheological and sensory quality.

All enzymes were obtained in powder form and dispersed in the salt prior to addition to the curd.

Proteinase and peptidase activity of the enzyme preparations

To assess the proteinase activity of FlavourAge-FR and DCA 50 the enzymes were assayed on 0.4% azocasein (Sigma) in 0.2 mol/l Tris-HCl buffer at pH 7.5 according to the method of Garcia de Fernando and Fox (1989). The reaction was stopped with 2% trichloroacetic acid after 30 min incubation at 30 °C, and the absorbance measured at 440 nm. Aminopeptidase activity of the enzyme preparations was measured against the following *para*-nitroanilide substrates: lysine, leucine, aspartic acid, glutamic acid and proline. Enzymes were added to paranitroanilides in 0.2 mol/l Tris-HCl buffer pH 7.5, at a rate of 50 µl (at dilutions of 100, 10 and 1 mg/ml) to 450 µl substrate (2.2 mmol/l), and incubated for 30 min at 30 °C. The reaction was stopped by addition of

1.5 ml of 1.5 mol/l acetic acid; the degree of *para*-nitroanilide hydrolysis was measured by the change in absorbance at 410 nm. Post-proline dipeptidyl aminopeptidase activity was determined according to the method of Booth *et al* (1990). A sample (50 μ l) of enzyme was incubated with 450 μ l of 0.111 mmol/l Gly-Pro-AMC (Bachem, Bubendorf, Switzerland) in 50 mmol/l Tris-HCl pH 7.5 for 5 min at 30 °C. The reaction was terminated by the addition of 1 ml 1.5 mol/l acetic acid. The degree of hydrolysis was determined by measuring fluorescence of the samples, using excitation and emission wavelengths of 370 nm and 440 nm respectively. The extent of release of 7-amino-4-methyl coumarin (AMC) between 0 and 200 nmol per tube was computed. Activity is expressed as nmol AMC produced per min per ml of enzyme dilution. Results are shown in table II.

Enzyme application

DCA 50, a blend of peptidase and protease (Muir *et al*, 1992) and FlavourAge-FR were gifts from Imperial Biotechnology (London) and Chr Hansen Ltd (Little Island, Cork, Ireland), respectively. FlavourAge-FR was added at the dosage rate of 0.25 g/kg per weight of curd, which is the level recommended by Hansen for FlavourAge-FR. DCA 50 was added to the curd at the recommended level of 0.55 g/kg curd (Imperial Biotechnology, London).

Composition of cheeses

Grated cheese samples were analysed in duplicate for salt (IDF, 1979), fat (Gerber method IS 69, 1955), total nitrogen (IDF, 1986), and moisture (IDF, 1982); the pH of a paste, prepared by macerating 10 g grated cheese in 10 g H₂O, was measured using a pH meter 26 (Radiometer, Copenhagen, Denmark).

Proteolysis

Proteolysis was monitored by measuring the percentage of total nitrogen soluble in water (WSN), 75% ethanol(ALC-N) and 5% tungsto-phosphoric

acid (PTA-N) using the methods of Kuchroo and Fox (1982) and Stadhouders (1960).

Polyacrylamide gel electrophoresis was performed in a vertical cell (Pharmacia) according to the method of Andrews (1983), as modified by Shalabi and Fox (1987), using a running gel and a stacking gel. Cheese samples (100 mg) were dispersed in 1 ml of sample buffer (0.75 g Tris, 48 g urea and 0.4 ml conc HCl made up to 100 ml with distilled water; pH 7.6) and 0.2 ml of 2-mercaptoethanol. Bromophenol blue dye was added to the defatted samples at a rate of 50 μ l/ml; the prepared samples (25 μ l) were held at 40 °C with intermittent agitation for 20 min, then cooled to 4 °C and filtered through glass wool. Defatted samples (25 μ l) were applied to the gel slots. The power supply was set at 280 V and the current increased gradually to 150 mA.

Gels were stained using Amido black, prepared by dissolving 0.2 g naphthalene black 12B (BDH, Poole, Dorset, UK) in 200 ml glacial acetic acid/ethanol/distilled water (20/100/80, v/v) and were destained in a 7% solution of acetic acid in 5% methanol and stored in 7% acetic acid.

