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Gas Chromatographic Analysis of Free Carboxylic Acids in Foods Using a Micropacked Column

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ABSTRACT

A micropacked column packed with FFAP 4% on desilanized Volaspher A-2 is proposed for determining free carboxylic acids (C_2–C_{20}, and $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, i-C_4 and i-C_5) without derivatization.

The column has high efficiency and separates several carboxylic acids in extracts of food samples without a separate acid extraction. For very complex real mixtures, an additional extraction provides an excellent acid profile.

Thermal degradation was not observed for unsaturated acids and the accuracy and precision of the analysis were good.

INTRODUCTION

In recent years much attention has been given to the analysis of carboxylic acids. These compounds are ubiquitous in food and living organisms. For food processing, knowledge of the amounts of specific carboxylic acids can indicate possible adulteration or microbial activity. Some fatty acids are interesting as flavour factors (Suomalainen *et al.*, 1974; Clapperton, 1978) and also can be responsible for specific off-flavours (Hawthorne et al., 1986). Caprilic and capric acids are toxins for yeasts and consequently they can

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inhibit alcoholic fermentation (Geneix *et al.*, 1983). Moreover, knowledge of carboxylic acid profiles helps to determine whether a fat sample is derived from an animal or a vegetal source. Consequently, rapid and reliable methods of analysis of carboxylic acids are needed.

Gas chromatographic analysis of free carboxylic acids is difficult due to the low volatility of the longer chain acids and to the relatively high polarities of the shorter chain length acids. This often results in adsorption and ghosting. Derivatization has been widely used (Metcalfe & Wang, 1981; Kochar & Matsui, 1983; Martinez-Castro *et al.*, 1986) but discrimination (i.e. unequal reactivity of the various acids towards the derivatising reagent) can be a problem (Metcalfe & Wang, 1981). In any case the technique is time consuming and direct injection of free carboxylic acids should be further considered (Julin *et al.*, 1983).

Use of HPLC for free carboxylic acid analysis involves some problems in detection. Although derivatives have been measured using both UV and fluorescence detectors (Chaytor, 1987), some UV detector modifications have been considered that permit gradient elution chromatography which normally causes baseline instability (Stolywho *et al.*, 1985). Actually, refractive index retention has been most widely used although it is not compatible with gradient elution.

Supercritical Fluid Chromatography (SFC) is considered as a good choice to analyze thermally labile acids of low volatility (Novotny *et al.*, 1981; White & Houck, 1986). Nowadays high efficiencies with SFC are available and the technique has already proved its usefulness in the analysis of saturated and unsaturated carboxylic acids with packed (Hellgeth *et al.*, 1986) and capillary columns (Markides *et al.*, 1986). However, further work is necessary on possible ways of improving the selectivity of the technique.

Previously we reported our study of micropacked columns for gas chromatography (Reglero *et al.*, 1985). These columns provide high sample capacity as well as high efficiencies and can be considered as an alternative to thick film capillary columns for specific purposes (Herraiz *et al.*, 1987). Also micropacked columns have an important advantage: due to their relatively high optimal flow rates they are compatible with standard injection systems. Therefore, quantitative results are not affected by discrimination. Moreover, high efficiency micropacked columns can be prepared from a wide range of single or mixed phases. Micropacked columns have been successfully loaded with phases designed for specific separation (Reglero *et al.*, 1986).

In this work we report our results on the analysis of C_2–C_{20} (including $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, *i*-C_4 and *i*-C_5) free carboxylic acids, by using a micropacked column. Separations of carboxylic acids in extracts of real samples (grape must, wine, wine distillate, milk and cheese) are included as examples.

EXPERIMENTAL

Materials and instrumentation

The standards of saturated acids C_2–C_{18} were obtained from Polyscience Corporation and the unsaturated acids and arachidic acid from Fluka.

The chromagraphic system consisted of a Perkin-Elmer Model 8320 Gas Chromatograph with the required software to integrate peak areas. The chromatograph was equipped with a PTV injector operating as a standard hot injector (350°C) and with a FID (350°C).

