CHROM. 4980

Direct gas chromatographic estimation of lower alcohols, acetaldehyde, acetone and diacetyl in milk products

Data pertaining to the lower alcohols, aldehyde and ketone, their occurrence and concentration in milk and milk products are often mentioned in connection with the study of fermentation, flavour structure, stability of quality of milk products during storage, etc.

The determination of the above compounds is discussed in numerous papers. Practically all of these methods are based on two principal operations: isolation of the compounds from the samples (distillation, extraction) and then determination mostly by GLC. The isolation usually involves some loss especially of the easily volatile compounds. Direct GLC analysis of these compounds without processing the sample prior to its injection onto the column is difficult because the water content and other accompanying components in the sample and still other factors lower and affect the quality of chromatographic analysis. These problems are usual with liquid–solid column packing.

Uncoated porous polyaromatic materials as column packing are very suitable for direct determination of water, alcohols, glycols, certain gases, hydrocarbons and volatile acids[1]. Porous polyaromatic materials were used to advantage for direct GLC determination of the lower alcohols, volatile acids, etc., in microbial fermentation media and in rumen fluid[2–4]. Remarkable results were achieved by BAKER *et al.*[5] in determination of lower alcohols, acetone and acetaldehyde in blood by direct GLC analysis of untreated samples by using Porapak Q as column packing. Also in milk serum and in aqueous extracts of cheese, Polypack-2 in direct GLC determination of water-soluble fatty acids was used[6].

The results achieved in using porous polyaromatic materials in GLC for direct determination of some compounds prompted us to apply this materials also for direct GLC determination especially of the non-acidic fermentation products in milk and milk products.

Experimental

Apparatus and column. Fractovap Model GB (Carlo Erba, Milan) with FID was used. The glass column (160 cm long, I.D. 4 mm) was filled with 80–100 mesh Porapak Q and Porapak P (1:1). The column temperature was 105° and the inlet and detector were maintained at 125°. Nitrogen as carrier gas (1.5 kp/cm^2) was used. In the inlet part of the column, a removable glass tube (or glass beads) was installed providing protection against pollution of the chromatographic column with accompanying substances of sample.

Procedure. Methanol, acetaldehyde, acetone, ethanol, isopropanol, *n*-propanol and isobutanol were used for preparation of the standard solution. Anhydrous acetonitrile (b.p. 81–82°) as the internal standard was used. Standard curves were plotted for the substances covering the wide concentrations required for the material analysed.

For the determination of volatiles in milk and liquid milk products (or milk products homogenised with water), samples were adjusted to pH 7.5–8.0 (ref. 7).

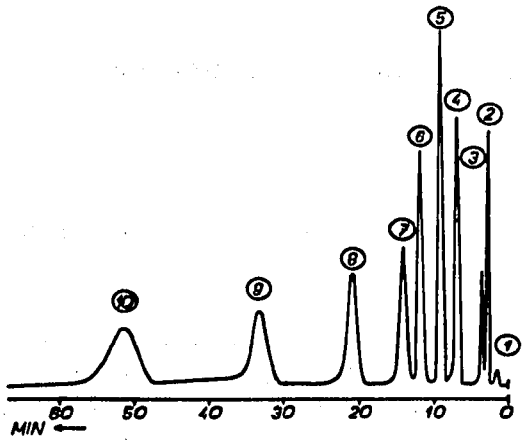

Fig. 1. Chromatogram of a standard solution with acetonitrile as internal standard. 1 = water; 2 = methanol; 3 = acetaldehyde; 4 = ethanol; 5 = acetonitrile; 6 = acetone; 7 = isopropanol; 8 = *n*-propanol; 9 = diacetyl; 10 = isobutanol.

Internal standard (0.05–0.40%) was used in addition depending on the levels of volatiles anticipated or encountered. In all determinations 5–10 μl of the standard solution or sample were injected directly into the gas chromatograph.