The water-soluble nitrogen (WSN) extracts of the low moisture cheeses after 60, 120 and 180 days ripening at 4 or 10 °C were analyzed by gel permeation fast protein liquid chromatography (FPLC) according to the method of O'Callaghan and Wilkinson (unpublished observations). Samples of the WSN extract (1.5 ml) were centrifuged in Eppendorf tubes at 13 000 g for 5 min, diluted (1:10) with 0.1 mol/l Tris-HCl containing 0.1 mol/l NaCl and 10 mmol/l NaNO₃ and filtered through a 0.45- μ m Millipore filter (Millipore, Bedford, MA, USA). The samples were applied to a Superose-12 column (Pharmacia), previously calibrated with various molecular weight standards. The optimal separation range for this column is in 10³-3 x 10⁵ Da.

Individual free amino acids (FAA) were determined in 100 μ l samples of 12% TCA filtrates prepared from the water-soluble extract of low moisture cheeses treated with FlavourAge-FR or DCA 50 after 60, 120 or 180 days ripening at 4 or 10 °C. Filtrates were analyzed using a Beckman 6300 Analyzer (Beckman, High Wycombe, Bucks, UK). Data were captured using a PC Minichrom system (VG Data Systems, Altrincham, Cheshire, UK); concentrations were expressed as nmol per ml filtrate which were subsequently converted to μ g per g of cheese.

Rheology

The force required to fracture the cheese (*i.e.* yield value) was measured on the Universal Testing Machine (Model 1112) using the method of Peleg (1976). Cylindrical samples ($r = 30$ mm; $h = 30$ mm) were cut from the cheese and equilibrated at 4 °C for 4 h prior to testing, and then compressed to 30% of initial height using a 50 kg load cell. A full scale load at 50 kg and cross-head speed of 5 cm/min were used. Yield value which represents the force (kgf) required to fracture the cheese is reported as the mean of triplicate analyses.

Sensory evaluation

Sensory evaluation was performed by an in-house trained panel of 8 graders. The cheeses were graded on a scale of 0 to 8, on the basis of flavour, aroma, texture and overall impression, where 8 represented an excellent cheese, 6 a good cheese, 4 an acceptable cheese, 2 a poor cheese and 0 as a totally unacceptable cheese.

The values reported are the mean grades awarded to the cheeses for overall impression.

RESULTS

The composition of the various cheeses is shown in table I. To investigate the accelerating effects of different enzymes in cheeses of different moisture contents, 2 sets of cheese were manufactured: i) high moisture/high pH cheeses which had $\approx 38\%$ moisture and a pH of 5.30; and ii) low moisture/low pH cheeses with $\approx 34\%$ moisture and a pH of 5.10. Evaluation of enzymes in high and low moisture cheeses designated H and L, respectively, should indicate the variability that may occur between effectiveness of enzyme and cheese composition (*cf* Fedrick *et al*, 1986; Guinee *et al*, 1991). The range of moistures in this study represents those found in commercial cheeses.

Proteinase and aminopeptidase activities of enzymes

The results of the various enzyme assays carried out on the preparations are shown

Table I. Chemical composition of experimental cheeses after 1 d. Cheese description: high (H) and low (L) moisture control, FlavourAge-FR-treated and DCA 50-treated cheeses.

Composition chimique des fromages expérimentaux après 1 j. Description du fromage: Contrôle à haute (H) et basse (L) humidité, fromages traités avec la FlavourAge-FR et DCA 50.

Treatment	Moisture (%)	NaCl (%)	S/M (%) ^a	Protein (%)	pH
Control (H)	38.30	2.2	5.7	25.1	5.27
Control (L)	34.90	1.7	4.8	24.0	5.08
DCA 50 (H)	37.60	2.2	5.7	24.0	5.32
DCA 50 (L)	34.60	2.0	5.7	23.7	5.07
FlavourAge-FR (H)	38.90	2.2	5.7	24.4	5.29
FlavourAge-FR (L)	35.09	1.8	5.1	24.0	5.09

^a S/M = salt-in-moisture. All results are means of duplicate analyses.

