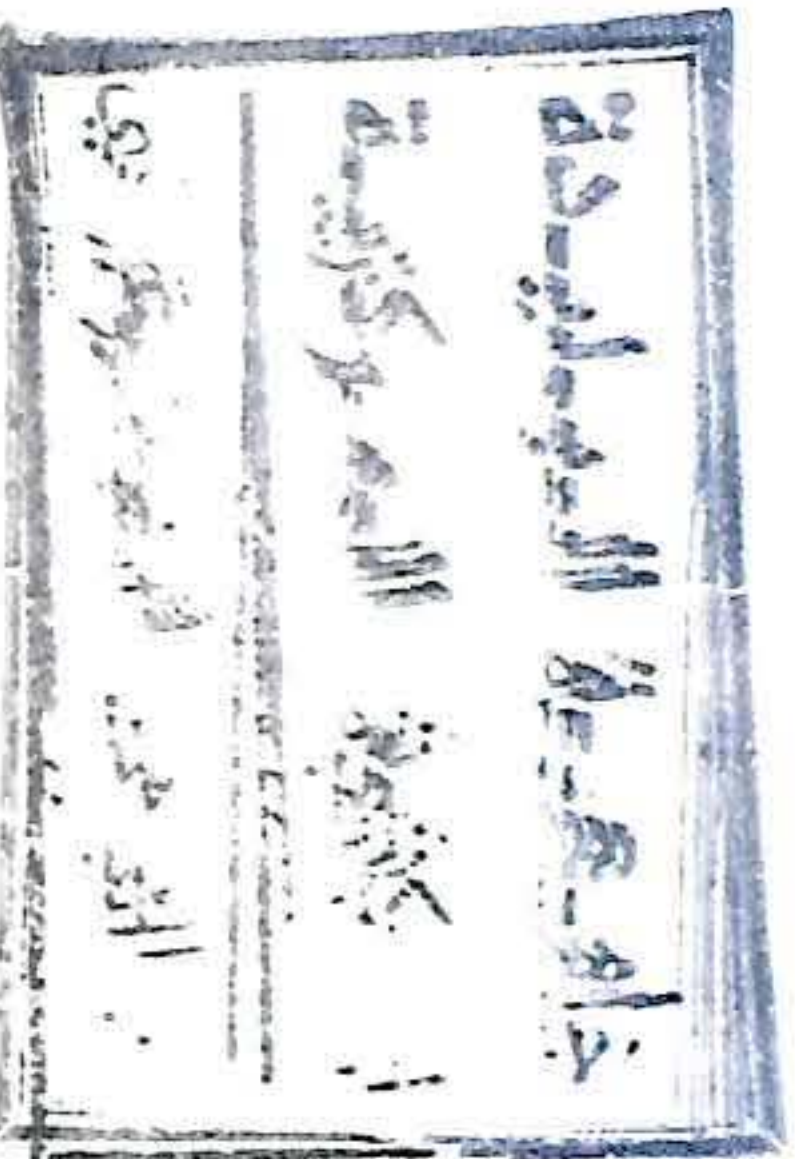


329 AGRO

329

Agro



THE BRITISH LIBRARY

This document has been supplied by, or on behalf of, The British Library Document Supply Centre, Boston Spa, Wetherby, West Yorkshire LS23 7BQ United Kingdom

WARNING: Further copying of this document (including storage in any medium by electronic means), other than that allowed under the copyright law, is not permitted without the permission of the copyright owner or an authorised licensing body.

N<sup>o</sup> 32/102.  
Chamle.



## Contribution of the Indigenous Microflora to the Maturation of Cheddar Cheese

P. L. H. McSweeney, P. F. Fox

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

J. A. Lucey, K. N. Jordan & T. M. Cogan

National Dairy Products Research Centre, Moorepark, Fermoy, County Cork, Republic of Ireland

(Received 30 March 1992; revised version accepted 5 October 1992)

### ABSTRACT

*Cheddar cheeses were made from raw milk, pasteurised milk (72°C, 15 s) or milk produced from skim milk which had been microfiltered using an Alfa-Laval MFS-1 MF unit and mixed with pasteurised cream (72°C, 30 s). Microfiltration (MF) reduced the total bacterial count (TBC) by >99% and MF cheesemilk had a lower TBC than pasteurised milk; counts of non-starter lactic acid bacteria (NSLAB) were <1/ml. Cheeses were ripened at 10°C. Commercial graders and a trained taste panel found the pasteurised and MF cheeses to be of high and equivalent sensory quality but the raw milk cheese rapidly developed a strong and atypical flavour and was downgraded. NSLAB counts for the MF, pasteurised and raw milk cheeses were 43,  $3.9 \times 10^2$  and  $1.47 \times 10^5$  cfu/g after pressing and  $9.3 \times 10^6$ ,  $1.12 \times 10^7$  and  $1.19 \times 10^8$  cfu/g after 11 weeks. The cheeses and their water-soluble extracts were indistinguishable by urea-polyacrylamide gel electrophoresis for up to 3 months, but slight differences were apparent at 6 months. Peptide profiles were similar for pasteurised and MF cheeses but different for the raw milk cheese. Water-soluble nitrogen levels in the cheeses were similar although amino acid nitrogen and free glutamic acid were consistently highest in the raw milk cheese. The raw milk cheese exhibited the highest concentration of free fatty acids throughout ripening. The non-starter microflora, consisting*

613

predominantly of *Lactobacillus* spp., differed in the raw and pasteurised cheeses. The results show that MF efficiently reduced the numbers of indigenous microorganisms in milk and cheese. The results also suggest that the indigenous microflora of milk markedly affects the quality of Cheddar cheese made from raw milk.

## INTRODUCTION

Cheddar cheese made from raw milk tends to develop a stronger flavour and generally ripens more quickly than cheese made from pasteurised milk (Price & Call, 1969). Changes in cheesemilk that may be caused by pasteurisation include denaturation of indigenous enzymes, slight denaturation of whey proteins and their interaction with caseins and destruction of the thermolabile members of the indigenous microflora, including non-starter lactic acid bacteria (NSLAB). It is possible that any of these, and perhaps other, changes influence the ripening process but alteration of the indigenous NSLAB microflora is probably most significant. To date, the exact role of NSLAB in cheese maturation and flavour development remains unclear.

As reviewed by Peterson and Marshall (1990), the starter bacteria in Cheddar cheese rapidly exhaust the growth substrate (lactose) and the starter cell numbers decline due to a combination of the absence of lactose, the low pH and the high sodium chloride (NaCl) concentration in the curd. Typically, the starter counts in cheese fall to ~1% of the maximum levels within 1 month. In contrast, NSLAB can increase from quite low numbers to become the dominant microorganisms in the cheese (e.g.  $10^8$  cfu/g). The non-starter flora of cheese is variable but consists mainly of members of the genera *Lactobacillus*, *Pediococcus* and *Micrococcus* (Peterson & Marshall, 1990). The non-starter flora of Irish Cheddar cheese appears to consist almost entirely of *Lactobacillus* species (Jordan, K. N., unpublished). Since lactobacilli dominate the flora of cheese throughout much of the ripening period, it seems likely that at least some of the many enzymes identified in the NSLAB (Khalid & Marth, 1990a) play a role in the biochemistry of cheese ripening.

A number of researchers have found differences between cheeses made from raw and pasteurised milk (Sherwood, 1936; Bullock & Irvine, 1956; Scarpellino & Kosikowski, 1962; Lau *et al.*, 1990, 1991); however, the reason(s) for the observed differences are not clear (Lau *et al.*, 1991). Sherwood (1936) treated young cheeses with chloroform in an attempt to inhibit the growth of NSLAB and concluded that the differences observed between the raw and pasteurised milk cheeses were due largely to heat-

induced chemical changes in milk constituents. In a more recent study, Lau *et al.* (1991) compared cheeses made from raw and pasteurised milk using modern analytical techniques. Both the extent and characteristics of proteolysis were altered by pasteurisation and the authors suggest that this could be a result of heat-induced whey protein-casein interactions which reduced the accessibility of the caseins to enzyme action since pasteurisation may cause a significant increase in the amount of whey protein retained in the cheese (Lau *et al.*, 1990). However, Lau *et al.* (1991), who did not study the microbiological conditions of milk or cheeses, did not preclude the possibility that the observed differences were a consequence of some inactivation of milk proteinases or NSLAB. They commented that their experimental procedure was not designed to establish the role of such proteinases or NSLAB in proteolysis.

Microfiltration (MF) is a membrane separation technique developed for use in the food and biotechnology industries. The membranes retain bacteria but allow casein micelles to pass through. Therefore, MF offers a very efficient technique for removing microorganisms from milk (Maubois, 1989). It can be used on a large scale to produce essentially sterile milk and provides a simpler approach than aseptic milking and pasteurisation (Fox, 1989) to prepare cheese milk free from NSLAB without concomitant heat-induced changes, in order to study the contribution of NSLAB to cheese ripening.

The objectives of the present study were to compare the ripening characteristics of Cheddar cheeses made from raw, pasteurised or MF milk in order to assess the significance of the NSLAB in cheese ripening without concomitant heat-induced changes.

## MATERIALS AND METHODS

### Cheese manufacture

Cheddar cheeses were manufactured in 450-litre vats according to a standard protocol from raw, pasteurised (72°C, 15 s) or MF milk at the National Dairy Products Research Centre, Moorepark, Fermoy, Co. Cork, Republic of Ireland. The MF cheese milk was produced by recombining raw skim milk which had been microfiltered using an Alfa-Laval MFS-1 cross-flow microfiltration unit (Alfa-Laval Filtration Systems, Lund, Sweden, with ceramic membranes, pore size: 1.4 µm; flow rate 100 litres/h at 30 kPa giving a flux rate of 500 litres/m<sup>2</sup>) and pasteurised cream (72°C, 30 s). Cream and MF skim milk were recombined to give the same fat percentage as the raw milk. Cheeses were ripened at 10°C. Composition of

the cheeses was determined in accordance with standard methods (AOAC, 1984).

Cheesemaking was performed in triplicate. The results presented here are for cheeses from one experiment which were analysed in detail.

### Microbiological analyses

The efficiency of the MF process for removal of bacteria from the milk was assessed by enumerating coliform (Violet Red Bile Agar, Oxoid, Basingstoke, UK), total bacteria (Tryptone–Glucose–Yeast Extract Agar, Oxoid, Basingstoke, UK) and NSLAB (*Lactobacillus* Selection Agar (LBS), Baltimore Biological Laboratories, Rockville, MD, USA) in the raw, pasteurised and MF milks.

During ripening, counts of coliform, starter (LM 17 agar; Terzaghi & Sandine, 1975) and NSLAB were determined in duplicate after the cheeses were removed from the press and after 6, 11, 16 and 27 weeks of ageing.

Fifty colonies each were selected at random from the LBS agar plates prepared from the 16-week-old raw and pasteurised cheeses, incubated aerobically and cultured in deMan-Rogosa-Sharpe (MRS) broth (deMan *et al.*, 1960). Pure isolates were obtained by streaking twice on MRS agar and the pure strains then transferred into MRS broth. The broth cultures were observed microscopically and the cells separated centrifugally from 1 ml aliquots and frozen in MRS broth, containing 25% glycerol.

Stock cultures were prepared by culturing the frozen strains overnight in MRS broth and classified by the following characteristics: colony morphology, thermophilic growth, utilisation of mannitol, raffinose, lactose and citrate, growth in litmus milk, absence of catalase, end-products of metabolism and the isomer of lactate produced. The strains were provisionally identified according to the taxonomic schemes of Sharpe (1981) and Kandler and Weiss (1984).