The column was connected to injector and detector by short capillary tubes (0·2 mm i.d.) of empty fused silica. The union with the injector was 5 cm long and with the detector was 15 cm long. In the latter case the fused silica tube passed through the stainless steel piece of the detector up to the flame and avoided contact of the sample with metallic surfaces.

The column was prepared according to a previously reported procedure (Reglero *et al.*, 1985). Desilanized Volaspher A-2 (Merck) 120–140 mesh was used as solid support with 4% (w/w) of FFAP loading as stationary phase. The pyrex tubing used (7·5 m × 0·6 mm i.d.) was previously deactivated by treatment with the stationary phase itself at 350°C for 24 h (Schomburg *et al.*, 1979).

The oven temperature was 90°C for 5 min; it then was raised at 5°C/min to 275°C for 10 min.

Hydrogen (P-10 grade) was used as carrier gas (15 kg/cm^2).

Sample preparation

For the grape must, wine and wine distillate, 250 ml of sample was continuously extracted with 150 ml of Freon 11 for 24 h. A 30 ml sample of the extract was subsequently concentrated at 32°C to 0·3 ml under a 30 cm Vigreaux column. An aliquot (5 μl) was injected into the GC in each case.

For wine and wine distillate, the acids were separated from the unconcentrated Freon 11 extract by shaking with a NaOH solution (pH = 10·5) The aqueous solution was then re-extracted with Freon 11 after it was acidified to pH = 1 with HCl (De Rijke & Ter Heide, 1983). The Freon 11 solution was concentrated and injected as above.

A 250 ml sample of cow's milk was precipitated with trichloroacetic acid (10%) and the filtrate was extracted with 150 ml Freon 11. The concentration and injection were carried out as above.

Blue (Gamonedo) cheese (10 g) was extracted under reflux with 2 ml of *n*-pentane (De Frutos, M. *et al.*, unpublished method). The pentane solution was concentrated to 1 ml and 5 μl sample was injected.

RESULTS AND DISCUSSION

Figure 1 shows the chromatogram of the separation of 21 saturated and unsaturated carboxylic acids with carbon number from 2 to 20. The separation was made using the micropacked column described in the experimental section. This column has an appreciable efficiency as well as a sufficient sample capacity (Table 1). No loss of efficiency was observed below 60 ng of compound injected. These qualities are useful to the column for the analysis of complex samples.

Figures 2 and 3 show the separation of some carboxylic acids in extracts of wine and wine distillate. Chromatograms of samples with acid separation treatments (Figs 2a and 3a) are simpler than those of full extracts (Figs 2b and 3b). However, in the latter case determination of the acids is possible because the column has sufficient resolution power. Consequently, the second extraction could be skipped to eliminate discrimination for this step.

