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Quantitative determination of mono- and oligosaccharides in potato tubers by gas-liquid chromatography

During the course of studies on the successive culture of potato root tip, one of the present authors found that potato tuber extract is essential for the cultivation, and the amounts of its sugar components are related to the growth of root tip. Although gas chromatographic determinations of trimethylsilyl ethers have recently been developed for sugar analysis[1-9], nothing has been reported concerning a mixture of carbohydrates containing oligosaccharides.

This paper reports an adaptation of the method of SWEELEY *et al.*[1] for the quantitative analysis of samples containing fructose, glucose, inositol, sucrose, melibiose and raffinose from potato tubers.

Materials and methods

Gas chromatograph. Apparatus: Shimadzu GC-4APF equipped with a flame-ionisation detector and integrator (Disc Instrument Inc.). Columns: 2-m stainless steel tube (3 mm I. D.) packed with 1.5 % SE-30 on 60–80 mesh Shimalite W.

Preparation of trimethylsilyl derivatives. To a mixture of carbohydrate (about 1 mg) and pyridine (0.2 ml) were added hexamethyldisilazane (0.1 ml) and trimethylchlorosilane (0.1 ml), and after heating at 60° for 10 min, the reaction mixture was evaporated to dryness under a stream of nitrogen. The residue was extracted with *n*-hexane (0.2 ml) and centrifuged. The supernatant (1 μl) was injected into the gas chromatograph.

Preparation of sample from potato tuber. Sliced potato tuber (500 g) was frozen at —20° for 5 h and extracted with H_2O at 2° for 24 h. The aqueous solution (500 ml) was deproteinized by heating, acidifying at pH 2 and adding 60 % MeOH. The filtrate was concentrated to about 50 ml *in vacuo* at 60°. The resulting solution was extracted with petroleum ether and neutralised by using Amberlite IR-120 and IRA-4B, then it was concentrated to 500 ml (1 ml = 1 g fresh weight). After 1 ml of internal standard solution (mannitol, 2.5 mg/ml) was added to this sample (1 ml), the mixture was evaporated to dryness *in vacuo* at 60°. The residue was silylated as described above.

Calculation. The following equation gives the weight (*Sw*, mg) of carbohydrate:

$$Sw = \frac{Sc \cdot Mw}{Mc \cdot F}$$

where

Sc is the total sum of the sugars, obtained by integrator;
Mc is the value for the internal standard (mannitol);
Mw is the weight (mg) of internal standard added;
F is the relative response of carbohydrate.

Results and discussion

Stationary phase. Several ordinary liquid phases such as SE-30, OV-1, OV-17 and neopentylglycol succinate were examined. For the simultaneous determination of the mixture of fructose, glucose, inositol, sucrose, melibiose and raffinose, the following chromatographic conditions were found to be suitable (Fig. 1): carrier gas, nitrogen at

TABLE I

RELATIVE RESPONSES OF CARBOHYDRATES (RELATIVE TO MANNITOL)

*Carbohydrate**	*TMS derivative*		*Relative response*
	Number of TMS group	*Carbon contents relative to mannitol*	
Fructose	5	0.875	0.672 ± 0.013
Glucose	5	0.875	0.778 ± 0.017
Inositol	6	1.000	1.101 ± 0.022
Sucrose	8	0.750	0.765 ± 0.018
Melibiose	8	0.750	0.548 ± 0.023
Raffinose	11	0.707	0.445 ± 0.018

* Trimethylsilyl ether

a flow rate of 50 ml/min; column temperature, maintained at 150° for 10 min and then programmed to increase at a rate of 6°/min; injection port temperature, 220°; detector temperature, 280°; column, 1.5 % SE-30 on Shimalite W (60–80 mesh).

Relative response. Mannitol was selected as an internal standard, since it is readily available, gives a single peak, is not found in potato tuber and has a favourable retention time for the present analysis. Table I shows the responses of the six sugars relative to mannitol. The carbon contents in each of these carbohydrates varied after the trimethylsilylation, and the observed differences between their relative responses may be due to this. The responses are likely to be proportional to the carbon contents in the trimethylsilyl ethers. It should therefore be noted that the term relative response must be included in the calculation formula when different carbohydrates are analysed by trimethylsilylation.