Tous les résultats sont la moyenne de 2 analyses.

in table II. FlavourAge-FR showed strong proteinase and leucine aminopeptidase activity (slight aminopeptidase activity was detected against lysine), no activity was detected against proline, aspartic acid or glutamic acid *para*-nitroanilides; post-proline dipeptidylaminopeptidase activity was not detected in the FlavourAge-FR preparation. DCA 50 appeared to have a low proteinase activity on azocasein but showed activity against all the aminopeptidase substrates and post-proline dipeptidylaminopeptidase was detected in this preparation. In addition to proteolytic activity, FlavourAge-FR also contains a unique lipase (Chr Hansen, Denmark). The effects of this lipase on the extent and type of lipolysis in Cheddar cheese has been reported by Arbige *et al* (1986). Apart from a study by Guinee *et al* (1991), to our knowledge, no other information exists on the proteinase/peptidase activities of Flavour-

Age-FR and the effects thereof on proteolysis, flavour and texture of Cheddar cheese of varying compositions.

Proteolysis of cheeses

Formation of soluble nitrogen

The formation of nitrogen (N) soluble in water, 75% ethanol or 5% PTA in cheeses ripened for 180 d at 4 and 10 °C is shown in figure 1. All N fractions increased at a diminishing rate with ripening time. At 4 °C, highest levels of WSN, ALC-N and PTA-N were found in FlavourAge-FR-treated cheeses at all stages of ripening. DCA 50 treatment generally resulted in slight increases in WSN and ALC-N levels, but 5% PTA-N levels were similar to those of the control cheese, while similar trends were obtained for cheeses ripened at 10 °C. In-

Table II. Proteinase and peptidase activities of DCA 50 and FlavourAge-FR.
Activité protéinasique et peptidasique de DCA 50 et de FlavourAge-FR.

Enzyme substrates	Enzyme concentration (mg/ml)	Activity (Δ OD/30 min) ^a	
		DCA 50	FlavourAge-FR
<i>Proteinase</i>			
Azocasein	6.2	0.05	0.50
<i>Aminopeptidase</i>			
Leu-pNA	100	0.494	0.543
Lys-pNA	10	0.773	0.0051
Pro-pNA	100	0.548	ND
Asp-pNA	100	0.486	ND
Glu-pNA	100	0.634	ND
<i>Post-proline-dipeptidyl aminopeptidase</i>			
Gly-Pro-AMC	10	132 ^b	ND

ND = not detected. ^a Activity is expressed as the change in OD (optical density) at 410 nm over 30 min. ^b Activity is expressed as nmol AMC produced per min per ml of enzyme concentration.

Non détecté. ^a L'activité est exprimée par le changement de densité optique à 410 nm de plus de 30 min. ^b L'activité est exprimée en nmol d'AMC produit par min et par ml d'enzyme.

creasing the ripening temperature from 4 to 10 °C generally resulted in higher values for all categories of soluble N for all cheeses (fig 1).