Standard curves were plotted from a water or milk solution of the standards. The ratio of each peak area to that of the internal standard acetonitrile was determined and plotted against the concentration ratio.

Results and discussion

Excellent separation of methanol, acetaldehyde, ethanol, acetone, isopropanol, *n*-propanol, diacetyl, isobutanol and acetonitrile was obtained by using a Porapak Q and Porapak P mixture as column packing (see Fig. 1).

Comparing the separation quality using Porapak Q or Porapak P separately and then their mixture, it can be seen that the separation of the tested compounds and the situation of the internal standard improved and the time of analysis got

TABLE I

RELATIVE RETENTION TIMES OF MILK PRODUCTS VOLATILES AS COMPARED TO ACETONITRILE ON PORAPAK Q, PORAPAK P AND THEIR MIXTURE

Compound	*Relative retention times*		
	Porapak Q	*Porapak P*	*Mixture (1:1)*
Methanol	0.30	0.22	0.31
Acetaldehyde	0.38	0.28	0.40
Ethanol	0.82	0.53	0.75
Acetonitrile	1.00	1.00	1.00
Acetone	1.46	0.93	1.31
Isopropanol	1.86	0.91	1.57
n-Propanol	2.73	1.47	2.36
Diacetyl	4.32	2.41	3.75
Isobutanol	7.01	2.93	5.74

TABLE II

RECOVERY OF VOLATILE COMPOUNDS FROM MILK

Compound	*Sample number*	*Present (mg)*	*Found (mg)*	*Recovered (%)*
Methanol	1	23.5	21.6	91.8
	2	10.8	11.3	105.0
	3	15.6	15.1	96.8
	4	7.8	7.6	97.5
Ethanol	1	131.3	121.6	92.6
	2	204.6	188.3	92.0
	3	69.7	68.5	98.3
n-Propanol	1	14.7	14.3	97.6
	2	23.4	24.6	105.4
	3	14.2	13.4	94.4
	4	12.8	12.9	100.9
	5	31.6	33.2	105.1
Isopropanol	1	13.3	12.4	93.3
	2	34.1	33.1	97.1
	3	13.8	13.9	100.6
	4	21.9	23.0	105.2
Acetone	1	15.3	16.1	105.3
	2	13.4	14.3	106.9
	3	28.4	27.1	95.5
	4	17.4	16.0	92.0
Acetaldehyde	1	20.5	20.0	97.6
	2	98.2	89.9	91.5
	3	101.7	99.0	97.4
	4	58.1	56.1	96.6
	5	80.3	73.2	91.2
Diacetyl	1	30.4	29.6	97.3
	2	32.3	32.6	100.8
	3	66.4	67.3	101.3

TABLE III

PRECISION OF SIMULTANEOUS DETERMINATIONS OF VOLATILE COMPOUNDS IN YOGHURT CULTURE

	Methanol(?) (mg%)	*Acetaldehyde (mg%)*	*Ethanol (mg%)*	*Acetone (mg%)*
	0.65	4.39	7.71	0.50
	0.64	3.99	7.94	0.44
	0.63	4.30	8.05	0.53
	0.53	4.20	7.14	0.42
	0.62	4.41	7.51	0.48
	0.65	4.18	7.71	0.41
	0.61	3.69	7.40	0.37
	0.49	3.87	7.01	—
	0.58	3.69	6.99	—
	0.53	3.68	6.99	—
Mean	0.59	4.04	7.44	0.45
Standard deviations (±)	0.06	0.29	0.40	0.05
Coefficient of variation (%)	10.1	7.2	5.4	11.1

TABLE IV

VOLATILE COMPOUNDS IN SOME MILK PRODUCTS

Milk product	*Volatile compounds (mg %)*						
	Methanol(?)	*Acetaldehyde*	*Ethanol*	*Acetone*	*Isopropanol*	*n-Propanol*	*Diacetyl*
Milk (cowy flavour)	—	0.61	3.33	79.54	—	—	—
Kefir culture	0.22	0.64	712.54	—	0.02	0.07	0.08
Cream culture	0.42	0.46	2.86	0.11	—	0.04	0.51
Yoghurt culture	—	1.55	0.91	—	0.15	—	—

shorter using the mixture (see Table I). The standard curves were linear in the whole range of the concentrations tested.