### Assessment of proteolysis

Water-soluble extracts of the cheeses were prepared according to the method of Kuchroo and Fox (1982). Water-soluble nitrogen (WSN) was determined by the macro-Kjeldahl method. The liberation of free amino acids was determined in duplicate on the water extract by the cadmium–ninhydrin method of Folkertsma and Fox (1992) and free glutamic acid was assayed using an enzyme kit with glutamate dehydrogenase and pig-heart diaphorase (Boehringer Mannheim, Mannheim, Germany). Aliquots of the water extracts were freeze-dried for analysis by reverse phase high-performance liquid chromatography (RP-HPLC) and electrophoresis.

Urea-polyacrylamide gel electrophoresis (PAGE) was performed on the cheeses and water-soluble extracts using the stacking gel system of Andrews (1983); the gels were stained using a modification of the method of Blakesley and Boezi (1977) with PAGE blue G-90.

RP-HPLC was performed using an LC-9A solvent delivery system with FCV-9AL flow control valve and DGU-2A degassing unit (Shimadzu Corp., Kyoto, Japan) and a Rheodyne model 7125 syringe-loading sample injector (Rheodyne Inc., Cotati, CA, USA) fitted with a 20  $\mu$ l sample loop. Ultrasphere-ODS guard (4.6 mm  $\times$  4.5 cm) and analytical (4.6 mm  $\times$  25 cm) columns (Beckman Instruments Inc., San Ramon, CA, USA) were used and detection was by means of an LC-75 spectrophotometric detector (Perkin Elmer, Norwalk, CT, USA) at 230 nm, interfaced with a C-R6A Chromatopac integrator (Shimadzu Corp., Kyoto, Japan).

Chromatographic conditions were as follows:

Solvent A: 0.1% trifluoroacetic acid (TFA, v/v, sequential grade, Sigma, St. Louis, MO, USA) in water.

Solvent B: 0.1% TFA (v/v) in CH<sub>3</sub>CN/water (50:50, v/v, HPLC grade acetonitrile: Rathburn Chemicals Ltd, Walkerburn, UK).

Samples (4 mg/ml) were dissolved in solvent A and filtered through a 0.45  $\mu$ m cellulose acetate filter (Sartorius GmbH, Gottingen, Germany). Filtrate (20  $\mu$ l) was applied to the column and eluted at a flow rate of 1 ml/min at 100% A for 5 min. A gradient of 0–100% B was then commenced (3.3% B/min) and the column was finally eluted with 100% solvent B for 5 min.

### Assessment of lipolysis

Total free fatty acids were determined by the copper soaps method of Bynum *et al.* (1984). Free fatty acid profiles of the cheese were determined by methylating diethyl ether extracts with tetramethyl ammonium hydroxide followed by GC analysis, as described by Martinez-Castro *et al.* (1986). The fatty acid methyl esters were resolved by a Pye Unicam PU 4500 gas chromatograph (Pye Unicam Ltd, Cambridge, UK), containing a 10% Silar 10-C column (210 cm), interfaced with a Shimadzu CR3A Chromatopac integrator (Shimadzu Corp., Kyoto, Japan).

### Sensory assessment

During ripening, cheeses were graded by a trained taste panel consisting of nine members at the National Dairy Products Research Centre on a 0–8 (0 — poorest: 8 — best) scale considering four attributes (appearance,

flavour and aroma, body, and overall quality). Cheeses were also assessed by two graders from the Irish Department of Agriculture and Food, using the same scale.

## RESULTS

### Microbiological analyses

#### *Milk*

Total bacterial, coliform and NSLAB counts for the milks are summarised in Table 1. MF reduced the indigenous microflora with a very high efficiency (>99.9%), producing an almost sterile milk. Although less so than MF, the efficiency of pasteurisation was also quite high. Both MF and pasteurised milk showed no lactobacilli in 1 ml test samples.

#### *Cheese*

The composition of the cheeses (Table 2) was within the range typical for Cheddar.

**TABLE 1**  
Bacterial Populations (cfu ml<sup>-1</sup>) in MF, Raw and Pasteurised Cheese Milk<sup>a</sup>

<i>Milk</i>	<i>TBC</i>	<i>Coliform</i>	<i>NSLAB</i>
MF skim milk and cream <sup>b</sup>	630	1	<1
Raw milk	63 000	320	191
Pasteurised milk	840	<1	<1

<sup>a</sup>Mean of duplicate analyses.

<sup>b</sup>Total bacterial counts for skim milk were 16 cfu ml<sup>-1</sup> upon exiting the MF unit and 380 cfu ml<sup>-1</sup> before adding cream.

**TABLE 2**  
Composition of Cheddar Cheeses made from MF, Raw and Pasteurised Milk<sup>a</sup>

<i>Milk</i>	<i>pH</i>	<i>NaCl</i> (%)	<i>Moisture</i> (%)	<i>Fat</i> (%)	<i>Protein</i> (%)	<i>Ash</i> (%)
MF	5.12	1.78	35.97	33.00	23.80	3.73
Raw	5.14	1.74	37.79	30.50	24.69	3.74
Pasteurised	5.10	1.68	37.63	31.00	24.25	3.69

<sup>a</sup>Mean of duplicate analyses.

The microbiological quality of the cheese during ripening is shown in Fig. 1. In agreement with earlier studies (Martley & Lawrence, 1972; Visser, 1977), starter counts decreased during ripening, by one to two log cycles in 27 weeks (Fig. 1(a)). Coliforms were virtually absent from the MF and pasteurised cheesemilk (Table 1); however, the cheeses made from these milks contained significant numbers of coliform bacteria, which decreased by two log cycles in the first 10 weeks of ripening (Fig. 1(c)). The raw milk cheese contained substantial numbers of coliforms at day 1, but these also declined during ripening. The numbers of NSLAB were low in the MF and pasteurised milk cheeses ex-press but their number increased markedly during ripening (Fig. 1(b)), with higher counts being consistently obtained in the raw milk cheese. Presumably, this is a reflection of higher numbers of lactobacilli in the raw milk. All of the NSLAB were identified as lactobacilli (Table 3). More species were found in the raw milk cheese than in the pasteurised milk cheese. Care must be taken in interpreting the data on starter counts (Fig. 1(a)), since NSLAB will also grow on the M17 medium used to count them and, if present in sufficiently large numbers, will result in overestimations. In the case of the MF and pasteurised milk cheese, this is not a problem since the counts of starter bacteria were  $> 10^8/g$  in most cases while the NSLAB were  $< 10^7/g$ . However, in the case of the raw milk cheese, counts of NSLAB and starter bacteria reached  $10^8/g$  within 11 weeks. This suggests that the counts of starter bacteria in this cheese may be overestimated.

One hundred isolates (50 from the raw milk cheese and 50 from the pasteurised cheese) were selected from the LBS agar plates used to count the NSLAB at 16 weeks. All the isolates were *Lactobacillus* but the species found in the raw milk cheese differed considerably from those in the pasteurised milk cheese (Table 3). *Lactobacillus casei* subsp. *casei* was the dominant species in the pasteurised cheese but the microflora of the raw milk cheese was more heterogeneous. Presumably, the lactobacilli in the pasteurised cheese originated from the cheesemaking equipment, environment, personnel, etc., whereas the lactobacilli in the raw milk cheese originated from these sources as well as from the milk.

### Sensory assessment

The scores for appearance, aroma and flavour, body, and overall acceptability are summarised in Table 4 for each of the three cheeses which were assessed by the two groups of judges; a 0–8 point scale (0 — poorest: 8 — best) was used for each attribute. The sensory qualities of the cheeses were not consistent between experiments but the ranking was similar, i.e. the cheeses made from pasteurised and MF milk were generally



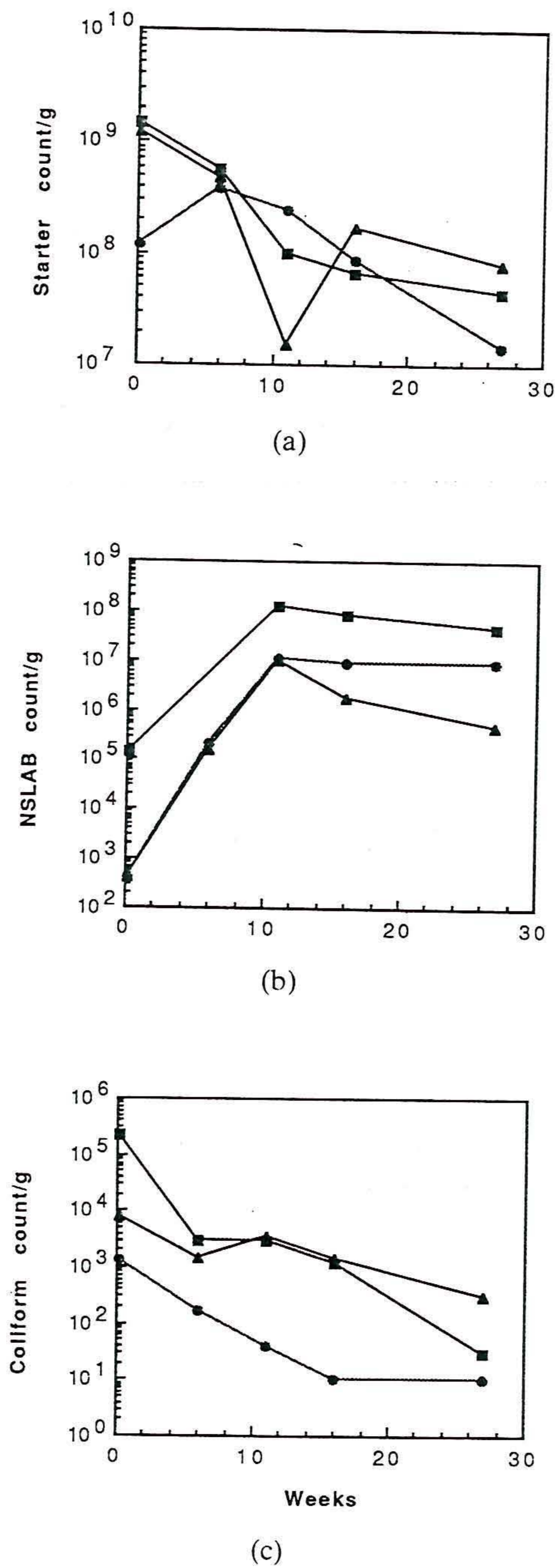


Fig. 1. Counts of (a) starter, (b) NSLAB, and (c) coliform bacteria during ripening of cheese made from (■) raw, (●) pasteurised, or (▲) microfiltered milk. Note the different scales on each ordinate.

**TABLE 3**  
Classification of Lactobacilli Isolated from Raw and Pasteurised Milk Cheeses<sup>a</sup>

Strain	Isolates (%)	
	Raw	Pasteurised
<i>L. plantarum</i>	8	0
<i>L. casei</i> subsp. <i>pseudopplantarum</i>	30	8
<i>L. casei</i> subsp. <i>casei</i>	38	88
<i>L. curvatus</i>	16	0
Others (including no identification)	8	4

<sup>a</sup>50 isolates—each were selected at random from raw and pasteurised milk cheeses isolated on LBS agar, incubated aerobically at 16 weeks.