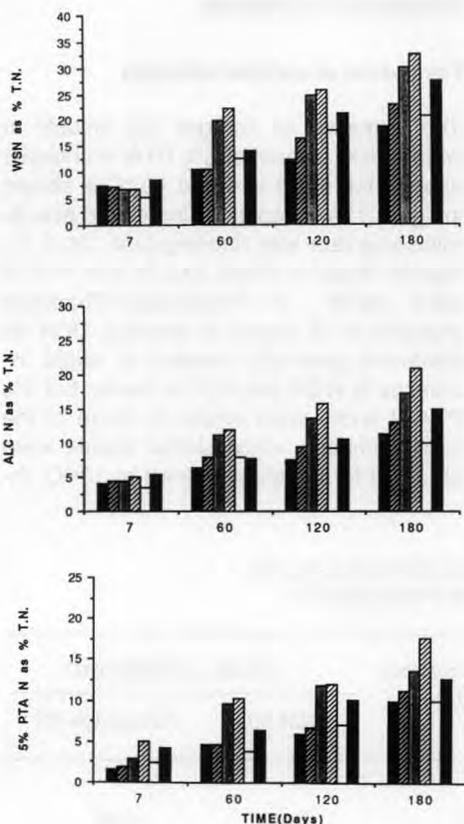


Fig 1. Formation of water soluble N (WSN); 75% ethanol soluble N (ALC-N) or 5% PTA-N (PTA-N) in low moisture Cheddar cheeses treated with FlavourAge-FR ripened at 4 (▨) or 10 °C (▧) and DCA 50 ripened at 4 (□) or 10 °C (■) and control ripened at 4 (■) or 10 °C (▧) for 180 d.

Formation de azote soluble dans l'eau (WSN); azote soluble dans 75% éthanol (ALC-N); 5% PTA-azote (PTA-N) dans des fromages de type Cheddar à basse humidité traités avec la FlavourAge-FR affinés à 4 (▨) ou 10 °C (▧) et avec DCA 50 affinés à 4 (□) ou 10 °C (■), et pour le contrôle affiné à 4 (■) ou 10 °C (▧) pendant 180 j.

No clear trend existed between cheese moisture and degree of proteolysis as detected by N solubility in any of the solvents.

Polyacrylamide gel electrophoresis (PAGE)

The level of proteolysis in the various cheeses as indicated by PAGE correlated well with the formation of WSN during ripening (figs 1, 2). At all stages of ripening, both at 4 or 10 °C, degradation of both α_{s1} - and β -caseins was much more extensive in the FlavourAge-FR-treated cheeses than in the DCA 50-treated or control cheeses. In the latter cheeses, there was little breakdown of β -casein, even after ripening for 180 d at 10 °C. α_{s1} -Casein was extensively degraded in the control and DCA 50-treated cheese at the end of maturation, especially at 10 °C (data not shown), but to a much lesser extent than in the FlavourAge-FR-treated cheese.

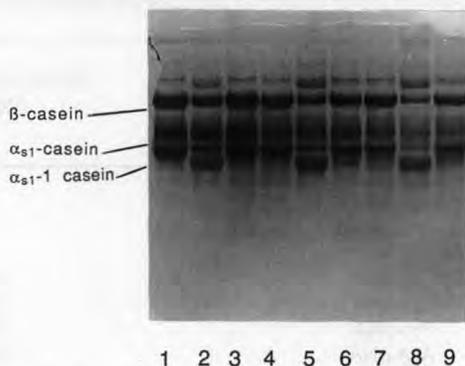


Fig 2. Gel electrophoretograms of low moisture control cheeses (lanes 3, 6, 9) or treated with DCA 50 (lanes 1, 4, 7) or FlavourAge-FR (lanes 2, 5, 8) and ripened at 4 °C for 60 (1, 2, 3), 120 (4, 5, 6) or 180 (7, 8, 9) d.

Gel d'électrophorèse des fromages à basse humidité de contrôle (lignes 3, 6 et 9), traités avec DCA 50 (lignes 1, 4 et 7) ou avec la FlavourAge-FR (lignes 2, 5 et 8) et affinés à 4 °C pendant 60 (1, 2, 3), 120 (4, 5, 6) ou 180 j (7, 8, 9).

Gel permeation FPLC

The molecular weight distribution of peptides of gel permeation FPLC profiles of WSN extracts from 120- and 180-d-old low-moisture cheeses, ripened at 4 °C, are shown in table III. Low molecular weight peptides (<1 000 Da) and free amino acids accumulated to the highest levels in FlavourAge-FR-treated cheeses, intermediate for DCA 50, and lowest in the control cheeses. According to Jarrett *et al* (1982), PTA-N contains free amino acids and very small peptides, *ie* molecular weight < 640 Da.

Free amino acids

The concentration of free amino acids (FAA) increased in all cheeses with ripening time, temperature (data not shown), and with enzyme treatment (fig 3); the difference between treatments was most pronounced after 180 d. FAA accumulated to highest levels in FlavourAge-FR-treated cheeses and the lowest concentrations were found in the control cheeses at both 4 or 10 °C at all sampling times. This is in agreement with the trends found for the

various soluble nitrogen fractions, *ie* WSN, ALC-N or PTA-N.

