Applications of capillary gas chromatography to the quality control of butter and related products

A. ANTONELLI*

Istituto di Industrie Agrarie, Università degli Studi, Via S. Giacomo 7, 40126 Bologna (Italy)

L. S. CONTE

MAF, Ispettorato Repressione Frodi, Ufficio di Bologna, Via S. Giacomo 7, 40126 Bologna (Italy)

and

G. LERCKER

Istituto di Tecnologie Alimentari, Università di Udine, Via Marangoni 97, 33100 Udine (Italy)

ABSTRACT

Analyses performed to establish the quality of butter are essentially based on the packed column gas chromatographic determination of fatty acid composition. One of the main problems is the determination of the short-chain fatty acids, particularly butyric acid. In previous work, comparing results obtained by on column injection with those obtained by split injection, with and without silanized glass-wool in the liner, at two different pre-set splitting ratios, it was found that a packed injector at the highest splitting ratio gives results equivalent to those obtained with on-column injection. When small amounts of butyric acid must be detected, less volatile esters are more suitable than those obtained by rapid transesterification with sodium butoxide. In this paper results obtained for the determination of the amount of butter in bakery products are reported. Results for both real samples and model systems gave highly significant values for the correlation coefficient for split and on-column injection on the basis of fatty acid butyl esters. Triglycerides were also determined in order to measure the amount of butter by means of trienanthine.

INTRODUCTION

James and Martin [1] first determined the fatty acid composition of milk by packed column gas chromatography in 1956, and subsequently capillary GC [2–15] was also used for this purpose. The main problem concerning butter analysis is the determination of the short-chain fatty acids (C_4–C_8), and this is increased when split injection is used even if simple devices can sometimes help in obtaining results close to those given by on-column injection [15].

The use of less volatile esters (*e.g.*, butyl or benzyl esters) might provide an interesting solution of the problem [11,12,16–21]. The rapid method proposed by Christopherson and Glass [22] can be used with sodium butoxide instead of sodium methoxide [16,17].

In recent years, a particular kind of butter has been produced in the European

Economic Community (EEC) namely "concentrated butter". This butter is employed only for bakery and confectionery products and to distinguish this product [23–25] some denaturants are added, *i.e.*, stigmasterol (stigmasta-5,22-dien-3β-ol), trienanthine (glycerol triheptanoate) and vanillin (4-methoxy-3-hydroxybenzaldehyde).

In this paper the calibration and application of a simple method for butter analysis and its determination in bakery products is described. For the latter purpose both butyric and enanthic acid were used. The level of the former was assumed to be 3.75% (average of 90 values in the literature [17]) and that of, the latter 1.1% as stated by the EEC [25].

We have already used packed column GC for the determination of butter fatty acids such as butyl esters [17]. Good results with packed columns were obtained when butyric acid was used as a measure of the amount of butter in bakery products, whereas less reliable results were obtained when enanthic acid was used for this purpose.

The application of capillary GC was studied with the following aims: (a) to improve the determination of enanthic acid; (b) to reduce the time of analysis; (c) to separate possible *trans* isomers in order to detect the presence of hydrogenated fats; and (d) determination of trienanthine.

EXPERIMENTAL

Standard solutions

Standard solutions of pure triglycerides in *n*-hexane and in olive oil were prepared with glycerol tributanoate at concentrations of 0.69, 13.2, 18.6 and 28.1 mg/ml for butyric acid determination, and glycerol triheptanoate (trienanthine) (K&K Rare and Fine Chemicals, Plainview, NY, U.S.A.) and glycerol trioctanoate (Fluka, Buchs, Switzerland) at a concentration of 1 mg/ml as internal standard (I.S.) for triglyceride determination.

Sodium butoxide

A 1 M solution of sodium butoxide in butanol was prepared following the method used by Zaugg [26] for the preparation of sodium ethoxide.

