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DIRECT GAS CHROMATOGRAPHIC DETERMINATION OF POLYALCOHOLS IN BIOLOGICAL MEDIA

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SUMMARY

A simple and rapid method for the direct gas chromatographic determination of free polyalcohols (sugar alcohols) is described. Several applications, which are of interest in the field of biochemical analyses, are described and discussed.

INTRODUCTION

For the qualitative determination of polyalcohols, a paper chromatographic method is most frequently used. However, in most solvent systems, tetritols run with tetroses, pentitols with pentoses, etc., and even in the absence of sugars, the separation of polyols such as arabitol, xylitol and ribitol is difficult. Other techniques such as column chromatography are not routinely employed for the identification of polyols, but thin-layer chromatography and paper electrophoresis may improve some separations (for a general review see ref. 1).

The quantitative determination of individual polyols can only be performed after elution from the chromatograms[2] or after column chromatography[3] followed by colorimetric estimation. Direct densitometric estimation on papers is unsatisfactory because no highly specific reagents are available. The gas chromatographic methods hitherto employed for polyol determination were based upon the formation of volatile derivatives. Thus, polyhydric alcohols were determined as methyl ethers[4], acetals and ketals[5], but mostly as acetates[6–11] or as trimethylsilyl ethers[12–14]. Quantitative determination of monosaccharides as their alditol acetates[11] has the advantage of the possible simultaneous estimation of some polyols and monosaccharides. Although the reduction of monosaccharides is a simple procedure[15], these methods are expensive and time consuming.

Methods of direct determination, such as those used for the determination of glycols and glycerol[16–23], are more interesting. Polyaromatic resins, such as Porapak, Polypak and Chromosorb 102, can be used very successfully for this purpose[24]. However, the direct determination of sugar alcohols has never been performed.

In this paper we would like to report the direct gas chromatographic identification and estimation of glycerol, erythritol, xylitol, arabitol, ribitol and mannitol. Biological media such as fermentation media were directly injected onto the column,

after removal of cells. Compared with other quantitative methods used for the estimation of these polyols in complex media, the present method is extremely simple, fast and much more reliable. Very short columns may be used.

MATERIALS AND METHODS

All gas chromatographic analyses were performed with a Carlo Erba Fractovap Model GB apparatus equipped with flame ionization detectors and thermal programming. The column packing material was Polypak 1, 120–200 mesh (Hewlett-Packard), and glass columns with lengths of 0.5 or 1 m and 6 mm O.D. were used. The oven temperature was 250° in isothermic analysis or was programmed from 150 to 250°. Detector and vaporizer temperatures were 350°. Nitrogen was used as the carrier gas.

RESULTS

Retention times for different polyhydric alcohols were determined on a 1 m column (Table I). Standard curves for the determination of glycerol, erythritol, D-arabitol and xylitol were established. On a 1 m column, standard curves for glycerol and erythritol are good, but for D-arabitol or xylitol the peaks are not sharp enough to allow accurate quantitative determinations (Fig. 1). On a 0.5 m column, however, a good standard curve was obtained for D-arabitol in the absence of xylitol. Fig. 2 gives an example of comparative separations of polyalcohols on a 1 m column and on a 0.5 m column. For the determination of polyalcohols in complex media, the media were injected directly without any preliminary treatment. This does not interfere with the determination of erythritol, arabitol or xylitol. Glycerol may also be determined provided a 1 m column is used and the concentration is not too low. When the concentration of glycerol in complex media is very low or when 0.5 m columns are used, other volatile components, with retention times below that of glycerol, interfere with the glycerol peak. In Fig. 3A the determination of glycerol after direct injection of a complex medium is easily performed as relatively high concentrations of polyalcohols were present in this medium compared with other volatile materials. Under other circumstances, the problem of separating glycerol from other volatiles may be solved by temperature programming and dual column analysis. The analysis is then performed on a 0.5 m column operating isothermically at 150° for 5 min. Compounds

TABLE I

RETENTION TIMES OF DIFFERENT POLYALCOHOLS

Polyalcohol	*Retention time (min)*
Ethylene glycol	1.80
Glycerol	4.69
Erythritol	14.10
Xylitol	30.30
D-Arabitol	38.10
L-Arabitol	38.10
Ribitol	39.90
D-Mannitol	38.10

with retention times lower than glycerol leave the column during this period. The temperature is then raised from 150° to 250° at a rate of 4°/min. Glycerol emerges at about 180°, followed by erythritol and later by arabitol. Fig. 3B indicates a programmed analysis of a complex medium under these conditions, where concentration of polyalcohols is low compared with other volatile materials.