The concentrations of free glutamic acid and leucine, which showed dramatic increases in concentration between 120 and 180 d, especially in DCA 50-treated cheeses, reached much higher levels than those of all other amino acids. While both glutamic acid and leucine were present at somewhat higher levels in FlavourAge-FR-treated at 120 d, leucine attained higher concentrations in the DCA 50-treated cheese at 180 d. The latter also contained the highest concentrations of free proline after 180 d ripening.

Rheological analysis

Yield values (kgf) generally decreased with ripening time (data not shown). Lowest yield values were consistently obtained for FlavourAge-FR-treated cheeses at all stages of ripening at 4 or 10 °C. DCA 50-treated cheeses had yield values which were generally similar to, or slightly less than, those of the control cheeses. Lower yield values for enzyme treated-cheeses

Table III. Molecular weight distribution profile in water soluble extracts of 120- or 180-d old low moisture cheeses ripened at 4 °C.

Distribution des poids moléculaires des peptides de l'extrait soluble dans l'eau de fromages à basse humidité affinés à 4 °C pendant 120 et 180 j.

Cheese	Ripening Temp (°C)	Age (d)	Molecular weight (kDa) distribution (% of total area)				
			<1	1-5	5-10	10-25	>25
Control (L)	4	180	11.0	28.0	19.0	16.0	26.0
FlavourAge-FR	4	180	25.0	27.0	14.0	17.0	17.0
DCA 50	4	180	22.0	26.0	13.0	17.0	23.0
Control	4	120	9.0	24.0	22.0	16.0	30.0
FlavourAge-FR	4	120	25.0	24.0	15.0	16.0	21.0
DCA 50	4	120	16.0	21.0	18.0	16.0	29.0

would be expected because of the more extensive protein degradation, especially in FlavourAge-FR-treated cheeses (figs 1, 3) (*cf* Creamer and Olson, 1982; Luyten *et al*, 1987).

Sensory analysis

Mean grading scores of all cheeses during the 180-day ripening period are summarized in table IV. Generally, grading scores