Fatty acid composition

A Carlo Erba Mega Series 5300 gas chromatograph, equipped with split–splitless and on-column injectors with a 30 m × 0.32 mm I.D. fused-silica capillary column, coated with RTX-2330 cyanopropylsilicone phase (0.25 μm) (Restek, U.S.A.), was used. The carrier gas (hydrogen) flow-rate was 3.0 ml/min, the splitting ratio (when applied) 1:80, the oven temperature was programmed from 50 to 200°C at 5°C/min and the flame ionization detector and injector (for split injection only) temperature were 210°C. The chromatogram were recorded and the relative peak areas calculated with Spectra-Physics Model 4290 integrators working at 1 V full-scale.

Triglyceride determination

A Carlo Erba Fractovap 4200 gas chromatograph, equipped with a split–splitless injector a flame ionization detector with a 10 m × 0.32 mm I.D. fused-silica capillary column, coated with RTX-1 (0.15 μm) (Restek), was used. The carrier gas

(helium) flow-rate was 2.2 ml/min, the splitting ratio 1:60, the oven temperature was programmed from 200 to 340°C at 8°C/min and the flame ionization detector and injector temperature were 340°C. The chromatograms were recorded and the relative peak areas calculated with Spectra-Physics Model 4290 integrators working at 1 V full-scale.

Sample preparation

For fatty acid determination, 0.5 g of butter or lipid extract from bakery products [17] were weighed in a screw-capped tube, then 0.75 ml of sodium butoxide solution and 5 ml of *n*-hexane were added. After vigorous vortex shaking for 30 s, the sample was centrifuged at 2200 *g* for 10 min and washed three times with 2 ml of distilled water. Finally, 1 μl of the organic layer was injected into the gas chromatograph. The reaction yield (85%) was reported in a previous paper [17]. Triglyceride determination was performed by dissolving 30 mg of fat in 0.5 ml of toluene; 25 μl of this solution were added to the same volume of I.S. (glycerol trioctanoate) solution. Finally, 1 μl of organic layer was injected into the gas chromatograph.

RESULTS AND DISCUSSION

In previous work [15] we studied the chromatographic behaviour of butter fatty acids as methyl esters obtained according to Christopherson and Glass [22]. The outcome of split (silanized glass-wool packed or not) and on-column injections were compared on the basis of their relative standard deviations (R.S.D.s) as a function of the numbers of carbon atoms. Further, two different pre-set splitting ratios and several reaction times were investigated.

From this previous study, the on-column and split injection mode with the packed liner appeared to be a more accurate method for the determination of short-chain fatty acids than the split mode without a packing in the liner. In fact the R.S.D.s for on-column and split injection with a packed liner were < 10% when higher splitting ratios were used [15]. The transmethylation reaction seemed not to be influenced by reaction times between 20 and 300 s.

Even though these results suggested that split injection was advantageous, problems connected with dirt in the liner packing necessitated frequent changes of the liner. The use of less volatile esters as described here gives similar results without a liner packing. Further, it is possible to limit the losses of the most volatile fatty acids, eluting as butyl esters, by means of capillary columns coated with relatively thermostable stationary phases without an appreciable reduction in resolution.

To evaluate the quantitative chromatographic behaviour of butyl esters of short-chain fatty acids, mainly butyric and enanthic acid, standard solutions of glycerol tributanoate as described under Experimental were analysed with the same column and different injection techniques (split and on-column). Each sample was analysed three times for both injection techniques. The analysis of variance (ANOVA) on the R.S.D.showed no significant differences, *i.e.*, the accuracies of the two methods of sample introduction were identical.

Regression analysis (split *versus* on-column injection) was applied to verify the agreement of these data. The results showed a highly significant correlation ($r = 1.00$) and also that the relative equation fully explains their relationship ($F = 5.817 \cdot 10^{-5}$).

TABLE 1

RESULTS OF DETERMINATION OF BUTTER CONTENT IN REAL SAMPLES

The determinations were performed with butyric (C_4) and enanthic acid (C_7) in both split and on-column modes.