**TABLE 4**  
Grades for Quality of Cheddar Cheeses made from MF, Raw and Pasteurised Milks

Attribute	Cheese <sup>a</sup>								
	MF			Raw			Pasteurised		
	1	2	3	1	2	3	1	2	3
Appearance	5.0	1.27	6.0	5.1	1.32	5.5	5.9	1.16	6.0
Flavour and aroma	4.8	1.12	7.0	3.7	1.82	4.0	4.9	1.50	7.0
Body	4.6	1.05	4.0	4.6	1.51	4.0	5.0	1.32	4.0
Overall quality	4.7	1.0	6.0	4.1	1.54	4.25	4.0	1.05	6.0

<sup>a</sup>1 — Mean. 2 —  $\pm$  standard deviation of scores by a nine member taste panel at 3 months (0 — poorest; 8 — best). 3 — Average of two Irish Department of Agriculture and Food graders at 4 months.

similar and different from that of the raw milk cheese, which was always the most strongly flavoured and was always regarded as atypical. The MF and pasteurised milk cheeses were considered to be of high quality and received generally equal grades from both sets of graders. Department of Agriculture and Food graders awarded somewhat higher grades to both cheeses than did the taste panel. The raw milk cheese was downgraded by both groups of graders, mainly on the basis of flavour and aroma which were considered atypical of Cheddar cheese currently available on the Irish market. The authors, who were not members of the taste panel, agreed that the raw milk Cheddar was atypical; however, they considered that this intensely flavoured cheese may be attractive to connoisseurs of traditional Cheddar cheese.

### Assessment of proteolysis

Urea-PAGE electrophoretograms of the three cheeses were similar at each sampling time (Fig. 2). However, differences were apparent in urea-PAGE of WSN of the raw milk cheese at 6 months (Fig. 3), although the levels of WSN were essentially the same in all cheeses (Fig. 4).

Analysis of the water-soluble extracts of the cheeses by the cadmium-ninhydrin method (which is highly sensitive for free amino acids), showed that the raw milk cheese contained considerably higher concentrations of free amino acids than the MF and pasteurised milk cheeses throughout ripening (Fig. 5). The concentration of free glutamic acid in the raw milk cheese was also higher than that in the other cheeses (Fig. 6). These results suggest a greater depth of proteolysis in the raw milk cheese, resulting from greater peptidase activity, presumably originating from the NSLAB.

RP-HPLC elution profiles of water-soluble extracts of the cheeses revealed considerable differences. Profiles of the cheeses at 2 days were similar, with only minor differences visible (Figs 7(a)–(c)). In each case, three major peaks (with elution times of  $\sim 3$ , 4.5 and 15 min) and minor

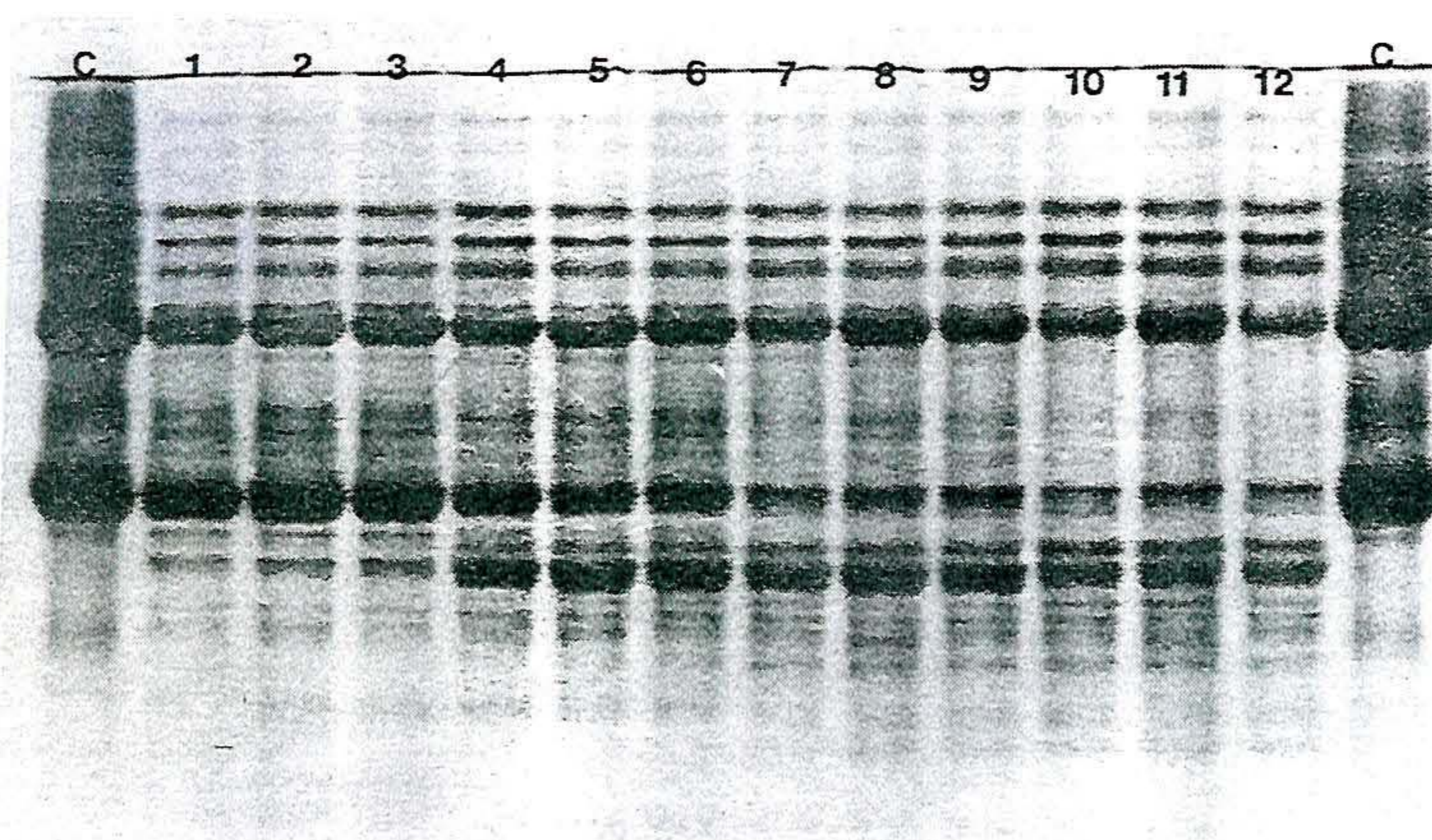
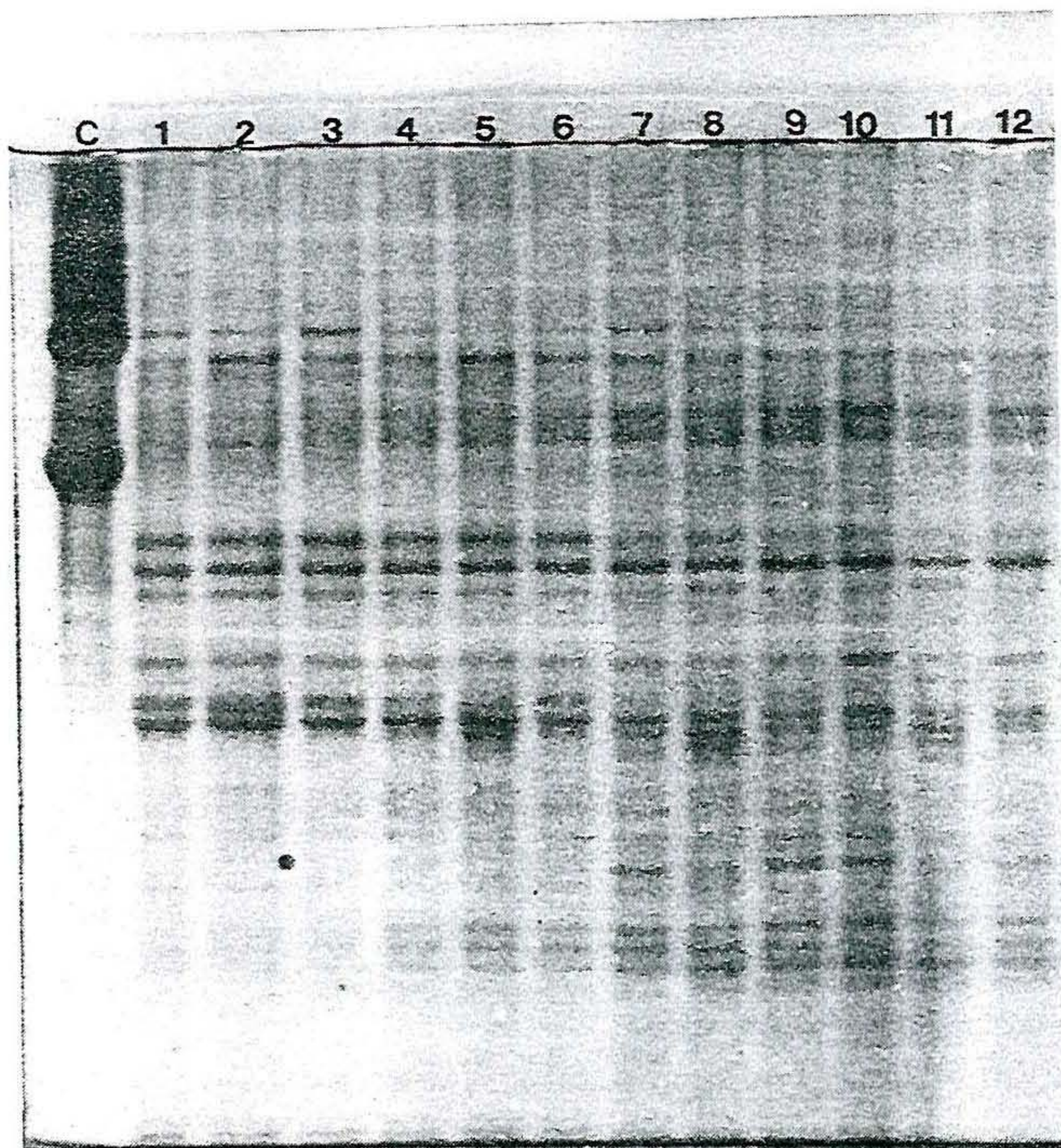


Fig. 2. Urea-PAGE of Na-caseinate (C) and Cheddar cheeses made from MF, raw or pasteurised milk at 2 days (1, 2, 3), 2 weeks (4, 5, 6), 3 months (7, 8, 9), and 6 months (10, 11, 12).



**Fig. 3.** Urea-PAGE of Na-caseinate (C) and water-soluble extracts of Cheddar cheeses made from MF, raw or pasteurised milk at 2 days (1, 2, 3), 2 weeks (4, 5, 6), 3 months (7, 8, 9), and 6 months (10, 11, 12).

peaks at  $\sim 7$  min and in the region 18–28 min were present. Much of the peptide material was eluted in the hydrophobic region (30–40 min).

At 3 months, the RP-HPLC profiles of WSN from the MF and pasteurised cheeses were essentially similar but that from the raw milk cheese was quite different, both qualitatively and quantitatively (Figs 7(d)–(f)). Qualitatively, the principal differences were in the peptides that eluted between 24 and 28 min and to a lesser extent between 32 and 36 min (i.e. hydrophobic peptides). The MF and pasteurised milk cheeses contained considerably higher concentrations of peptides eluting in both of these regions than the raw milk cheese and different peptides were also present — at least the relative proportions of the peptides present were markedly different. The raw milk cheese contained higher concentrations of the peptides with elution times of 16, 22 (two peaks) and 24 min.