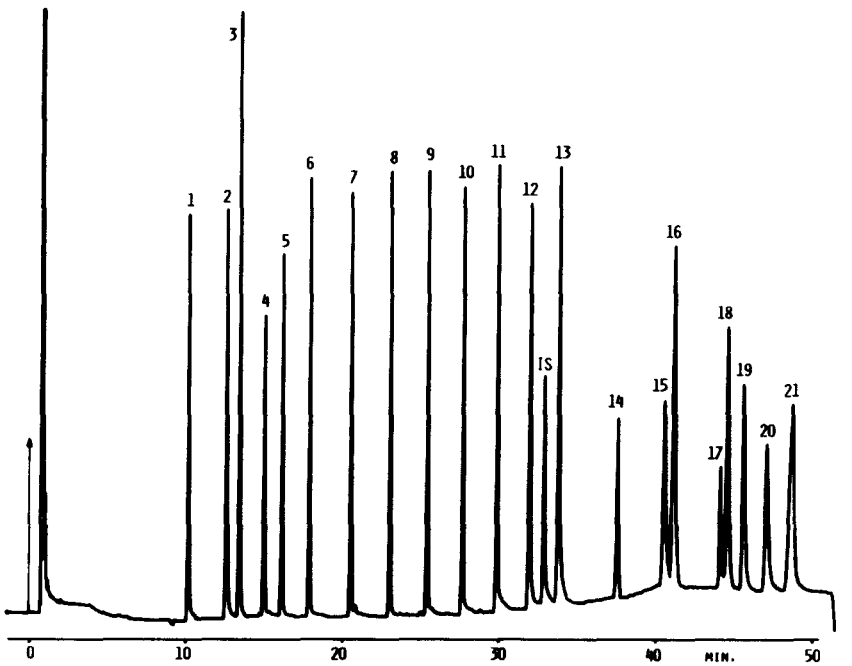

Fig. 1. Chromatogram of a synthetic solution of carboxylic acids. See conditions in the text. Peak identification: 1. acetic acid (C_2); 2. propionic acid (C_3); 3, isobutyric acid (i-C_4); 4. butyric acid (C_4); 5. isovaleric acid (i-C_5); 6. valeric acid (C_5); 7. caproic acid (C_6); 8. oenantic acid (C_7); 9. caprilic acid (C_8); 10. pelargonic acid (C_9); 11. capric acid (C_{10}); 12. undecanoic acid (C_{11}); 13. lauric acid (C_{12}); 14. myristic acid (C_{14}); 15. palmitic acid (C_{16}); 16. palmitoleic acid ($C_{16:1}$); 17. stearic acid (C_{18}); 18. oleic acid ($C_{18:1}$); 19. linoleic acid ($C_{18:2}$); 20. linolenic acid ($C_{18:3}$); 21. arachidic acid (C_{20}); IS internal standard (10-undecenoic acid).