Sample No.	Declared butter (%)	Split injection[a]				On-column injection			
		Butter (%) (C_4)	R.S.D. (%)	Butter (%) (C_7)	R.S.D. (%)	Butter (%) (C_4)	R.S.D. (%)	Butter (%) (C_7)	R.S.D. (%)
1	7.5	8.59 ± 0.15	1.75	9.16 ± 0.00	0.00	7.25 ± 0.15	2.07	8.24 ± 0.47	5.70
2	6.0	5.59 ± 0.33	5.90	8.80 ± 0.13	1.48	6.04 ± 0.04	0.66	6.72 ± 0.22	3.27
3	13.0	16.17 ± 0.46	2.84	15.42 ± 0.38	2.46	13.12 ± 1.01	7.70	13.82 ± 0.75	5.43
4	2.00	2.35 ± 0.11	4.68	2.75 ± 0.16	5.82	1.80 ± 0.14	7.78	2.25 ± 0.14	6.22

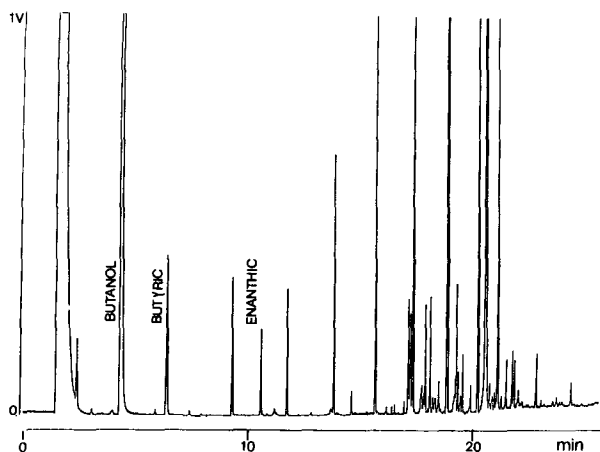[a] Results are means ± S.D. ($n = 3$).

Fig. 1. Fatty acid composition as butyl esters of a lipid extract of a biscuit on carbon chain.

It is also evident that split injection tends to give slightly increased results as the value of the slope is 1.12.

When the method was applied to real samples this behaviour was confirmed. Butter contents reported on the label, together with our results, are given in Table I. Regression analysis (split *versus* on-column injection) of the results showed that when calculations were performed with enanthic acid, the two injection modes were equivalent, confirmed by the correlation coefficient ($r = 0.891$), whereas when butyric acid was used for the same purpose, on-column injection gave more accurate results (Table I). Otherwise the regression parameters were very high in both instances. It might be that the two determinations can be considered to be complementary, because the natural variation of butyric acid may be balances by the technical addition of enanthic acid, and *vice versa*.
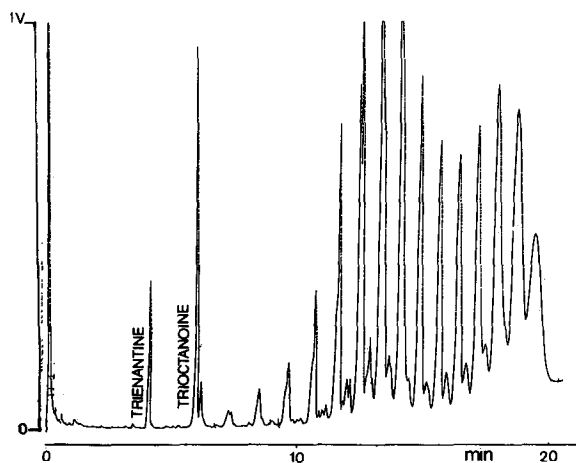

Fig. 2. Triglyceride analysis of a lipid extract of a biscuit.

TABLE II
RESULTS OF DETERMINATION OF BUTTER CONTENT IN REAL SAMPLES

The determinations were performed with trienanthine in the split mode.

Sample No.	Butter content (%)		
	Declared	Found[a]	R.S.D.
1	7.5	7.37 ± 0.14	1.90
2	6.0	7.40 ± 0.65	8.78
3	13.0	13.57 ± 0.32	2.36
4	2.00	2.53 ± 0.13	5.14

[a] Mean ± S.D. ($n = 3$).