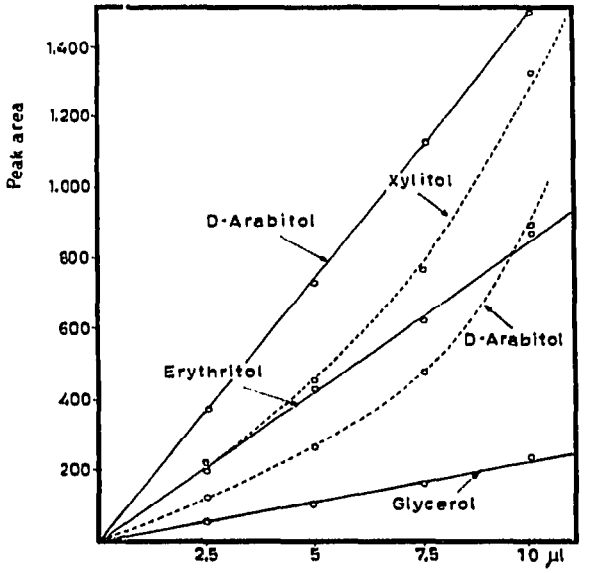

Fig. 1. Calibration curves for glycerol, erythritol, D-arabitol and xylitol. The dashed lines for arabitol and xylitol (200 µmoles/ml) were obtained with a 1 m column, whereas the solid line for arabitol (400 µmoles/ml) was obtained with a 0.5 m column. Solid lines for glycerol (66 µmoles/ml) and erythritol (200 µmoles/ml) were obtained with a 1 m column. The operating temperature was 250°, and nitrogen was used as the carrier gas. The peak area is expressed as height × width at half height.

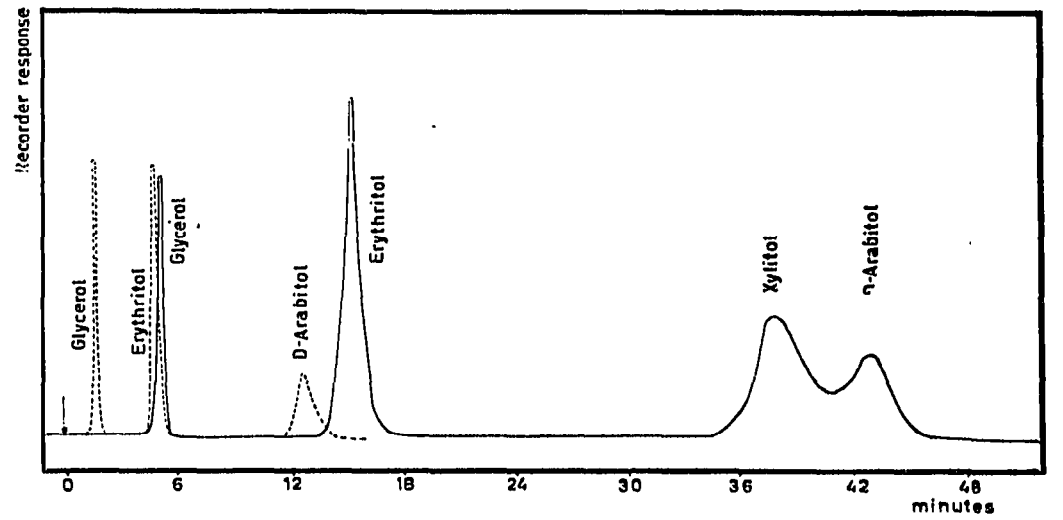

Fig. 2. Separation of glycerol, erythritol, D-arabitol and xylitol on a 1 m column and on a 0.5 m column. Other details are reported in the text or are as in Fig. 1.

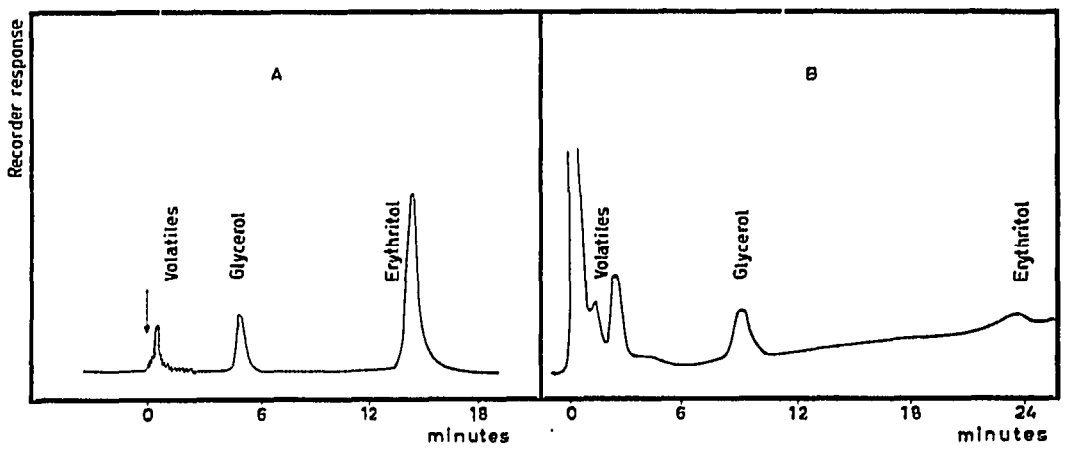

Fig. 3. A, Analysis of a complex medium with relatively large amounts of glycerol and erythritol as compared with volatiles having retention times lower than glycerol. A 1 m column was used. Other details are given in the text. B, Analysis of a complex medium with low amounts of glycerol and erythritol as compared with other volatiles. A 0.5 m column was used, but the temperature was programmed as described in the text. 10 μl of media were injected without preliminary treatment.