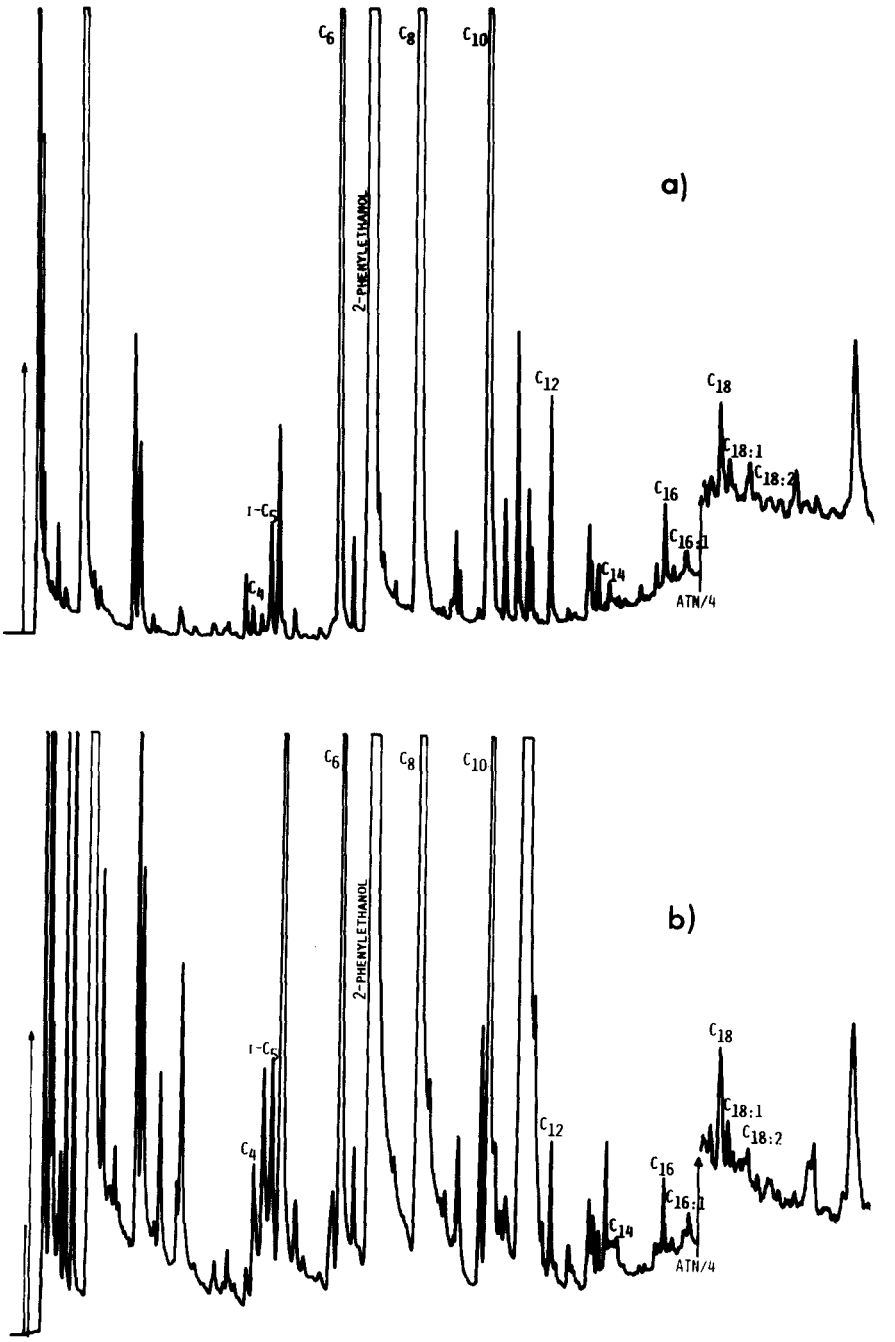

Fig. 2. Chromatograms of an extract of wine. (a) Extract after acid separation. (b) Full extract. See conditions in the text.