The elution profiles at 6 months (Figs 7(g)–(i)) confirmed that the MF and pasteurised cheeses were similar (although quantitative differences are

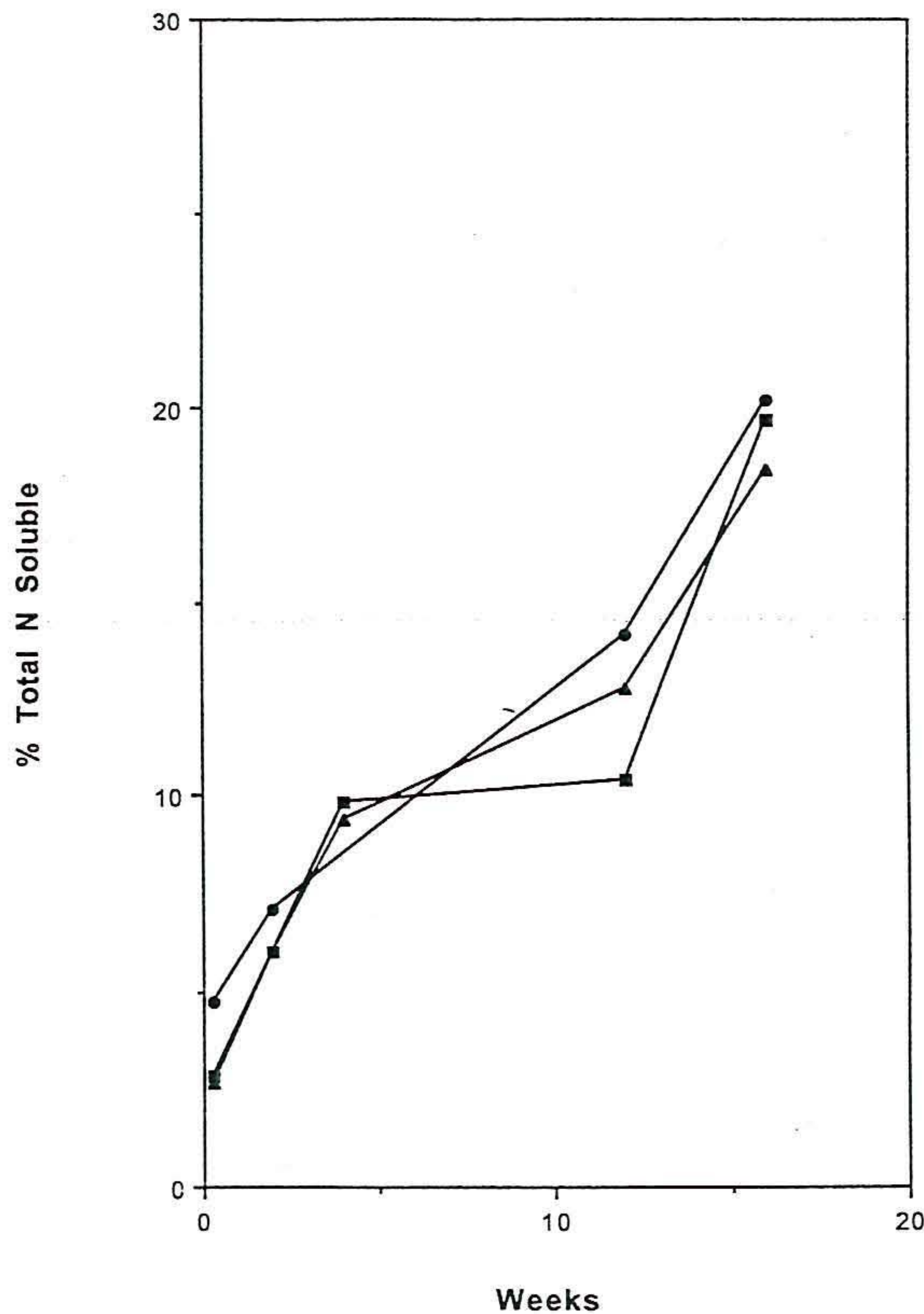


Fig. 4. Formation of water-soluble nitrogen in Cheddar cheeses made from (■) raw, (●) pasteurised, or (▲) microfiltered milk analysed in duplicate by the macro-Kjeldahl method.

apparent in the peak eluting at 14 min) and that the profile of the raw milk cheese was considerably different from the others. Differences were apparent throughout the region 16–32 min and there were quantitative differences in the relative proportions of the peptide eluting at 14 min.

#### Assessment of lipolysis

Total lipolysis (assessed by the copper soaps procedure) was the greatest in the raw milk cheese at all stages throughout ripening (Fig. 8); at 6 months, the concentration of the free fatty acids in the raw milk cheese was nearly twice that in the other cheeses. The MF cheese showed greater lipolysis than the pasteurised milk cheese, but a greater rate of increase in free fatty acid concentrations was apparent in the pasteurised milk cheese and both cheeses were equivalent at 6 months.

The profiles of individual saturated free fatty acids in the 6-month-old

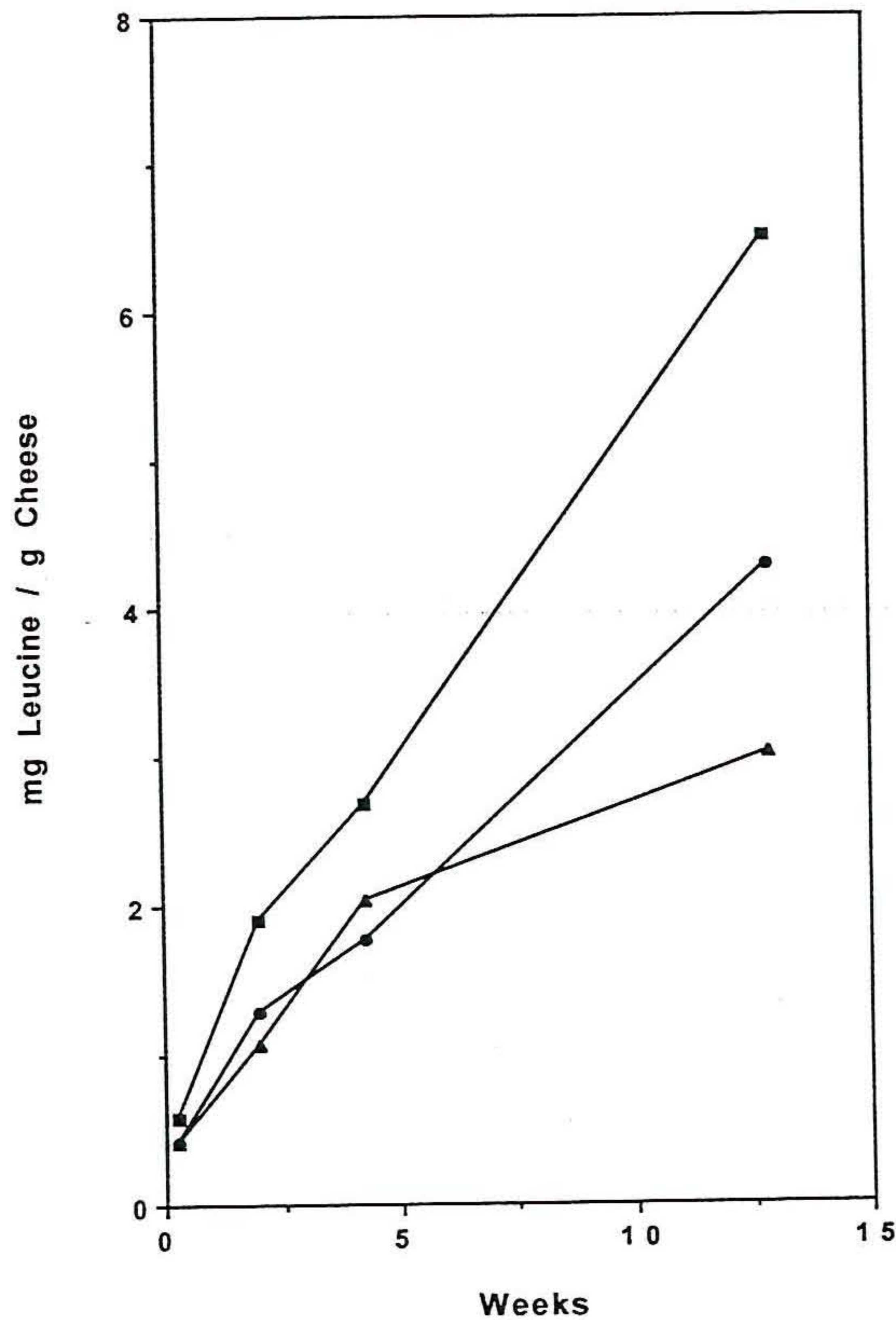


Fig. 5. Formation of total free amino acids in Cheddar cheeses made from (■) raw, (●) pasteurised, or (▲) microfiltered milk (as determined by the cadmium-ninhydrin assay). Values are means of duplicate analysis.

cheeses are shown in Fig. 9. The raw milk cheese contained the highest concentration of all free fatty acids. Caprylic ( $C_{8:0}$ ) and caproic ( $C_{6:0}$ ) were the dominant free fatty acids found.

## DISCUSSION

The results of this study demonstrate considerable differences between cheese made from raw milk and those made from pasteurised or MF milk. The pasteurised and MF cheeses were found to be very similar in terms of sensory quality, peptide profiles by RP-HPLC and urea-PAGE, amino acid nitrogen, free glutamic acid, water-soluble nitrogen and free fatty acids. The raw milk cheese matured more quickly (as determined by the formation of total free amino acids, glutamic acid and fatty acids) and

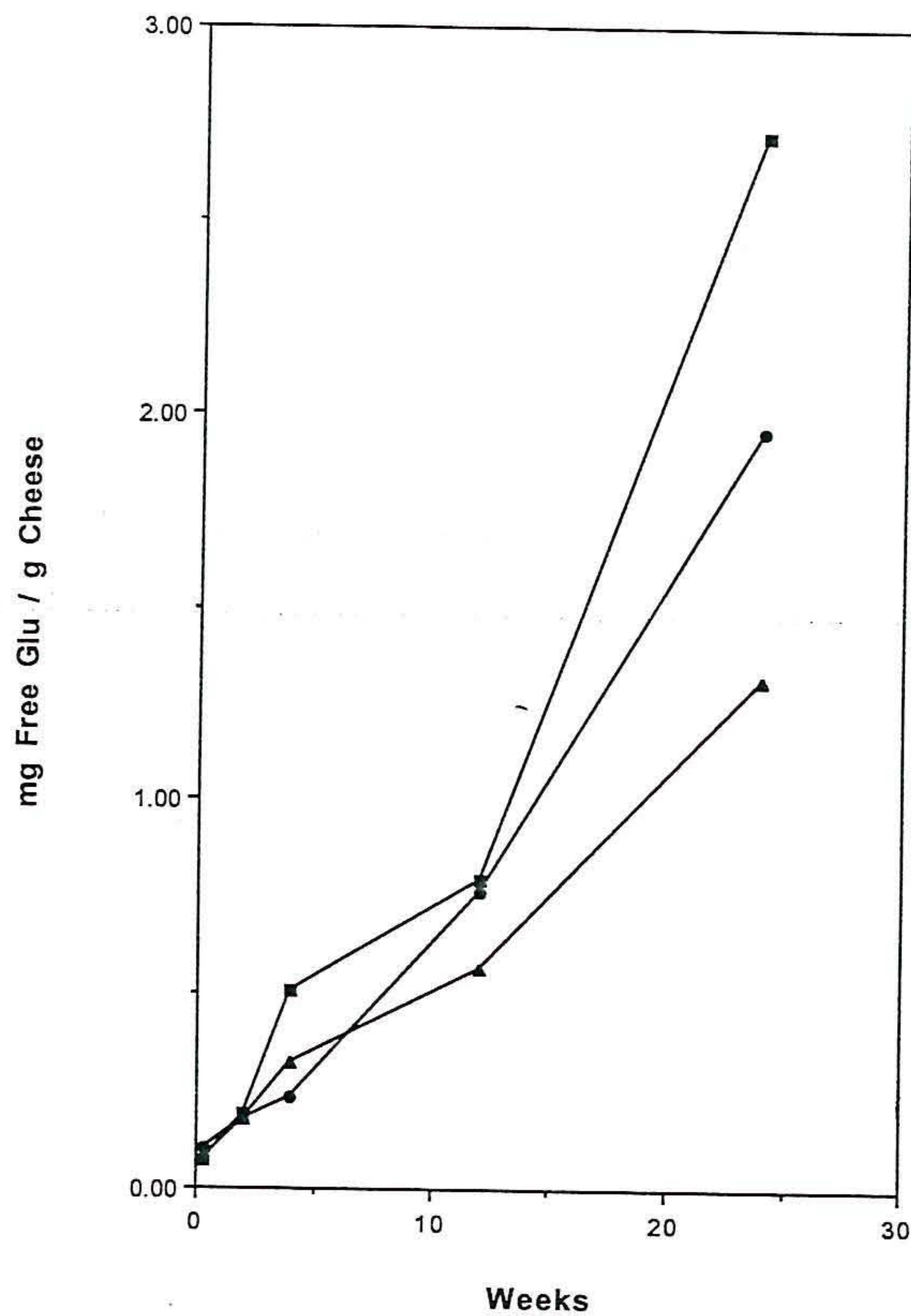


Fig. 6. Formation of free glutamic acid in Cheddar cheeses made from (■) raw, (●) pasteurised, or (▲) microfiltered milk. Values are means of duplicate analysis.