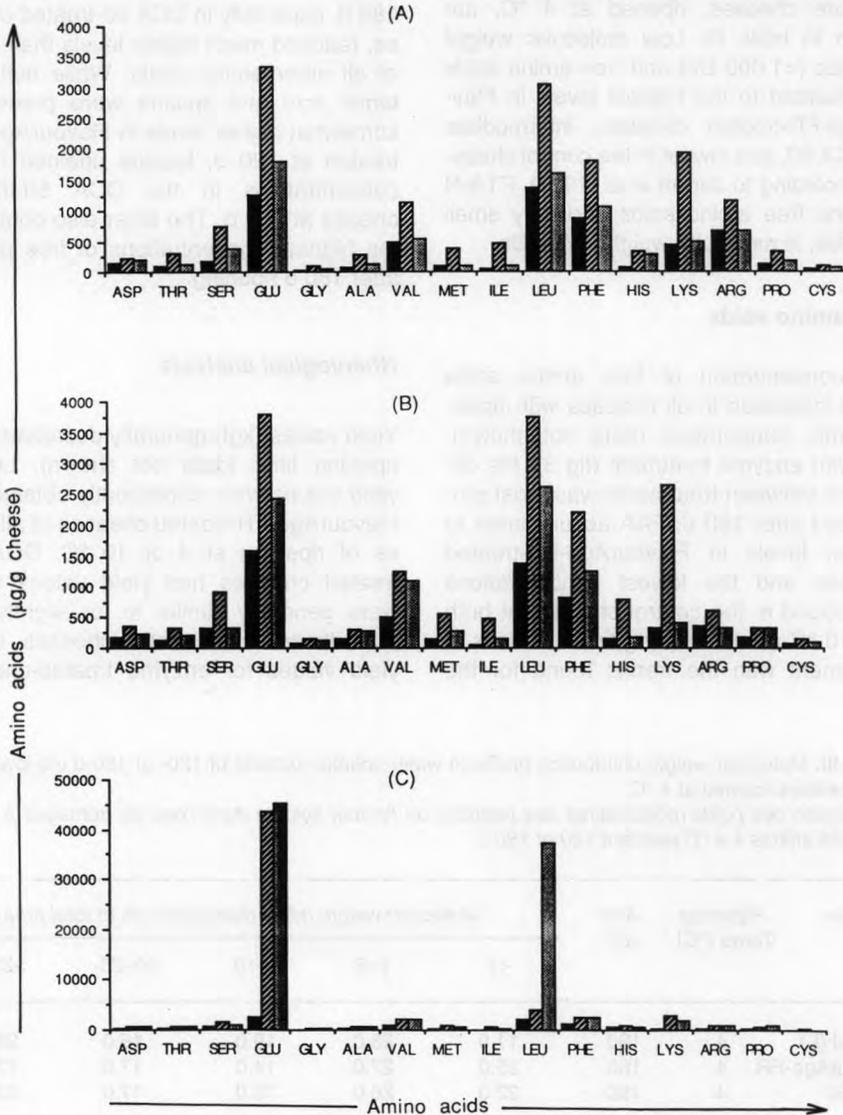


Fig 3. Free amino acids in low moisture cheeses treated with FlavourAge-FR (▨), DCA 50 (■) and control (■) and ripened at 4 °C for (A) 60, (B) 120 or (C) 180 d.

Acides aminés libres présents dans les fromages à basse humidité traités avec la FlavourAge-FR (▨), DCA 50 (■), et contrôle (■) affinés à 4 °C pendant (A) 60, (B) 120 et (C) 180 j.

Table IV. Mean grading scores for overall impression of high (H) or low (L) moisture control, FlavourAge-FR- and DCA 50-treated cheeses ripened at 4 or 10 °C for 60 (a), 120 (b) or 180 (c) d.

Moyenne des notes décernées pour l'impression générale des contrôles à haute (H) ou basse (L) humidité, des fromages traités à la FlavourAge-FR et DCA 50 affinés à 4 ou 10 °C pendant 60 (a), 120 (b) et 180 (c) j.

	Ripening temp (°C)	Treatment		
		Moisture	Control	Flavour- Age-FR
a				
L	4	3.7	4.1	3.7
L	10	3.5	3.6	3.7
H	4	3.8	4.0	4.2
H	10	3.7	3.7	3.8
b				
L	4	5.8	5.6	6.0
L	10	5.1	4.1	4.2
H	4	5.0	4.6	4.9
H	10	4.0	3.2	5.1
c				
L	4	4.0	3.7	4.2
L	10	2.8	3.7	3.2
H	4	4.5	2.8	3.4
H	10	3.8	2.5	4.0

for cheeses ripened at 4 or 10 °C reached maximum values at 120 d and decreased thereafter to varying degrees depending on ripening temperature, moisture content and enzyme treatment. Increasing the ripening temperature from 4 to 10 °C generally resulted in lower grading scores for all cheeses at all sampling times, except in the case of high moisture DCA 50-treated cheeses where flavour scores after 120 and 180 d ripening increased slightly with ripening temperature. Increased ripening temperature was paralleled by a more intense cheese flavour (characteristic of ma-

ture Cheddar) which was generally not very acceptable to the panel members who generally expressed a preference for mild-flavoured cheeses. This may have reflected itself in lower scores for cheeses, especially those which had received enzyme treatment and ripened for > 120 d.

No clear trend was found between moisture level and grading score; however, at 120 d the higher moisture cheeses generally received lower grades than the lower moisture cheeses.

After ripening for 60 d there was little or no difference between the grading scores of the control and enzyme-treated cheeses. Grading scores were consistently lower for the FlavourAge-FR-treated cheese at ripening times \geq 120 d; typical defects noted for these cheeses included: sourness, softness, acidity, soapiness, bitterness and rancidity. At all sampling times over the 180-d ripening period, at 4 or 10 °C, there was no consistent difference between the grades for the control and DCA 50-treated cheeses.