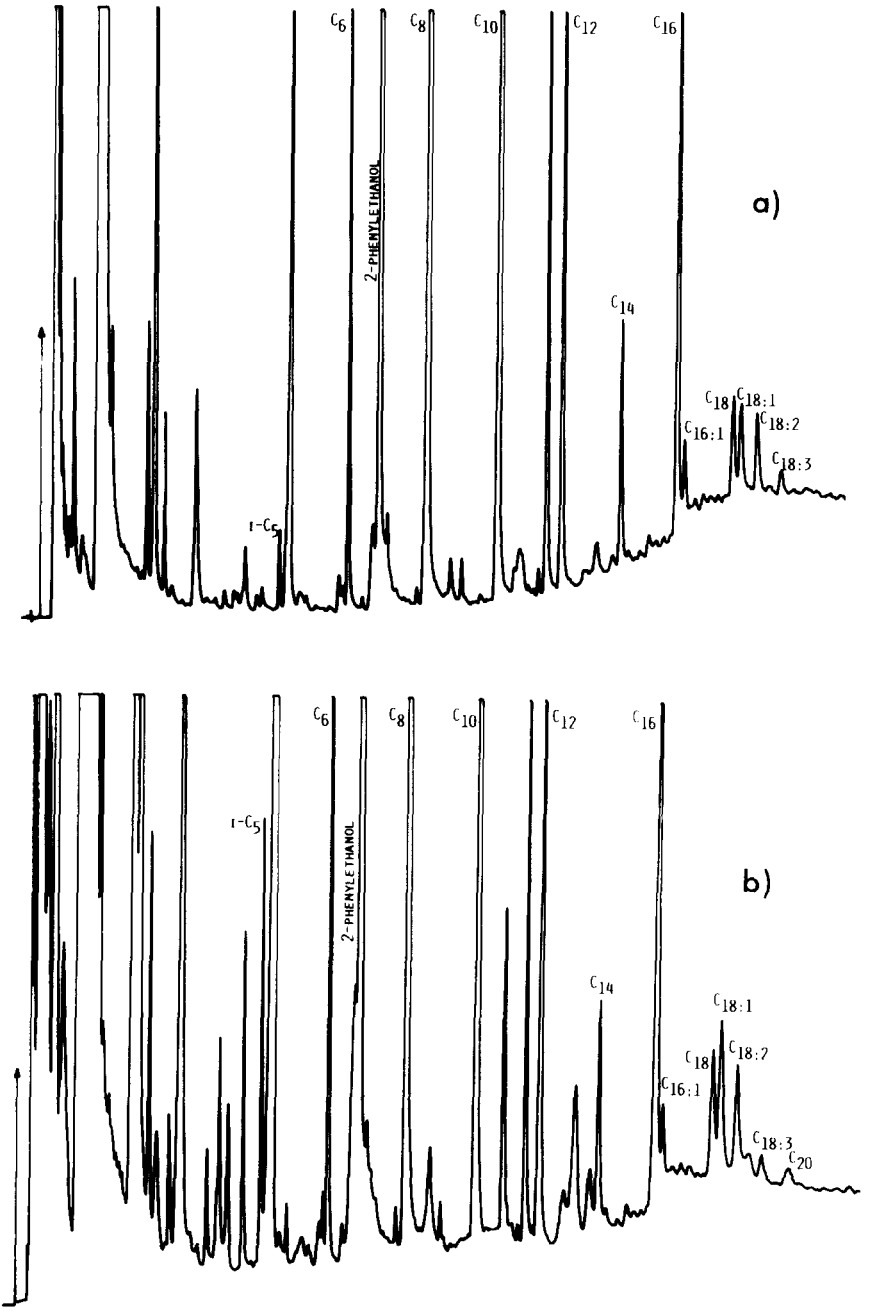

Fig. 3. Chromatograms of an extract of wine distillate. (a) Extract after acid separation (b) Full extract. See conditions in the text.

TABLE 1

Sample Capacity and Efficiency of the Micropacked Column for Caprilic Acid at 200°C ($k = 3{\cdot}7$). Carrier Gas Hydrogen at 12·3 cm/s

Nanograms injected	$\bar{H}$ (*mm*)
10	0·26
40	0·28
60	0·28
100	0·49
140	0·50
220	0·71
500	0·76

2-Phenylethanol appears in the acid fraction despite its relatively low acidity. This phenomenon has been noted in specific separations of sulfur compounds and also aldehydes and ketones from wine extracts (Almy, J., unpublished data).

Some authors (Markides *et al.*, 1986) reported that thermal degradation of unsaturated acids is an important limitation in the GC analysis. In our conditions we have not found signs of such degradation. In Table 2 are given percentages of recovery for palmitoleic, oleic, linoleic and linolenic acids in relation to each corresponding saturated acid (palmitic or stearic), whose recovery was taken as 100%. At 275°C isothermal and 350°C injector and detector, percentages of recovery are very similar for saturated and unsaturated acids. The analysis time was 16 min. Deactivation of the tube and the union of the column to injector and detector with fused silica connections probably prevented degradation or adsorption.

TABLE 2

Per Cent Recoveries ($n = 3$) for the Acids $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ in Relation to their Corresponding Saturated Acids (C_{16}, C_{18}) whose Recoveries were taken as 100% in all Cases. (Initial sample containing 10 ng of each compound. Injector and detector at 350°C, column isothermal at 275°C)

Nanograms of compound	*Per cent recovery*			
	$C_{16:1}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
13	99·85	100·1	99·96	100·8
16	99·04	99·94	100·0	99·51
20	100·5	99·39	97·53	99·53
30	100·1	99·16	97·65	98·72
60	100·1	97·94	97·98	100·0

TABLE 3
Precision and Accuracy of the Analysis for the Test Mixture of Carboxylic Acids (40 mg/litre each compound)

	C_2	C_3	i-C_4	C_4	i-C_5	C_5	C_6	C_7	C_8	C_9	C_{10}
CONC[a]	5·00	4·66	4·99	4·67	4·87	5·60	4·69	4·92	4·66	4·62	4·88
M[b]	4·91	4·45	4·86	4·58	4·81	5·52	4·73	5·09	4·64	4·77	4·89
C.V.%[c]	3·91	3·04	1·47	0·72	0·78	1·27	1·36	1·58	2·71	1·78	2·48
C.V.%[d]	2·38	3·01	1·46	0·89	1·04	0·86	1·50	1·97	2·60	2·46	2·42

	C_{11}	C_{12}	C_{14}	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	C_{20}
CONC[a]	4·58	4·77	4·68	4·68	4·61	4·64	4·60	4·76	4·59	4·54
M[b]	4·52	4·68	4·72	4·71	4·85	4·79	4·86	4·84	4·55	4·48
C.V.%[c]	3·41	2·68	1·70	3·39	3·94	1·07	1·13	1·93	1·75	1·42
C.V.%[d]	2·31	2·21	1·67	3·17	4·41	3·02	1·86	2·18	1·25	3·72

[a] Relative amount (%) in the test solution, by weight.
[b] Average values of the amount (%) obtained ($n = 5$).
[c] Coefficient of variation for the normalized values obtained ($n = 5$).
[d] Coefficient of variation for the ratio of the absolute peak areas to that for 10-undecenoic acid (internal standard).