rapidly developed a strong flavour. The finding that the raw milk cheese ripened more quickly agrees with observations of Price and Call (1969) and Lau *et al.* (1991) and the accelerated formation of free amino acids agrees with the results of Bullock and Irvine (1956). Slight differences in the peptide profiles of the cheeses were indicated by urea-PAGE while RP-HPLC showed considerable differences between the peptide profiles of the pasteurised and MF cheeses, which were almost identical, and those of the raw milk cheese. Only the larger peptides in the water extract of Cheddar stain under the electrophoretic conditions used (O'Sullivan & Fox, 1990). Hence, the principal difference between the cheeses was in respect to small peptides (detectable by RP-HPLC but not by PAGE) and free amino acids, indicating a higher extent of proteolysis in the raw milk cheese, presumably due to proteinases and peptidases from NSLAB.

MF reduced the indigenous microflora of the cheese milk to very low levels without heat treatment. The MF cheese was similar in all respects to

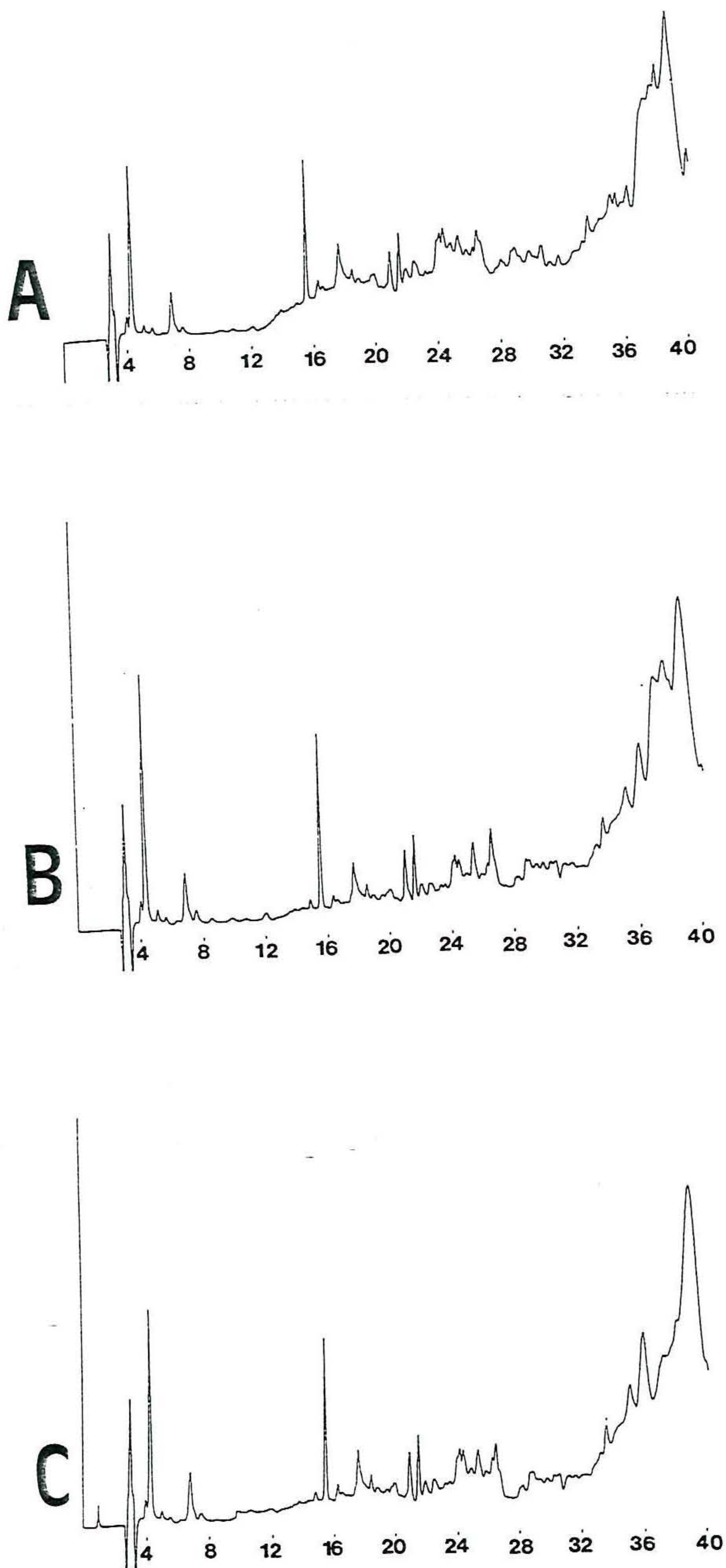


Fig. 7. Reversed-phase HPLC peptide profiles of water-soluble extracts from Cheddar cheeses made from MF, raw or pasteurised milks at (a, b, c) 2 days.



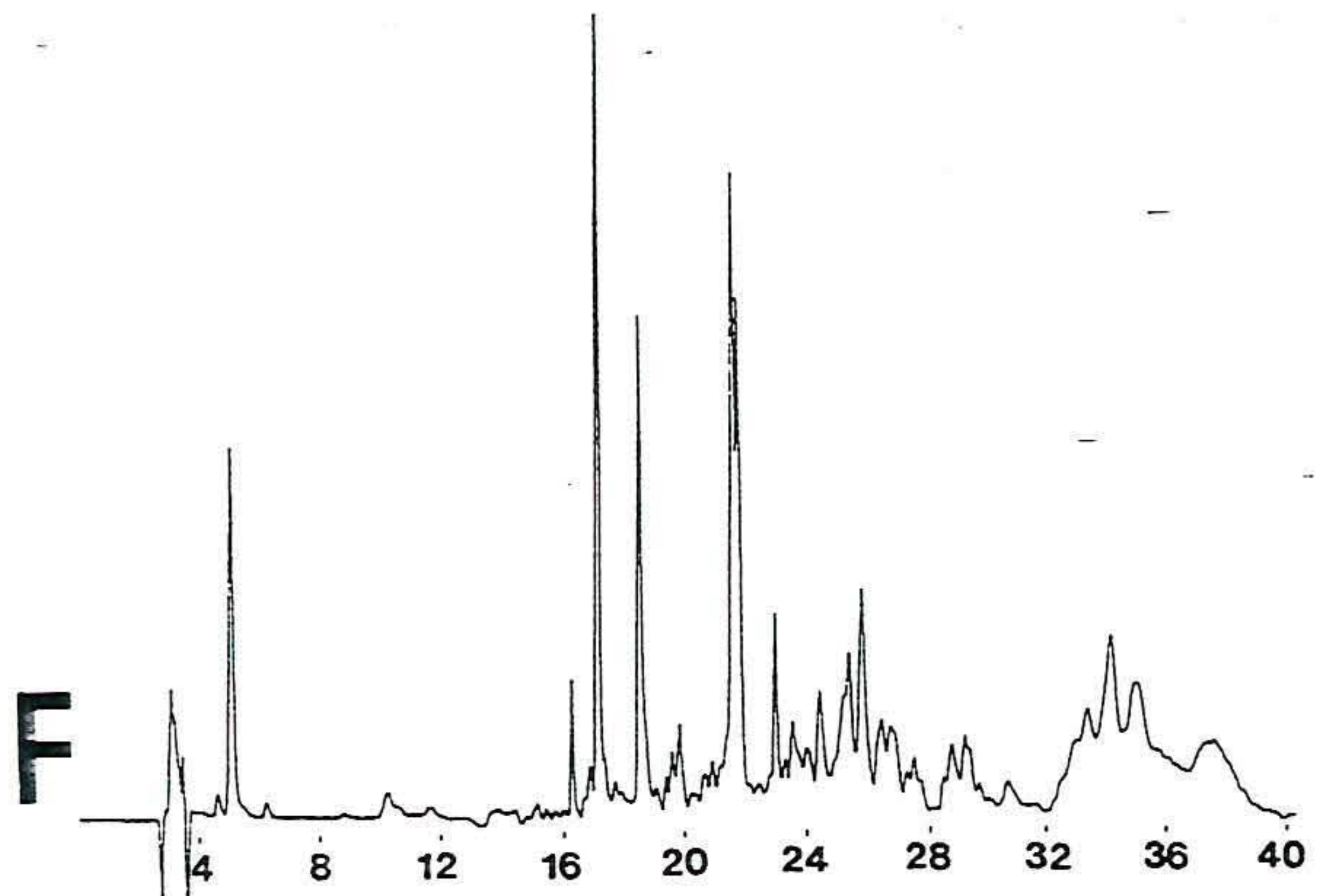
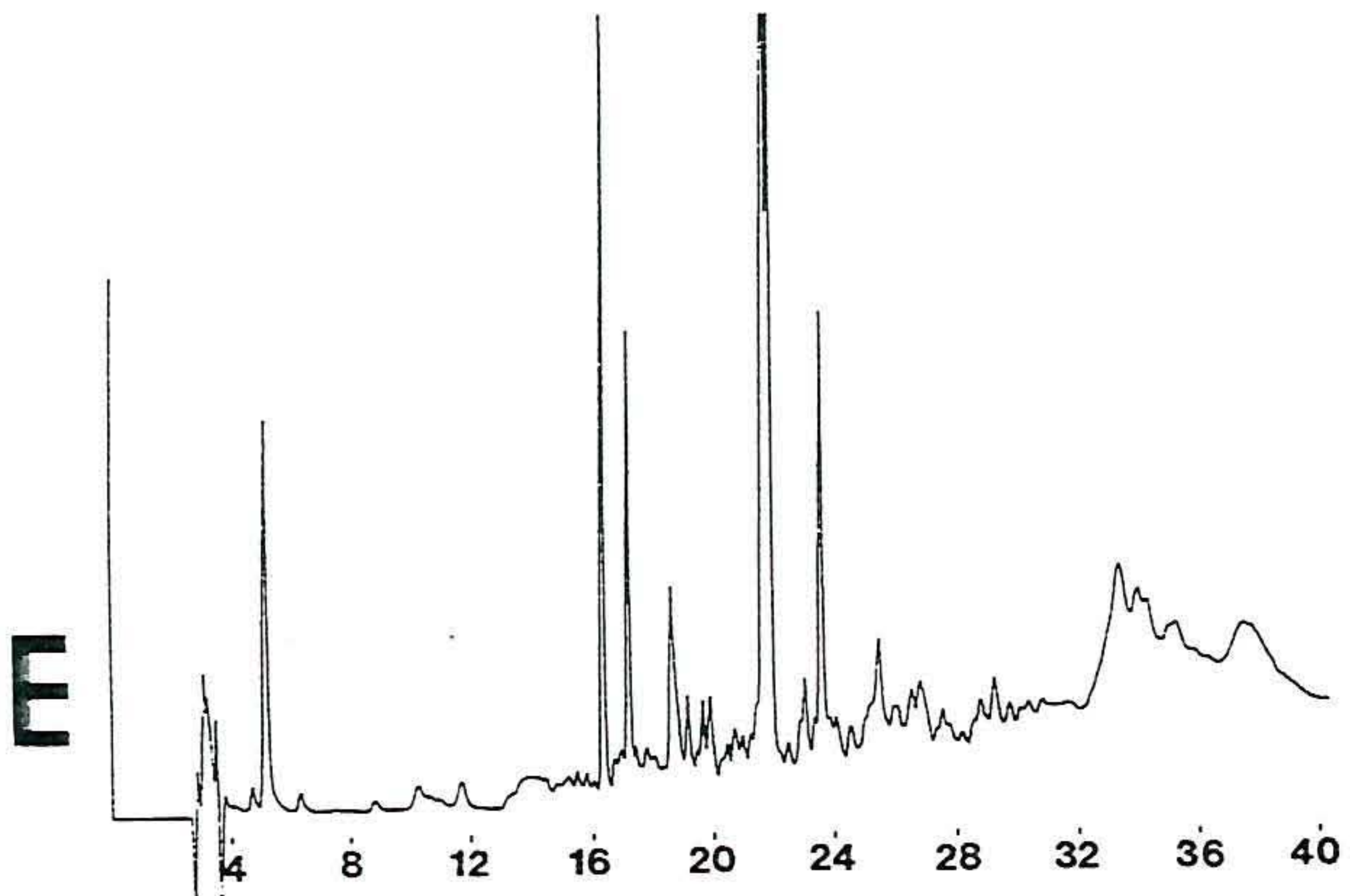
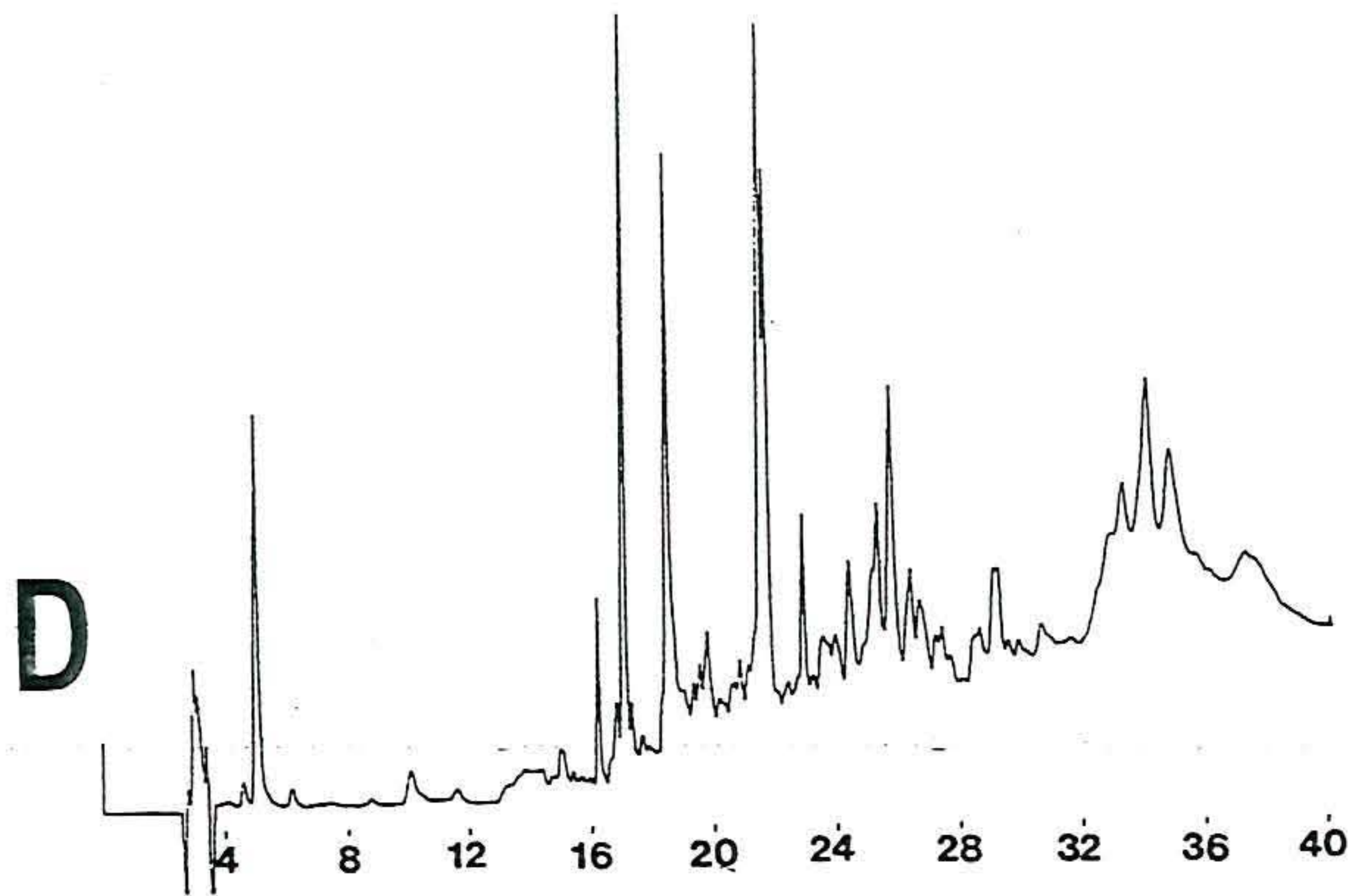


