A Gas Chromatographic Method for the Determination of Sugars in Foods

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A number of mono-, di-, and trisaccharides have been separated, identified, and quantitatively determined by a gas chromatographic method applicable to foods and applied specifically to wheat and wheat products. The sugars were extracted by conventional means, then reacted with hydroxylamine hydrochloride to convert reducing sugars to their oximes. The mixture of nonreducing sugars and reducing sugar oximes was silylated to form TMS ethers which were quantitatively determined by gas chromatography on SE-30. A combination of isothermal and programmed oven temperatures was used to separate arabinose, ribose, fructose, galactose, glucose, sucrose, lactose, maltose, and raffinose in a single chromatogram. Evaluations of linearity of response and recovery of standards added to bread were made, and responses to the internal standard inositol determined. The use of the oxime decreased the number of tautomers of some sugars; the procedure used gave a mixture whose tautomeric equilibria were consistent and stable. Data on the sugar content of wheat, wheat flour, bread, and wheat flakes cereal are given.

A growing awareness of the differences in the nutritional effects of different sugars has created a need for information on the amounts of these sugars in foods. Most available data on total carbohydrates have been calculated by difference from values for fat, moisture, nitrogen, and ash. There is also some information on the amounts of reducing and nonreducing sugars in foods. Data on the individual sugars are not extensive, however, principally because of methodological problems. Most methods for specific sugars are chromatographic, including column, paper, ion-exchange (Whistler and Wolfrom, 1962), and thin-layer (Stahl, 1965), all requiring colorimetric estimations on separated fractions. These methods are not wholly satisfactory since they are often either excessively time-consuming, insensitive, or nonspecific.

In recent years gas chromatography has been adapted to the separation and determination of volatile derivatives of sugars (Sloneker, 1968; Wells *et al.*, 1964). Trimethylsilyl (TMS) ethers are easily prepared and chromatographed but commonly give multiple peaks due to tautomeric forms of the reducing sugars. The shifting equilibria among these forms and the difficulty of separating those of some of the important sugars create problems in developing quantitative methods. Fructose, a common food sugar, may exist in up to five forms (Curtius *et al.*, 1968), depending on the sample treatment and the state of the equilibrium. The problem of tautomers may be eliminated by reducing the sugars to their acyclic alditols and chromatographing volatile acetates or TMS ethers (Sawardeker *et al.*, 1965). This procedure is, in general, satisfactory for aldo sugars, but not for mixtures containing both fructose and glucose. Since each of these sugars reduces to sorbitol (fructose yields, in addition, mannitol), quantitation is difficult.

The possibility of reducing the number of tautomers by converting the sugars to oximes before forming the TMS ethers was investigated by Sweeley *et al.* (1963). They found that the resolution of the sugar oximes studied was not adequate for analytical purposes, although there was a decrease in the number of peaks on the ethylene glycol succinate columns used. This approach has been reevaluated here, using different liquid phases and more efficient columns as a method suitable for foods containing both glucose and fructose. Since the work was undertaken as a part of a survey of the nutrients in wheat and wheat products, the additional sugars arabinose, ribose, galactose, sucrose, lactose, maltose, and raffinose were also included. These nine sugars, individually and in mixtures, were oximated to convert reducing sugars to oximes, then all hydroxyls were silylated to form TMS ethers. Quantitative glc was carried out on SE-30, but retention data have also been determined on OV-17 and OV-25. The method was also evaluated for precision, accuracy, and quantitative recovery of added sugars.

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MATERIALS AND METHODS

The sugars used as standards, the internal reference inositol, and all reagents were commercial products. Gas chromatographic separations were carried out on an F&M Model 402 gas chromatograph equipped with dual flame detectors and operated in the dual mode for temperature programming. All quantitative determinations were made on 1% SE-30 columns, using a combination of isothermal-programmed-isothermal oven temperatures (Figure 1).