Carbohydrates in potato tuber. The neutral sample solution obtained as described in the experimental section gave the chromatogram seen in Fig. 2 under the standard

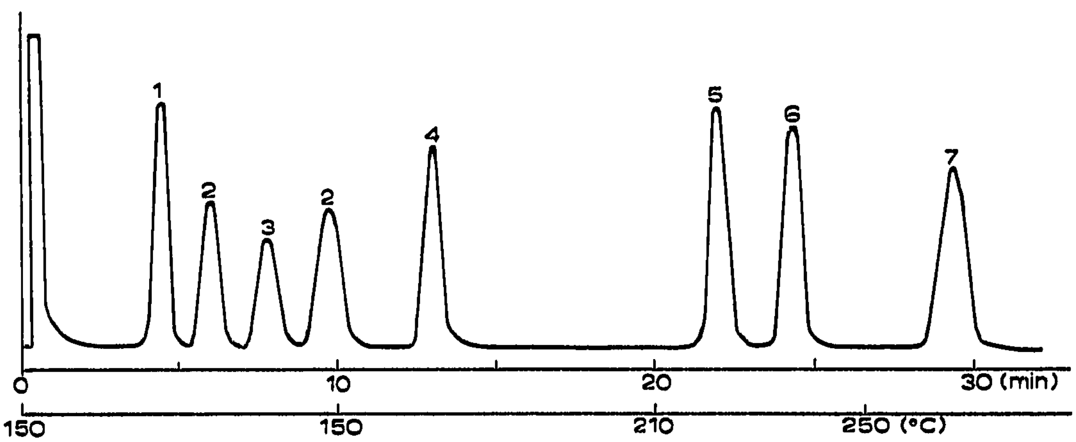

Fig. 1. Gas chromatogram of trimethylsilyl derivatives of an artificial mixture of seven carbohydrates. Column; 1.5 % SE-30 on Shimalite W (60–80 mesh); column temperature programmed from 150 to 280°; injection port temperature, 220°; detector temperature, 280°; flow rate of carrier gas, N_2, 50 ml/min. Components: (1) fructose, (2) glucose, (3) mannitol, (4) inositol, (5) sucrose, (6) melibiose, (7) raffinose.

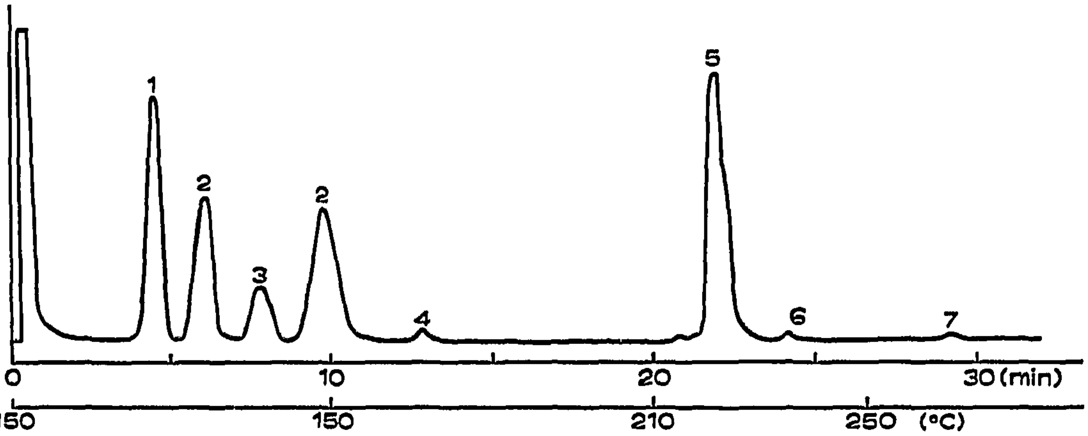

Fig. 2. Gas chromatogram of trimethylsilyl derivatives of carbohydrates from potato tuber. Chromatographic conditions as in Fig. 1. Components: (1) fructose, (2) glucose, (3) mannitol, (4) inositol, (5) sucrose, (6) melibiose, (7) raffinose.

conditions. The observed contents (mg/g of fresh weight) of carbohydrates in potato tuber were as follows: fructose, 3.40; glucose, 4.89; inositol, 0.09; sucrose, 4,44; melibiose, 0.12; and raffinose, 0.08. It is of interest that the sample had no detectable amount of galactose, which represents one of the sugar units in oligosaccharides such as melibiose and raffinose.

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