Fig. 7.—*contd.* (d, e, f) 3 months.

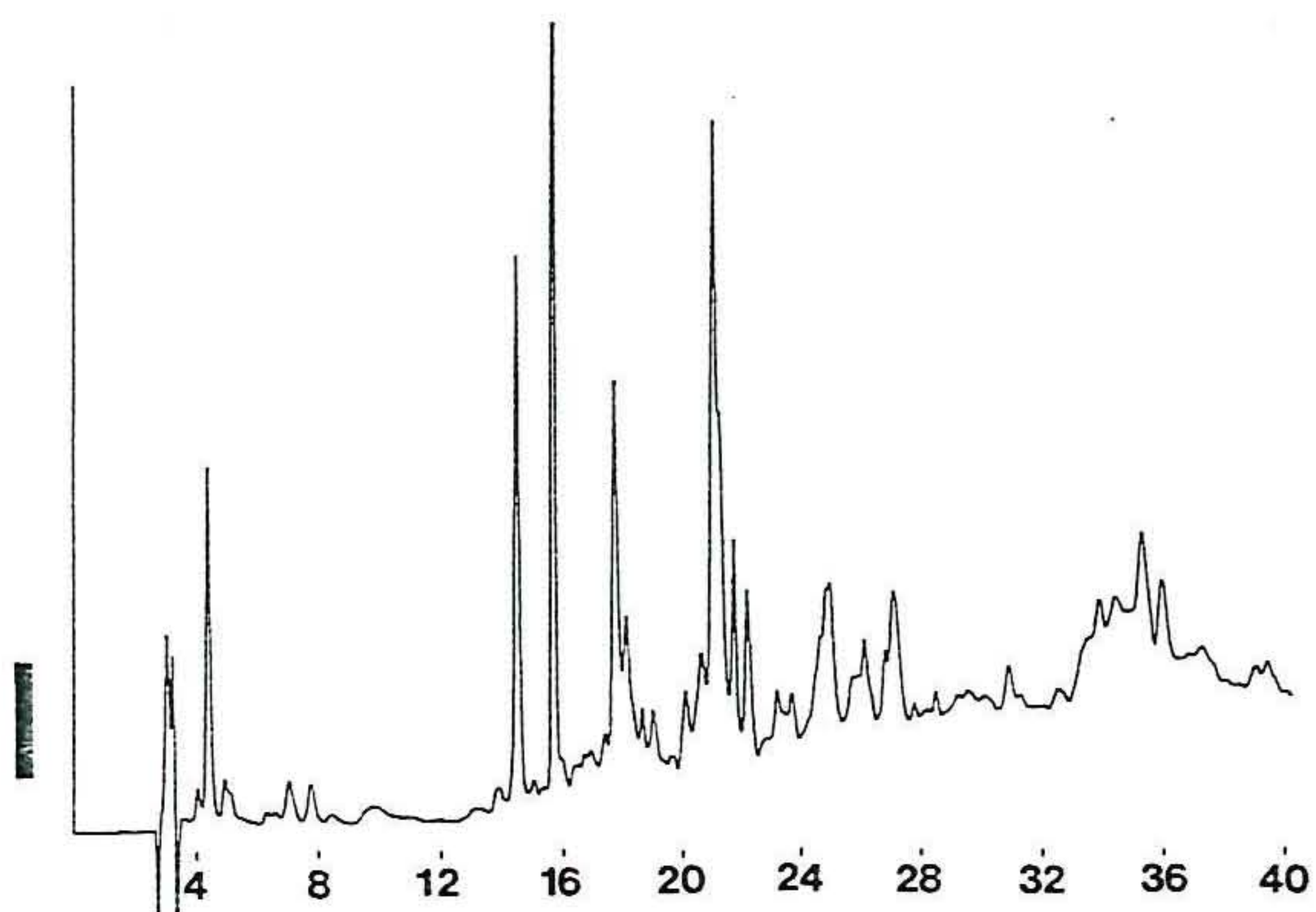
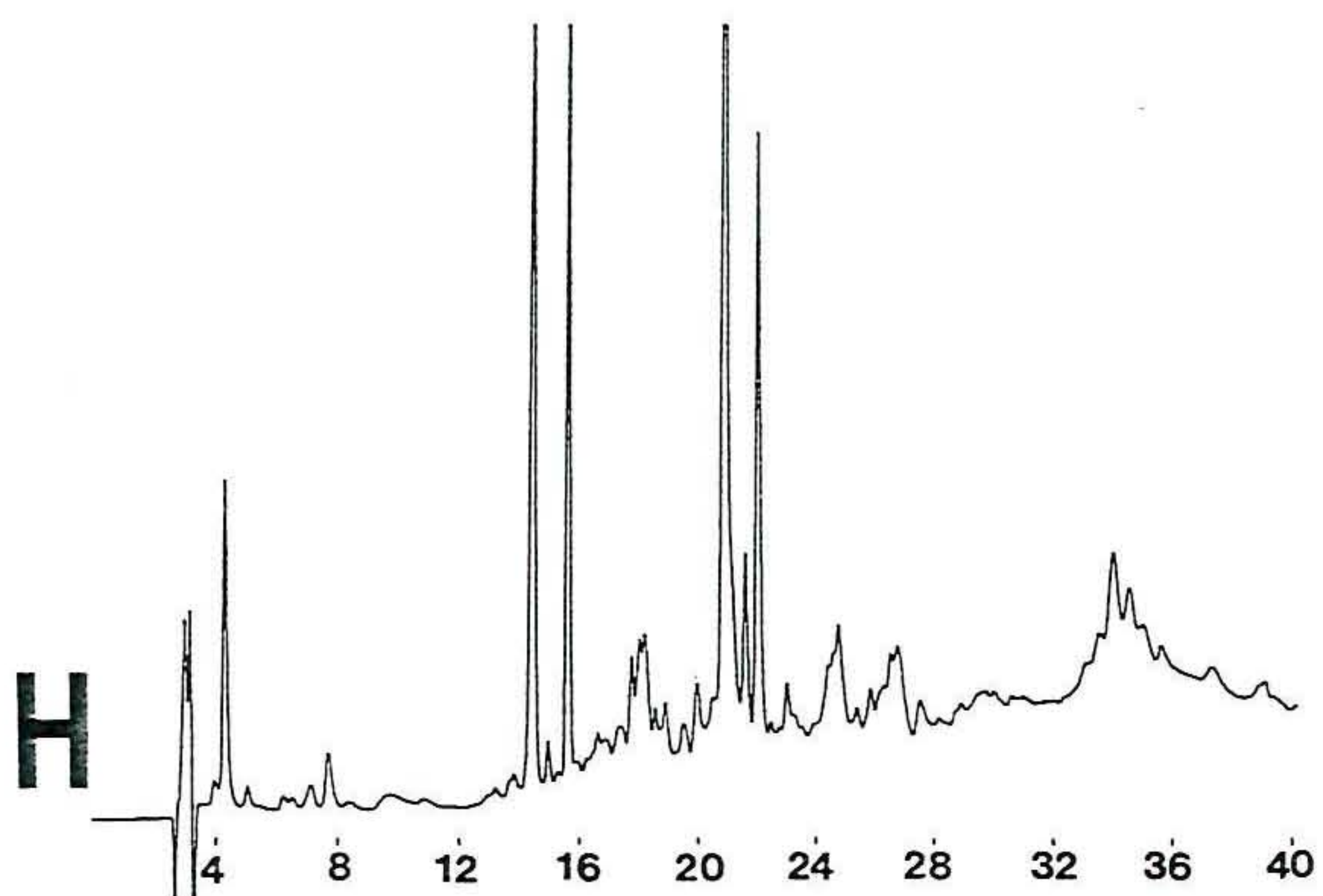
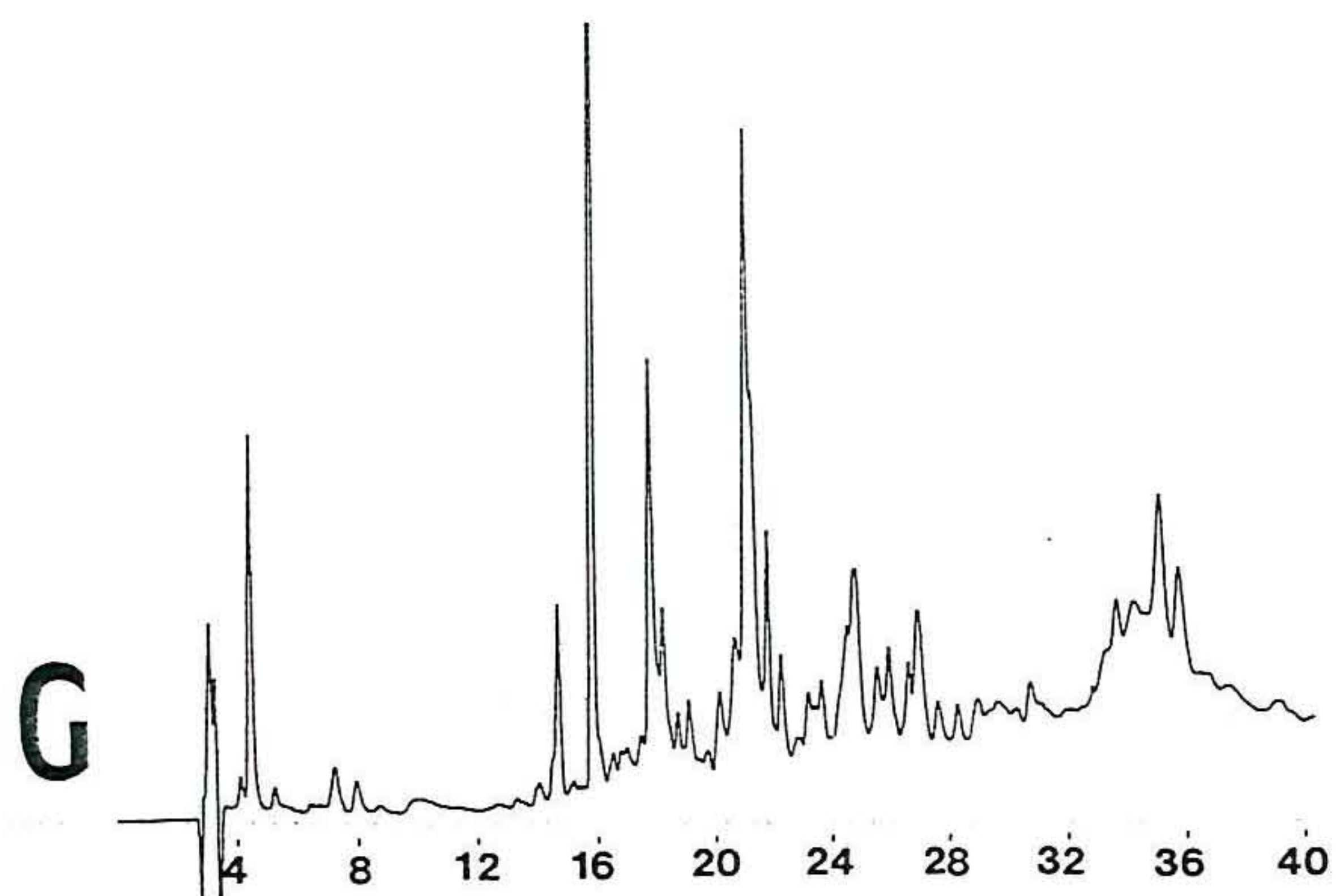