The sugars were extracted from an accurately weighed sample with 80% ethanol by allowing it to stand for 24 hr, with occasional swirling (Eheart and Mason, 1965). The alcohol extract was filtered and the residue washed with 80% ethanol until the washings gave a negative Molisch test. The filtrate was diluted to a known volume and an aliquot corresponding to 50 to 500 mg of sample or 0.25 to 5 mg of each sugar was mixed with 2 mg of inositol and evaporated to dryness in a water bath at 50° C with the aid of a gentle stream of nitrogen. After all solvent was removed, 1 ml of a solution of hydroxylamine hydrochloride in pyridine (25 mg per ml) was added and the mixture heated at 70 to 80° C for 30 min. Pyridine was removed by evaporating to dryness at 60° C in a stream of nitrogen; the last traces of water were removed as an azeotrope by adding a drop of benzene and again evaporating to dryness (Davison and Young, 1969). It was essential that all water be removed and rigorously excluded from this point on, since the sugar oxime TMS ethers were otherwise extremely unstable; this was particularly true of lactose and maltose. The mixture of sugar oximes and unreacted nonreducing sugars was dissolved in

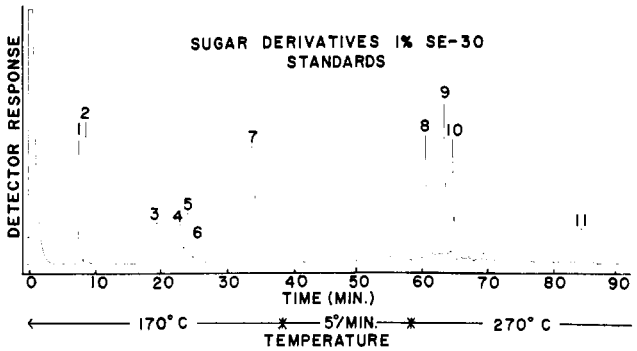

Figure 1. Chromatogram of oxime TMS ethers of reducing sugar standards and TMS ethers of nonreducing sugar standards. Column: 15 ft × 1/8 in. o.d., stainless steel, packed with 1% SE-30 on Gas Chrom Q (100–120 mesh). Oven temperature program: Isothermal at 170° C for 38 min; programmed at 4° C per min to 270° C; isothermal for 35 min. Detector temperature: 290° C. Injection port temperature: 290° C. Carrier gas: Helium, at 50 ml per min. The peak identities are: 1, arabinose; 2, ribose; 3, fructose; 4, galactose; 5, glucose; 6, galactose plus glucose; 7, inositol; 8, sucrose; 9, lactose; 10, maltose; 11, raffinose

0.5 ml of dry pyridine (stored over KOH pellets). Trimethylsilyl ethers were prepared by adding 0.5 ml of a mixture of pyridine/hexamethyldisilazane/trimethylchlorosilane (10/9/6). This solution was allowed to stand for 1 hr, then centrifuged. A 0.5-ml aliquot of the clear supernatant was transferred to a 3-ml vial, the excess reagent evaporated in a stream of nitrogen at 40° C, 0.5 ml of reagent grade isooctane added, and the residue dissolved by agitation on a vibrating mixer. The sample was then ready for injection onto the glc column. The replacement of pyridine with isooctane was not absolutely essential if the sample was to be chromatographed immediately. The derivatives were, however, unstable in the TMS reagent if kept for more than 48 hr; in isooctane they could be kept for 3 to 4 weeks. Moreover, the shorter solvent peak of isooctane was desirable when the sample contained low molecular weight sugars that elute on the pyridine peak.

Only nine sugars have been examined, selected because they they are known to occur in wheat or wheat products (Hlynka, 1964). They include seven reducing and two nonreducing sugars; the mixture chromatographed, therefore, consisted of TMS ethers of both oximated reducing sugars and the nonreducing sugars that do not form oximes. The chemical nature of the peak eluted was irrelevant, since it was only necessary that it be empirically related to a specific sugar. Five of the sugars, arabinose, ribose, sucrose, lactose, and raffinose, gave single well-separated peaks in the system used

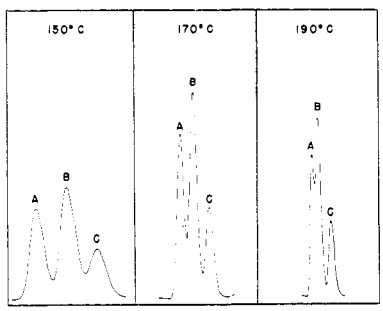

Figure 2. Relative retention of glucose and galactose oxime TMS ether tautomers as effected by temperature: A, galactose; B, glucose; C, galactose plus glucose