DISCUSSION

The addition of FlavourAge-FR led to extensive breakdown of α_{s1} - and β -caseins to lower molecular weight peptides and amino acids as indicated by gel electrophoresis, nitrogen solubilities in water, 75% ethanol and 5% PTA, and by free amino acid analysis and FPLC of the water soluble cheese extracts. DCA 50 acted primarily on α_{s1} -casein and generated levels of soluble N, lower molecular weight peptides and free amino acids intermediate between those obtained in the control and FlavourAge-FR-treated cheeses.

Enzyme-treated cheeses were softer than the control as detected by sensory evaluation and yield measurement, especially in the case of FlavourAge-FR-treated

cheeses, at both 4 or 10 °C at all stages of the 180-d ripening period.

This study indicates that the addition of exogenous enzymes to the curd, *ie* Flavour-Age-FR or DCA 50 at levels of 0.25 and 0.55 g/kg curd respectively, does not appear to accelerate the development of typically good flavour in Cheddar cheeses ripened at 4 or 10 °C. Bitterness, absent in control and DCA 50-treated cheeses but present in FlavourAge-FR-treated cheeses, may be attributed to the excessive, non-specific action of FlavourAge-FR on both α_{s1} - and β -caseins and the consequent accumulation of bitter peptides above threshold values required for perception, in the presence of limiting peptidase activity. In this context, it is worth noting that the addition of DCA 50, which had a relatively high peptidase to proteinase activity and a high post-proline dipeptidylaminopeptidase activity did not result in bitterness (table II). The possible contribution of the latter enzyme to debittering of peptides has been noted by Booth *et al* (1990) and Zevaco *et al* (1990).

The efficiency of any enzyme added to the curd to accelerate cheese ripening will depend on the following factors: enzyme dosage, ratio of peptidase to proteinase activity, uniform distribution of the enzyme in the curd (to avoid 'hot spots' of proteolytic activity), stability of the enzyme in the curd, substrate specificity, amount of enzyme lost on salting/pressing and storage temperature of the cheese. FlavourAge-FR may be considered unsuitable because of the relatively high proteinase to peptidase activity, along with an extensive action on α_{s1} - and β -caseins generating flavour and textural defects. DCA 50 appears to lack sufficient proteinase activity to generate a pool of peptide substrates for conversion to amino acids by the relatively high peptidase activity in this preparation. DCA 50 may be more effective at a higher dosage level at the temperatures of 4 or 10 °C, used in this study.

The following improvements could be implemented to improve the efficiency of these enzymes; in the case of Flavour-Age-FR, a lower dosage to the curd combined with the addition of a significant level of peptidase activities to the preparation so as to de-bitter any peptides produced during ripening. In the case of DCA 50, the inclusion of a higher level of proteinase in the preparation and/or a higher dosage rate per se may improve the performance. A cocktail of Flavour-Age-FR and DCA 50 at a ratio of \approx 20–30: 80–70, respectively, may be more successful than either enzyme treatment on its own.

REFERENCES

- Andrews AT (1983) Proteinases in normal bovine milk and their action on caseins. *J Dairy Res* 50, 45-55
- Arbige MV, Freund PR, Silver SC, Zelko JT (1986) Novel lipase for Cheddar cheese flavour development. *Food Technol* 40, 91-98
- Aston JW, Fedrick IA, Durward IG, Dullely JR (1983) Effect of elevated ripening temperatures on proteolysis and flavour development in Cheddar cheese. 1. Higher initial storage temperatures. *N Z J Dairy Sci Technol* 18, 143-151
- Aston JW, Giles JE, Durward IG, Dullely JR (1985) Effect of elevated ripening temperatures on proteolysis and flavour development in Cheddar cheese. *J Dairy Res* 52, 565-572
- Bartels HJ, Johnson ME, Olson NF (1987a) Accelerated ripening of Gouda cheese. 1. Effect of heat-shocked *Lactobacillus helveticus* on proteolysis and flavour development. *Milchwissenschaft* 42, 83-88
- Bartels HJ, Johnson ME, Olson NF (1987b) Accelerated ripening of Gouda cheese. 2. Effect of freeze-shocked *Lactobacillus helveticus* on proteolysis and flavour development. *Milchwissenschaft* 42, 139-144
- Booth M, Donnelly WJ, Ni Faholain I, Jennings PV, O'Cuinn G (1990) Proline-specific peptidases of *Streptococcus cremoris* AM2. *J Dairy Res* 57, 79-88