Fig. 7.—*contd.* (g, h, i) 6 months.

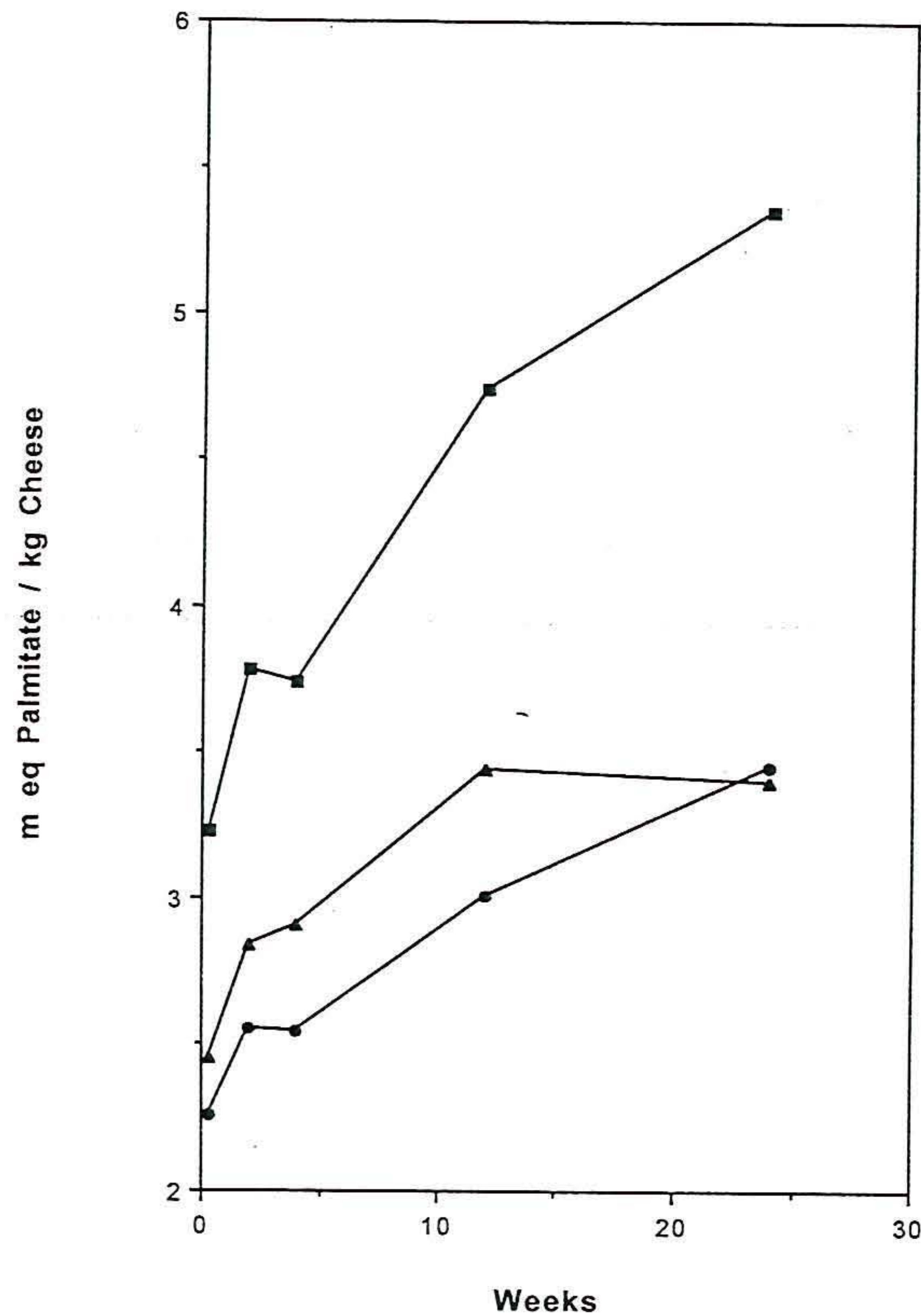


Fig. 8. Liberation of free fatty acids in Cheddar cheese made from (■) raw, (●) pasteurised, or (▲) microfiltered milk as determined by the copper soaps method.

the cheese made from pasteurised milk; the differences observed between these cheeses and the cheese made from raw milk are presumably due to the NSLAB, which were consistently higher in the raw milk cheese. This suggests that the non-starter microflora has a significant effect on proteolysis, lipolysis and overall quality of Cheddar cheese made from raw milk. This conclusion is at variance with that of Sherwood (1936). A number of authors have used lactobacilli as adjunct cultures to the starter *Lactococcus*; generally, a positive effect of lactobacilli on flavour was obtained although some authors found very little improvement (Peterson & Marshall, 1990).

*Lactobacillus* spp. dominated the non-starter flora of the cheeses and the strains found in the raw milk cheese were much more heterogeneous than those present in the pasteurised milk cheese. The flora of the raw milk cheese contained *L. casei* subsp. *casei*, *L. casei* subsp. *pseudopantarum* and *L. curvatus* while the pasteurised milk cheese contained mainly *L. casei*