As for the precision of the analysis, Table 3 shows the mean values ($n = 5$) and the coefficients of variation of the percentage obtained for each compound (the response factor for each carboxylic acid was calibrated previously). Coefficients of variation for the ratios of the absolute peak areas to that for an internal standard are also included. The internal standard was 10-undecenoic acid whose concentration, 40 mg/litre, was similar to the other acids.

The method provides good precision. The majority of the compounds have coefficients of variation lower than 2%. Furthermore, there was good agreement between the mean values of percentages obtained and those known in the test solution.

Figures 4, 5 and 6 show some examples of separation of carboxylic acids in some food samples: organic extracts of grape must, milk and cheese. In these samples many of the carboxylic acids previously reported (Van Straten & Maarse, 1983) were identified.

The performance of FFAP in separating the acids and the advantages of using a micropacked system suggest that this kind of column would be useful in SFC analysis of labile carboxylic acids of low volatility. In fact, micropacked columns exhibit a good balance between performance and pressure drop, with a low cost of one theoretical plate in terms of head pressure (Reglero *et al.*, 1985). In this work the column provides nearly 2000

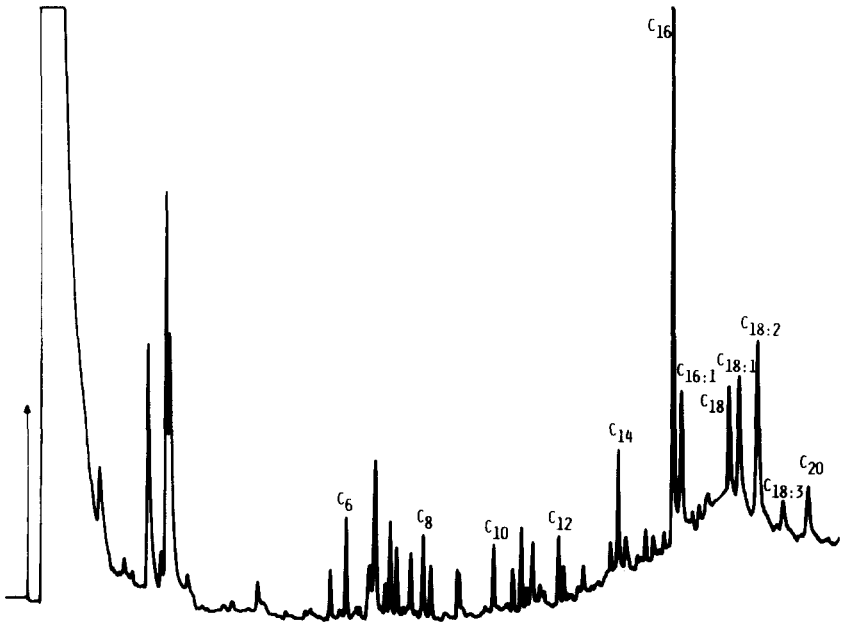

Fig. 4. Chromatogram of an extract of grape must. See conditions in the text.