- Creamer LK, Olson NF (1982) Rheological evaluation of maturing Cheddar cheese. *J Food Sci* 47, 631-636, 646
- Fedrick IA, Cromie SJ, Dullely JR, Giles JE (1986) The effects of increased starter populations, neutral proteinase and elevated temperature storage on Cheddar cheese manufacture and maturation. *N Z J Dairy Sci Technol* 21, 191-203
- Fox PF (1989a) Proteolysis during cheese ripening and manufacture. *J Dairy Sci* 72, 1379-1400
- Fox PF (1989b) Acceleration of cheese ripening. *Food Biotechnol* 2, 133-185
- Garcia de Fernando GD, Fox PF (1989) Factors affecting growth and extracellular proteinase production by a *Micrococcus* sp. *Ir J Food Sci Technol* 13, 162
- Grieve PA, Dullely JR (1983) Use of *Streptococcus lactis* lac- mutants for accelerating Cheddar cheese ripening. 2. Their effect on the rate of proteolysis and flavour development. *Aust J Dairy Technol* 38, 49-54
- Guinee TP, Wilkinson MG, Mulholland ED, Fox PF (1991) Influence of ripening temperature, added commercial enzyme preparations and attenuated mutant (Lac⁻) *Lactococcus lactis* starter on the proteolysis and maturation of Cheddar cheese. *Ir J Food Sci Technol* 15, 27-52
- Hayashi K, Revell DF, Law BA (1990) Effect of partially purified serine proteinases produced by *Brevibacterium linens* on the accelerated ripening of Cheddar cheese. *J Dairy Sci* 73, 579-583
- IDF (1979) *Cheese and Processed Cheese. Determination of Chloride Content: Potentiometric Titration Method*. IDF standard 88
- IDF (1982) *Determination of the Total Solids Content (Cheese and Processed cheese)*. IDF Standard 4A
- IDF (1986) *Determination of Nitrogen Content (Kjeldahl Method) and Calculation of Crude protein Content*. IDF Standard 20A
- Irish Standard (1955) *Determination of the Percentage Fat in Cheese*. Irish Standard, 69
- Jarrett WD, Aston JW, Dullely JR (1982) A simple method for estimating free amino acids in Cheddar cheese. *Aust J Dairy Technol* 37, 55-58
- Kuchroo CN, Fox PF (1982) Soluble nitrogen in cheese: comparison of extraction procedures. *Milchwissenschaft* 37, 331-335
- Law BA, Wigmore A (1982) Accelerated cheese ripening with food grade proteinases. *J Dairy Res* 49, 137-146
- Law BA, Wigmore A (1983) Accelerated ripening of Cheddar cheese with a commercial proteinase and intracellular enzymes from starter streptococci. *J Dairy Res* 50, 519-525
- Luyten H, van Vliet T, Walstra P (1987) Note on the shortness of the consistency of Dutch-type cheese. *Neth Milk Dairy J* 41, 285-288
- Muir DD, Banks JM, Hunter EA (1992) Sensory changes during maturation of fat-reduced Cheddar cheese: effect of enzymically active attenuated starter cultures. *Milchwissenschaft* 47, 218-222
- Peleg M (1976) Texture profile analysis parameters obtained by an Instron Universal Testing Machine. *J Food Sci* 41, 721-722
- Stadhouders J (1960) The hydrolysis of protein during the ripening of Dutch cheese. Enzymes and bacteria involved. *Neth Milk Dairy J* 14, 83-110
- Shalabi SI, Fox PF (1987) Electrophoretic analysis of cheese: comparison of methods. *Ir J Food Sci Technol* 11, 135-151
- Zevaco C, Monnet V, Gripon JC (1990) Intracellular X-prolyl dipeptidyl peptidase from *Lactococcus lactis* spp *lactis*: purification and properties. *J Appl Bacteriol* 68, 357-366