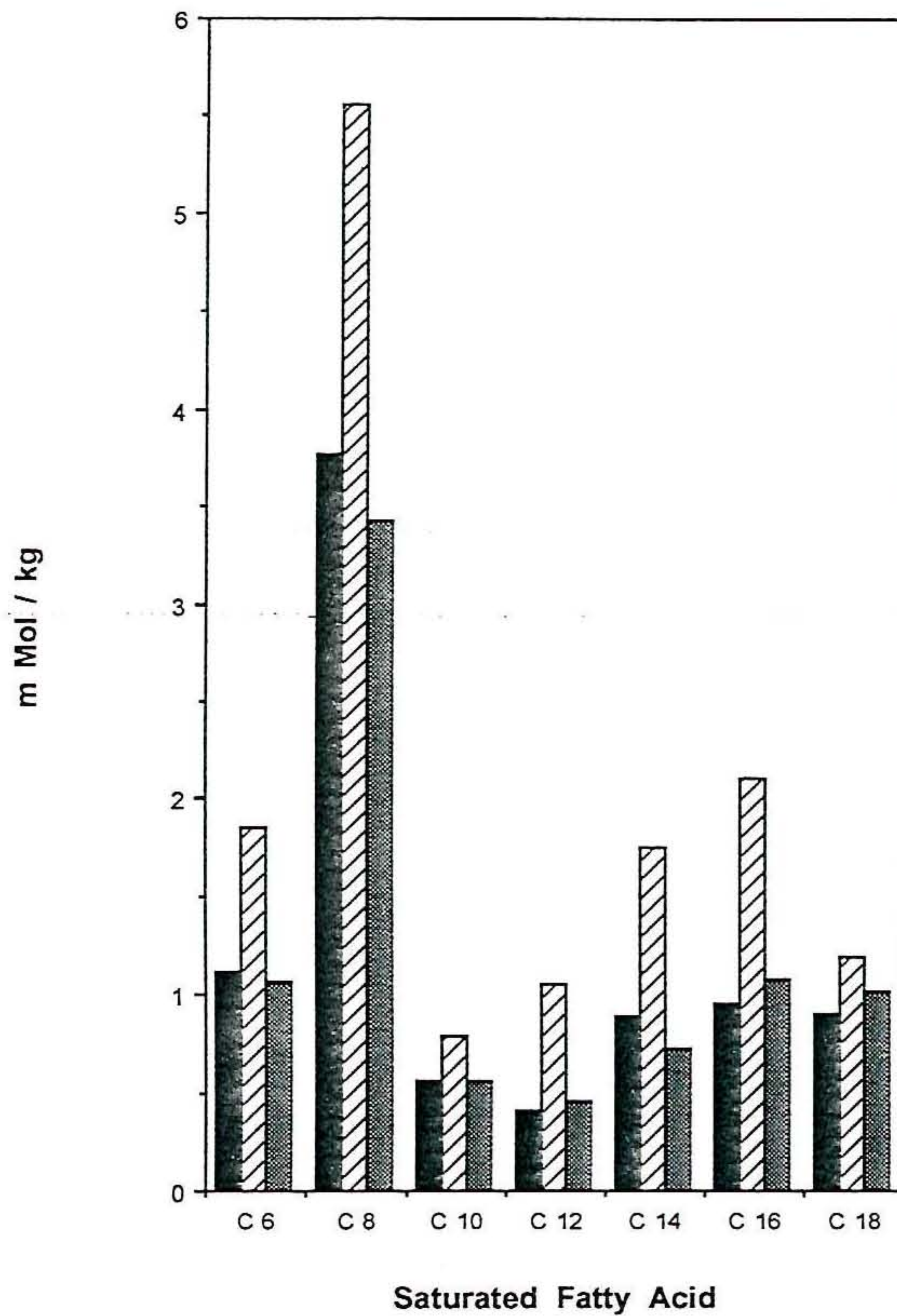


Fig. 9. Partial free fatty acid profile of Cheddar cheeses made from (▨) raw, (▤) pasteurised, or (■) microfiltered milk.

subsp. *casei*. A variety of proteolytic and peptidolytic enzymes have been identified in the NSLAB isolated from cheese (Khalid & Marth, 1990*a, b*; Peterson *et al.*, 1990) and the differences in proteolysis observed in this study presumably reflect differences in the microflora, both quantitatively and qualitatively. It is likely that the lactobacilli present in the pasteurised milk cheese were the result of post-pasteurisation contamination (Peterson & Marshall, 1990).

The coliform levels in the MF and pasteurised cheeses were rather high initially but all counts, including the raw cheese, decreased during ripening. It is unlikely that levels of coliform of  $10^4$ /g had any effect on proteolysis.

The MF cheese was manufactured from milk prepared by recombining raw skim milk and pasteurised cream to give a fat percentage similar to that of the raw milk. Many of the indigenous enzymes in milk are concentrated on the fat globule membrane (Kitchen, 1985) and some of these would have been inactivated or attenuated by pasteurisation. It is thus possible that

indigenous enzymes in the cream phase could have been responsible for the differences observed. However, preliminary results of a further study involving the manufacture of Cheddar cheese from raw skim milk and pasteurised cream show that little differences in flavour are detectable between this and raw milk Cheddar cheeses, indicating that the fat globule enzymes may not have been responsible for the flavour differences observed between the raw and pasteurised milk cheeses found in this study.

### CONCLUSIONS

The importance of the non-starter microflora in Cheddar cheese for proteolysis, lipolysis and flavour development was demonstrated in this study. Differences observed between the raw and pasteurised milk cheeses were ascribed to the elimination of the non-starter microflora rather than heat-induced changes in indigenous enzymes. Differences in proteolysis observed were due to peptidases of the non-starter lactobacilli. The microflora of the raw and pasteurised milk cheeses differed but all strains identified were lactobacilli. Presumably, the lactobacilli in the pasteurised milk cheese resulted from post-process contamination while those in the raw milk cheese originated also from the milk. Cheddar cheeses made from pasteurised and MF milk were similar in all respects studied.

### ACKNOWLEDGEMENT

The authors wish to thank Mr F. Drinan and Mr E. Mulholland for technical assistance.

### REFERENCES

- Andrews, A. T. (1983). Proteinases in normal bovine milk and their action on caseins. *J. Dairy Res.*, **50**, 45-55.
- AOAC (1984). *Official Methods of Analysis of the Association of Official Analytical Chemists*, 14th Edn, ed. S. Williams. Association of Official Analytical Chemists Inc., Arlington, VA.
- Blakesley, R. W. & Boezi, J. A. (1977). A new staining technique for proteins in polyacrylamide gels using Coomassie Brilliant Blue G250. *Anal. Biochem.*, **82**, 580-1.
- Bullock, D. H. & Irvine, O. R. (1956). A chromatographic study of Cheddar cheese ripening. *J. Dairy Sci.*, **39**, 1229-35.
- Bynum, D. G., Senyk, G. F. & Barbano, D. M. (1984). Determination of free fatty

- acid content of Cheddar cheese by a copper soaps method. *J. Dairy Sci.*, **67**, 1521–4.
- deMan, J. C., Rogosa, M. & Sharpe, M. E. (1960). A medium for the cultivation of lactobacilli. *J. Appl. Bacteriol.*, **23**, 130–5.
- Folkertsma, B. & Fox, P. F. (1992). Use of the Cd-ninhydrin reagent to assess proteolysis in cheese during ripening. *J. Dairy Res.*, **59**, 217–24.
- Fox, P. F. (1989). Proteolysis during cheese manufacture and ripening. *J. Dairy Sci.*, **72**, 1379–400.
- Kandler, O. & Weiss, N. (1984). Regular, non-sporing Gram-positive rods. In *Bergey's Manual of Systematic Bacteriology* (1st edn, Vol 2), eds P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Williams & Williams, Baltimore, MA, USA, pp. 1208–43.
- Khalid, N. M. & Marth, E. H. (1990a). Lactobacilli — their enzymes and role in ripening and spoilage of cheese: a review. *J. Dairy Sci.*, **73**, 2669–84.
- Khalid, N. M. & Marth, E. H. (1990b). Proteolytic activity by strains of *Lactobacillus plantarum* and *Lactobacillus casei*. *J. Dairy Sci.*, **73**, 3068–76.
- Kitchen, B. J. (1985). Indigenous milk enzymes. In *Developments in Dairy Chemistry — 3*, ed. P. F. Fox. Elsevier Applied Science Publishers, Barking, UK, pp. 239–79.
- Kuchroo, C. N. & Fox, P. F. (1982). Soluble nitrogen in Cheddar cheese: comparison of extraction procedures. *Milchwissenschaft*, **37**, 331–5.
- Lau, K. Y., Barbano, D. M. & Rasmussen, R. R. (1990). Influence of pasteurization on fat and nitrogen recoveries and Cheddar cheese yield. *J. Dairy Sci.*, **73**, 561–70.
- Lau, K. Y., Barbano, D. M. & Rasmussen, R. R. (1991). Influence of pasteurization of milk on protein breakdown in Cheddar cheese during ripening. *J. Dairy Sci.*, **74**, 727–40.
- Martinez-Castro, I., Alanso, L. & Juarez, M. (1986). Gas chromatographic analysis of free fatty acids and glycerides of milk fat using tetramethylammonium hydroxide as catalyst. *Chromatographia*, **21**, 37–40.
- Martley, F. G. & Lawrence, R. C. (1972). Cheddar cheese flavour. II: characteristics of single strain starters associated with good or poor flavour development. *NZ J. Dairy Sci. Technol.*, **7**, 38–50.
- Maubois, J.-L. (1989). *Applications of Membrane Techniques in the Dairy Industry — Proposals for a new Group of IDF Experts* (Bulletin 244). International Dairy Federation, Brussels, Belgium, pp. 26–9.
- O'Sullivan, M. & Fox, P. F. (1990). A scheme for the partial fractionation of cheese peptides. *J. Dairy Res.*, **57**, 135–9.
- Peterson, S. D. & Marshall, R. T. (1990). Nonstarter lactobacilli in Cheddar cheese: a review. *J. Dairy Sci.*, **73**, 1395–410.
- Peterson, S. D., Marshall, R. T. & Heymann, H. (1990). Peptidase profiling of lactobacilli associated with Cheddar cheese and its application to identification and selection of strains for cheese-ripening studies. *J. Dairy Sci.*, **73**, 1454–64.
- Price, W. V. & Call, A. O. (1969). Cheddar cheese: comparison of effects of raw and heated milk on quality and ripening. *J. Milk Food Technol.*, **32**, 304–11.
- Scarpellino, R. & Kosikowski, F. V. (1962). Evolution of volatile compounds in ripening raw and pasteurised milk Cheddar cheese observed by gas chromatography. *J. Dairy Sci.*, **45**, 343–8.
- Sharpe, M. A. (1981). The genus *Lactobacillus*. In *The Prokaryotes, A Handbook*

- on Habitats, Isolation and Identification of Bacteria* (Vol 2), eds M. P. Starr, H. Stolp, H. G. Truper, A. Balows & H. G. Schlegel. Springer-Verlag, Berlin, Germany, pp. 1653–79.
- Sherwood, I. R. (1936). Observations on the ripening of cheeses made from raw and pasteurised milk. *J. Dairy Res.*, **7**, 271–83.
- Terzaghi, B. E. & Sandine, W. E. (1975). Improved media for lactic streptococci and their bacteriophages. *Appl. Microbiol.*, **29**, 807–13.
- Visser, F. M. W. (1977). Contribution of enzymes from rennet, starter bacteria and milk to proteolysis and flavour development in Gouda cheese. 1. Description of cheese and aseptic cheesemaking techniques. *Neth. Milk Dairy J.*, **31**, 120–33.



## Evaluation Instrumentale et Sensorielle de Certaines Propriétés Texturales de Fromages à Pâte Molle

D. Hennequin & J. Hardy

Laboratoire de Physicochimie et de Génie Alimentaires, ENSAIA, 2 Avenue de la Forêt de Haye, 54500 Vandoeuvre Lès Nancy, France

(Received 5 March 1992; revised version received and accepted 31 July 1992)

### ABSTRACT

*The textural properties of 19 soft cheese varieties have been studied by instrumental and sensory techniques. Significant correlations have been observed between the sensory assessment of cheese firmness and strength recorded by penetrometry and uniaxial compression. Regression models validate the instrumental methods used. The penetrometric method is well adapted to soft cheese measurement. Indeed, very good correlations have been obtained and the utilization of this technique proves to be quick and easy.*

*Cheese composition has also been studied in order to determine the contribution of physicochemical parameters in the textural properties. The analysis of cheese varieties data by stepwise regression showed that pH, dry matter, fat and calcium contents are the main parameters which explain the textural properties of soft cheeses.*

### RESUME

*Les propriétés texturales de 19 variétés de fromages à pâte molle ont été étudiées par voie instrumentale et par voie sensorielle. Des corrélations significatives ont été observées entre l'évaluation sensorielle de la fermeté des fromages et les forces enregistrées par les techniques de pénétration et de compression uniaxiale. Les modèles de régression obtenus valident les méthodes instrumentales employées.*

*La composition des fromages a aussi été étudiée afin de déterminer les*