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THERMAL DEGRADATION OF SUGARS IN A GAS CHROMATOGRAPHIC INJECTION PORT

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SUMMARY

The degradation products of glucose formed during gas chromatographic analysis of fermentation liquids of *Streptococcus mutans*, *S. mitis* and *S. salivarius* were investigated.

A total of 18 sugars from mono- to trisaccharides were also examined by direct injection of aqueous solutions of the carbohydrates into a gas chromatograph packed with porous polymer Chromosorb 101. The main degradation products, including acetic acid and furfural derivatives, were identified by gas chromatography–mass spectrometry.

INTRODUCTION

Many studies on the gas chromatographic (GC) determination, without isolation, of volatile fermentation products of dental plaque, oral bacteria and saliva, which have a connection with dental caries and periodontal disease, have already been published[1–10].

We found that several peaks observed in the chromatogram are due to decomposition products of glucose that influence the quantitative analysis of fermentation products in culture broth of several streptococci.

It is well known that carbohydrates can be transformed by acid, base, amine and heat[11–12]. Recently, Popoff and Theander[15–17] showed that sugars such as hexose, pentose and hexuronic acid in aqueous solution could give a number of aromatic compounds under fairly mild conditions. Therefore, decomposition of sugars during GC under much more severe conditions might be well anticipated.

Thermal degradation of mono-, oligo-, and polysaccharides by pyrolysis GC[23–25] and pyrolysis mass spectrometry[26] has been investigated, but no work on the degradation of fermentation broth constituents in the GC injection port or on such degradation for structural analysis of sugars has been reported.

We examined 18 carbohydrates from mono- to trisaccharides and found that the degradation patterns depend on the concentration of sugars and on the structure of the monosaccharide.

EXPERIMENTAL

Apparatus

Gas chromatography. A Hitachi Model 063 gas chromatograph with a hydrogen flame ionization detector was used under the following conditions. Carrier gas, nitrogen at a flow-rate of 35 ml/min; detector temperature, 280°, injection port temperature, 270°; glass column, 2 m × 3 mm I.D.; packing, Chromosorb 101, 80–100 mesh (Johns-Manville, Denver, Colo., U.S.A.).

Before use, the column was conditioned overnight at 250° under a stream of nitrogen (flow-rate, 10 ml/min). The column temperature programmed from 172° to 200° at 2°/min and then maintained at 200°.

Gas chromatography–mass spectrometry (GC–MS). A Hitachi K-53 gas chromatograph and a Hitachi RMU-6E mass spectrometer were used. The following conditions were applied: carrier gas, helium at a flow-rate of 30 ml/min; glass column, 2 m × 3 mm I.D.; packing, Chromosorb 101, 80–100 mesh; ion source temperature, 250°; ionizing voltage, 70 eV; target current 70 μA.

Materials

The carbohydrates studied (Wako, Osaka, Japan) are listed in Table I. Furfural derivatives (Tokyo Kasei Kogyo, Tokyo, Japan) as authentic samples were used for GC–MS. C_1 to C_6 fatty acids, ethyl alcohol and lactic acid were purchased from Tokyo Kasei Kogyo.

Preparation of samples

All reagents were standard grade and were used without further purification. *Streptococcus mutans*, *S. mitis* and *S. salivarius* were maintained in mitis-salivarius agar and cultured in Todd-Hewitt broth (Difco Lab., Detroit, Mich. U.S.A.). Fermentation samples were prepared by removing the supernatant fluid from centrifuged cells and adjusting to pH 2 with 1 *M* phosphoric acid; 2 μl of the solutions was injected into the gas chromatograph.

Sample solutions of sugars at various concentrations were prepared with distilled water.

RESULTS AND DISCUSSION

A standard mixture of ethyl alcohol, C_1 to C_6 fatty acids and lactic acid was separated completely in a short time by temperature-programmed gas chromatography using a porous polymer Chromosorb 101 (Fig. 1). However, the GC analyses of the fermentation products from glucose-broth cultures of *Streptococcus mutans*, *S. mitis* and *S. salivarius* showed in addition to acetic and lactic acids two large peaks (A and B in Fig. 2) which had different retention times than those of the standard mixture shown in Fig. 1. Peaks A and B were identified as furfural and methyl furfural, respectively, by GC–MS comparison with authentic samples (Fig. 3).