Precision of the method described was determined by the recovery percentage of the compounds added to analysed samples of milk. Results are given in Table II. Isobutanol was not evaluated because we did not find it in detectable concentration in any of our materials tested. Precision of simultaneous determinations of volatiles in a yoghurt culture by using the described method is presented in Table III. Using this method we also tested some of fermented milk products. The results are given in Table IV. The typical chromatogram of volatile compounds of kefir culture is presented in Fig. 2.

The modification of the inlet part of the column (removable glass tube or glass beads) increases the lifetime of the column. It is recommended to clean (changing the removable tube or beads) this inlet part of column after about 25–30 injections.

Previous gas chromatographic methods for the determination of volatile compounds in milk and milk products required specimen processing—isolation of volatile compounds from the tested material—prior to their analysis by GLC. By this method no processing is required prior to the injection. This is accomplished by the use of polyaromatic materials as column packing and a modified glass inlet part of the column

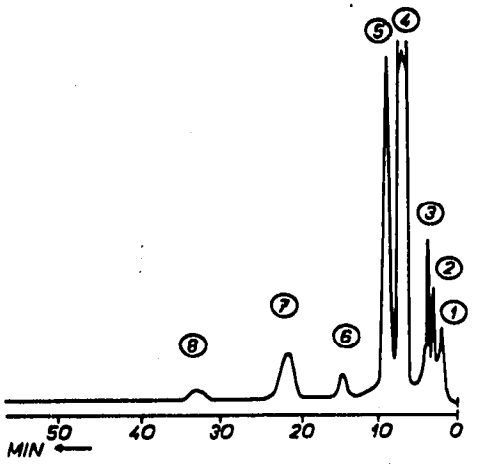

Fig. 2. Chromatogram of kefir culture. 1 = water, attenuation $10^2/1$; 2 = methanol, attenuation $10^2/1$; 3 = acetaldehyde, attenuation $10^2/1$; 4 = ethanol, attenuation $10^2/256$; 5 = acetonitrile, attenuation $10^2/64$; 6 = isopropanol, attenuation 10/1; 7 = *n*-propanol, attenuation 10/1; 8 = diacetyl, attenuation 10/1.

trapping the milk and milk product nonvolatile substances and protecting the column. The application of this method is limited by the lower boundary of concentrations of the analysed compounds occurring in the sample. It depends on the sensibility of the apparatus used. In our case for example even 0.08 mg% diacetyl, 0.04 mg% *n*-propanol, 0.02 mg% isopropanol and 0.1 mg% acetone could be determined.

The work suggests the use of this method also for the determination of other compounds as described and shows the possibility of its application to other materials than milk products. For this purpose it is necessary to change the analytical conditions and to use respectively some other combination of column packing polymer materials.

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The determination of picloram in fescue by gas–liquid chromatography*

Several residue methods for the determination of picloram (4-amino-3,5,6-trichloropicolinic acid) in soil and a variety of crops employ extraction with aqueous base and electron capture gas–liquid chromatography (GLC) of the methyl ester or the pyrolysis product of this herbicide. Recently we have reported a method for the determination of picloram in soil, which uses a closed-tube decarboxylation[1].

Picloram is extensively used to eradicate brush and other broad-leaved plants on grazing ranges[2]. We therefore decided to apply our method to the analysis of fescue. However, several serious analytical difficulties became apparent.

Basic extracts of fescue caused emulsions of considerable stability. The formerly used silica gel column failed to separate larger amounts of co-extractants from the

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