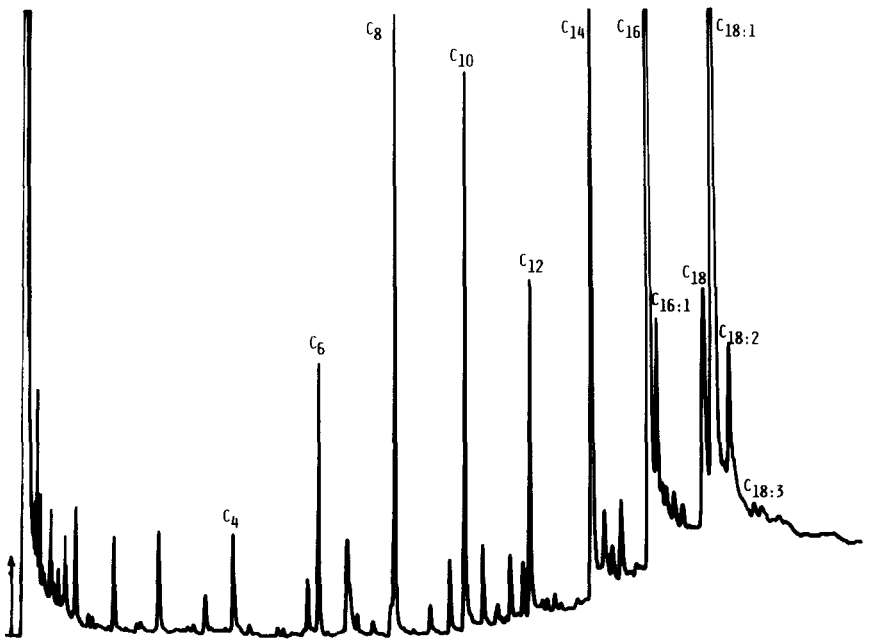

Fig. 5. Chromatogram of an extract of cow's milk. See conditions in the text.

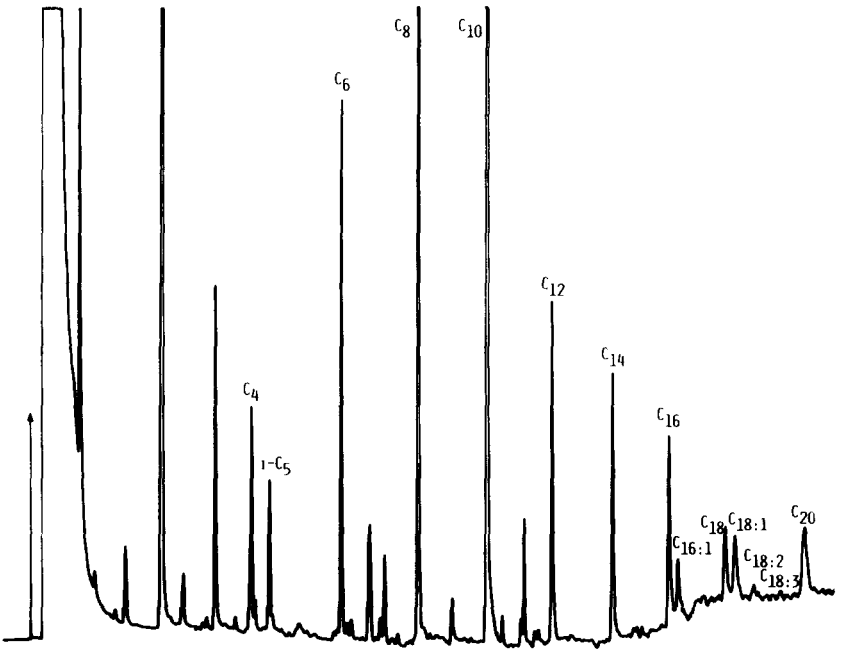

Fig. 6. Chromatogram of an extract of blue (Gamonedo) cheese. See conditions in the text.

theoretical plates for each kg/cm^2 of head pressure. Consequently, the use of this column in SFC would not lead to excessive differences in fluid densities along the column. Therefore, no loss of resolution due to variation of solute retention all through the column should be observed.

CONCLUSION

A 7·5 m × 0·6 mm of 4% FFAP micropacked column provides good results for the separation and quantification by gas chromatography of saturated and unsaturated carboxylic acids of carbon number from 2 to 20 in a reasonable analysis time (50 min). No effects of adsorption on thermal degradation were observed.

On the other hand, the characteristics of the micropacked column used appear to be useful for SFC analysis of labile carboxylic acids of low volatility.

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