As it is unlikely that these peaks are the metabolites produced by the bacteria, we assumed that they were formed during GC by thermal degradation of glucose remaining in the medium used.

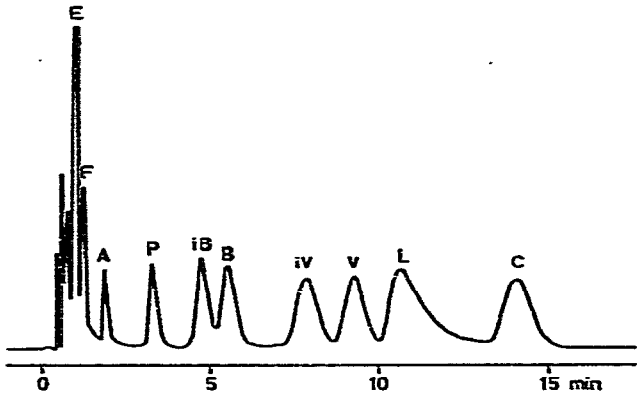

Fig. 1. Chromatogram of a mixture of 0.005 *M* acetic (A), propionic (P), isobutyric (iB), butyric (B), isovaleric (iV), valeric (V), and caproic (C) acids, 1.0 *M* formic acid (F), 0.0025 *M* ethyl alcohol (E) and 0.04 *M* lactic acid (L) on a Chromosorb 101 column temperature-programmed from 172° to 200° at 2°/min and then maintained at 200°. Injection port temperature, 270°; detector, 280°; sample volume, 0.6 μl; carrier gas, nitrogen at a flow-rate of 35 ml/min; attenuation, 20 × 1.

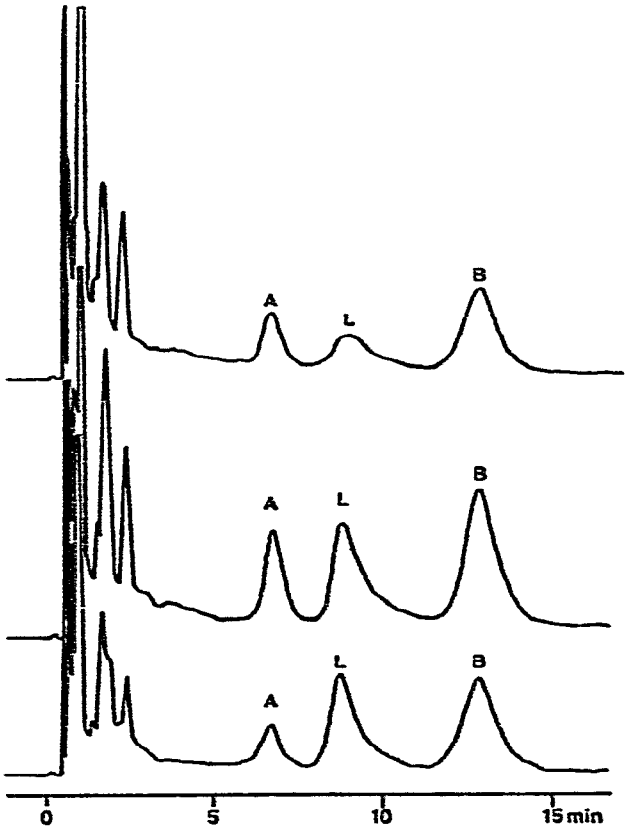

Fig. 2. Chromatograms of fermentation products in 5% glucose-Todd-Hewitt broth culture of *Streptococcus mutans* (bottom), *S. mitis* (middle) and *S. salivarius* (upper). L = lactic acid.

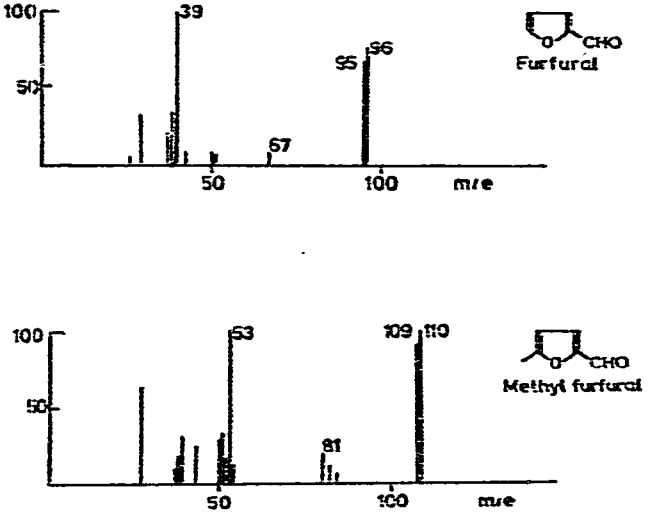

Fig. 3. Mass spectra of peak A (furfural) and peak B (methyl furfural) at 70 eV obtained by GC–MS.