APPLICATIONS AND DISCUSSION

Screening of cultures for the production of polyhydric alcohols

GLC provides a fast and quantitative screening method for the production of polyhydric alcohols by microorganisms. Since much work is being done to solve this problem (for general reviews, see refs. 25–27 among others), direct injection of culture media onto GLC columns will be very helpful in this field of research. For comparison the analysis of eight erythritol- and glycerol-producing strains was performed by paper chromatography, followed by elution and colorimetric analysis of the eluates, and by the direct gas chromatographic procedure. In the latter procedure 10 μl of fermentation media were injected directly onto the column after centrifugation of the cells, and analysed with thermal programming as described above. Measurements of retention times confirmed the nature of the polyols produced. When the results are compared quantitatively with those obtained by paper chromatography, very good agreement is found. However, a comparison of the time necessary for analysis shows that more than 40 h are required for a quantitative analysis of the eight media by paper chromatography, while the GLC method takes only 5 h. Moreover, results obtained by the latter method should be more reliable as the media undergo no preliminary treatment. The results of this analysis are given in Table II. The table demonstrates that the very different ratios of glycerol to erythritol do not interfere with the analyses.

Preliminary results had also indicated that several strains of *Candida utilis* may produce arabitol. Using the GLC method it was quickly established that the amounts were very low, and in most cases fermentation media had to be concentrated before injection. Although the presence of residual glucose resulted in the formation of a syrupy residue, this could still be injected without interfering with the quantitative determination of arabitol. Amounts were in the range of 60 up to 600 μmoles arabitol per 100 ml.

TABLE II

ANALYSIS OF FERMENTATION MEDIA WITH RESPECT TO POLYALCOHOL FORMATION

The temperature was programmed to rise from 150° to 250° at 4°/min. Flow rate of N_2, 32.5 ml/min. 10 µl of fermentation media were injected directly onto the 0.5 m column.

Strain	*Glycerol (µmole/100 ml)*	*Erythritol (µmole/100 ml)*	*Ratio G/E*
1	8.425	1.315	6.40
2	5.426	1.205	4.50
3	9.394	1.509	6.22
4	6.230	2.070	3.00
5	964	9.999	0.096
6	812	129	6.29
7	28	172	0.16
8	840	139	6.04

It should be mentioned, however, that direct injection of syrupy residues leads to a rapid decrease in sensitivity with respect to arabitol detection. No explanation for this specific decrease in sensitivity can be given, although it is possible that at the top of the column degradation products specifically adsorb some of the components to be analysed.

Identification of polyhydric alcohols

The method has been used to confirm the identity of a polyol produced by a syrup-spoiling microorganism studied before[28], and of arabitol, erythritol or glycerol in several osmophilic yeasts described in the literature (see general reviews). Clearly the method should also be very useful in the identification of reaction products obtained in the enzymatic reduction by polyoldehydrogenases of aldoses or ketoses.

Kinetics of polyhydric alcohol oxidation

Several polyhydric alcohols are used for the production of ketosugars with the aid of *Acetobacter* species. MOSES AND FERRIER obtained D-xylulose from D-arabitol[29], and the reaction was followed by measuring the optical rotation of the solution as

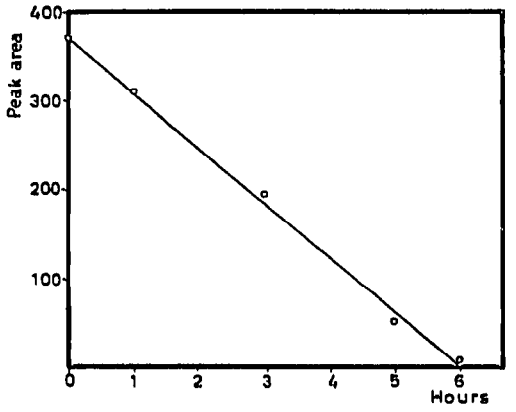

Fig. 4. Kinetics of D-arabitol oxidation with cells of *Acetobacter* species. 10 µl amounts of the reaction mixture (D-arabitol + cells of *Acetobacter suboxydans*) were directly injected at different time intervals, after centrifugation of the cells.

a function of time. Using their experimental conditions but a strain of *Acetobacter suboxydans* from our collection, we decided to follow the reaction through GLC estimation of residual arabitol as a function of time. Fig. 4 indicates that arabitol had completely disappeared after only 5 h. This fast analytical procedure thus allows the reaction to be stopped just after all the substrate has been used up and before other transformations of the ketosugar occur.

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