An example of fatty acid composition as butyl esters is shown in Fig. 1. The use of a cyanopropyl-bonded stationary phase allows the evaluation of the possible presence of *trans* isomers of unsaturated fatty acids. No significant amounts were detected in the samples examined.

As trienathine is added to concentrated butter as the pure triglyceride, its direct determination (Fig. 2) can be a rapid and useful means of establishing the amount of EEC concentrated butter in bakery products.

Results obtained in this way (Table II) show low R.S.D.s close to those obtained by fatty acid determinations. Further, the absolute data are in good agreement with the others.

The methods reported in this paper are similar in precision and accuracy, with no effect of differences in sample preparation or chromatographic conditions.

REFERENCES

1 A. T. James and A. J. P. Marin, *Biochem. J.*, 63 (1956) 144.
2 P. W. Parodi, *J. Diary Sci.*, 60 (1977) 1550.
3 V. Antila and V. Kankare, *Milchwissenschaft*, 38 (1983) 478.
4 H. T. Badings and C. De Jong, in J. Rijks (Editor), *Proceedings of the 5th International Symposium on Capillary Chromatography, Riva del Garda, April 26–28, 1983*, Elsevier, Amsterdam, 1983, p. 543.
5 W. Butte, *Chromatographia*, 261 (1983) 142.
6 G. Lercker, C. Zullo and A. Tortorelli, *Riv. Ital. Sostanze Grasse*, 64 (1987) 321.
7 L. Matter, *Lebensm. Gerichtl. Chem.*, 42 (1988) 26.
8 P. Kalo, P. Elo and M. Antila, *Milchwissenschaft*, 43 (1988) 416.
9 P. Lund, *Milchwissenschaft*, 43 (1988) 159.
10 M. J. Prager, *J. Assoc. Off. Anal. Chem.*, 72 (1988) 418.
11 D. P. Casper, D. J. Schingoethe, R. P. Middaugh and R. J. Baer, *J. Dairy Sci.*, 71 (1988) 1267.
12 R. P. Middaugh, R. J. Baer, D. P. Casper, D. J. Schingoethe and S. W. Seas, *J. Dairy Sci.*, 71 (1988) 3179.
13 R. J. Bear, K. M. Tieszen, D. J. Schingoethe, D. P. Casper, W. A. Eisenbeitz, R. D. Sharer and R. M. Cleark, *J. Dairy Sci.*, 72 (1989) 1424.
14 L. Matter, D. Schrenker, H. Husmann and G. Schomburg, *Chromatographia*, 27 (1989) 31.
15 L. S. Conte, R. Zironi and G. Lercker, in P. Sandra and G. Redant (Editors), *Proceedings of the 10th International Symposium on Capillary Chromatography, Riva del Garda, May 22–25, 1989*, Hüthig, Heidelberg, 1989, p. 889.
16 J. L. Iverson and A. J. Sheppard, *J. Assoc. Off. Anal. Chem.*, 60 (1977) 284.
17 L. S. Conte, A. Antonelli and A. Minguzzi, *Boll. Chim. Igien.*, 41 (1990) 347.

18 E. P. Jones and V. L. Davidson, *J. Am. Oil Chem. Soc.*, 42 (1965) 121.
19 P. O. Bethge and K. Lindström, *Analyst (London)*, 99 (1974) 137.
20 P. W. Jones and J. Kay, *J. Sci. Food Agric.*, 27 (1976) 1005.
21 A. T. G. Steverink, *Analyst (London)*, 109 (1984) 170.
22 S. W. Cristopherson and R. L. Glass, *J. Dairy Sci.*, 52 (1969) 1289.
23 EEC Regulation No. 232/75, EEC Official Journal 31.1.75, N.L. 24/45, Luxembourg.
24 EEC Regulation No. 1932/81, EEC Official Journal 14.7.81, N.L. 181/6, Luxembourg.
25 EEC Regulation No. 3143/85, EEC Official Journal 12.11.85, N.L. 298/9, Luxembourg.
26 H. E. Zaugg, in F. Fieser and M. Fieser (Editors), *Reagents for Organic Synthesis*, J. Wiley, New York, London, Sydney, 1st ed., 1967.