We examined 18 carbohydrates from mono- to trisaccharides in order to clarify the thermal degradation of sugars. The results are shown in Table I.

All the degradation and fermentation products were identified by comparing their retention times and mass spectra with those of authentic samples using GC and GC–MS. Especially the fermentation products of the streptococci were identified after purification on an ion-exchange resin column as acetic and lactic acids. The results will be reported elsewhere.

TABLE I

THERMAL DEGRADATION PRODUCTS OF CARBOHYDRATES

Class	*Sugars examined*	*Decomposition products*
Aldopentose	Arabinose Ribose Lyxose Xylose	Furfural
Aldohexose	Galactose Glucose Mannose	Acetic acid, furfural, methyl furfural, levulinic acid, hydroxymethyl furfural
Deoxysugar	Rhamnose	Methyl furfural
Ketohexose	Sorbose Fructose	Furfural, methyl furfural, hydroxymethyl furfural
Alditol	Sorbitol Mannitol	Not decomposed
Disaccharides	Sucrose Lactose Cellobiose Maltose	Reflecting structures of component monosaccharide
Trisaccharides	Raffinose Melezitose	Reflecting structures of component monosaccharide

Aldopentose gave only furfural as a decomposition product, whereas aldohexose gave acetic acid, furfural, methyl furfural, levulinic acid and hydroxymethyl furfural.

Rhamnose, a deoxy sugar, gave only methyl furfural, and ketohexose gave only furfural derivatives such as furfural, methyl furfural and hydroxymethyl furfural.

Alditol was not decomposed. Di- and trisaccharides gave degradation products reflecting the structures of the component monosaccharides.

Fig. 4 shows the degradation pattern of glucose. Injection of an aqueous solution of glucose into the gas chromatograph gave all the decomposition products of aldohexose described above at concentrations of 1% or more, but at 0.3% concentration the peaks of levulinic acid and hydroxymethyl furfural disappeared. No peak was observed under the same conditions at 0.001% concentration and below.

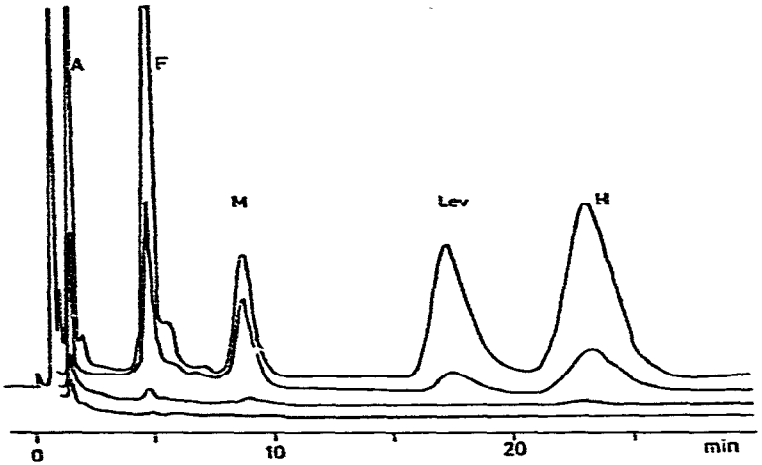

Fig. 4. Degradation pattern of glucose at various concentrations (5, 1.25, 0.3 and 0.15‰, respectively, reading downwards).

The other unidentified small peaks observed in Fig. 4 are now under investigation, although 56 volatile compounds, including aldehydes, ketones, diketones, etc., have already been identified in the case of the thermal degradation of glucose[22].

Both peak pattern and peak area are relatively reproducible. Therefore it is convenient for us to examine the degradation pattern at various concentrations whenever we prepare a column for quantification.

As carbohydrates in aqueous solutions, such as a culture medium, give degradation products during GC even in fairly low concentrations, it is necessary to prevent the degradation by removing the sugar from the sample, by lowering the temperature at the injection port, or by shortening the column.

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