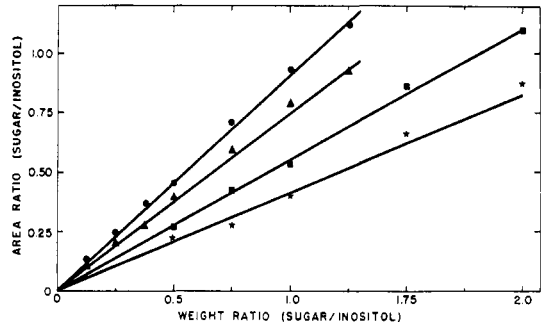

Figure 3. Response linearity of arabinose, glucose, maltose and raffinose. Sample size: arabinose and glucose, 0.125 to 1.25 μg; maltose and raffinose, 0.25 to 2.5 μg; inositol, 2 μg; ● arabinose; ▲ glucose; ■ maltose; ★ raffinose

and presented no quantitation problem. Fructose and maltose each gave two distinct partially separated peaks whose areas were summed for quantitative calculations. Glucose and galactose each gave two well-separated peaks easily distinguishable when only one of these sugars was present. When both were present in the same sample three peaks were obtained: the first galactose peak, the first glucose peak, and finally a third peak which was an unseparated combination of the second galactose and glucose peaks (Figure 2). This was the only pair examined that interfered to this extent. The problem of quantitation was met by using only the first tautomeric peak of each sugar.

Because of the use of partially separated peaks, it was not possible to measure areas by triangulation. A Disc integrator was used instead. The amount of each sugar present in the initial sample was calculated as follows:

$$\text{percent sugar} = \frac{C_c \times W_I \times 100}{K_c \times C_I \times W_s}$$

Where:

K_c = Correction factor; C_c = Integrator counts for the sugar peak; C_I = Integrator counts for the internal standard, inositol; W_I = Weight of inositol added to sample (mg); and W_s = Weight of sample in aliquot (mg).

RESULTS AND DISCUSSION

Because of the wide variation in the amounts of individual sugars in foods, one or more preliminary analyses were usually necessary to determine the optimum sample size and the attenuation required for each sugar peak. It was also necessary to chromatograph a sample without added inositol, since this compound occurs in many plant sources. The presence of inositol in the sample would require the selection of another internal standard. The linearity of response was of some concern because of the unfamiliar sugar oxime TMS ether derivatives and because of the necessity of using the combined areas of unresolved peaks as well as single tautomeric peaks. As a measure of the linearity, mixtures containing varying amounts of each sugar plus a fixed amount of inositol were derivatized and chromatographed under identical conditions. Plots of the peak area ratios (sugar/inositol) against the weight ratios (sugar/inositol) gave essentially straight lines. The results for arabinose, glucose, maltose, and raffinose are shown in Figure 3. The slopes of these curves as calculated by the method of least squares were used to correct for variations in response and to compensate for any losses. These values (K) for each sugar were: arabinose,

0.91; ribose, 0.91; fructose, 0.81; galactose, 0.60; glucose, 0.75; sucrose, 0.79; lactose, 0.60; maltose, 0.56: and raffinose, 0.42.

The recovery of added sugar was evaluated by analyzing a bread sugar extract before and after the addition of a known mixture of fructose, glucose, sucrose, lactose, and maltose. Calculated recoveries ranged from 88 to 104% (Table I).

A number of stationary phases were tested for their suitability for these separations, including OV-17 and OV-25. None was as satisfactory as SE-30. The glc retention data for the nine sugars on OV-17, OV-25, and SE-30, under conditions suitable for their identification, are given in Table II. Only high temperature phases were used because of the need to separate di- and trisaccharides. SE-30 columns of lower efficiency than those used here (TP = 5900, calculated on the inositol peak) would not be satisfactory for the analysis of mixtures containing both glucose and galactose, the pair whose separation is the most difficult. These two sugars present a further problem in that their relative separations are temperature dependent (Figure 2). Below 170° C the retention times for the monosaccharides were excessively long; above 170° C the first glucose peak and the first galactose peak began to overlap. Since these are the peaks used for quantitation, a temperature of 170° C was chosen for the initial isothermal period.

No attempt was made to determine the chemical and structural identities of the compounds chromatographed, other than relating them to specific sugars. Although syn and anti forms of the oximes and their TMS ethers are to be expected, there was no evidence of their separation on any of the phases used.

The method has been used to analyze representative samples of wheat and wheat products to demonstrate its usefulness as a practical analytical tool. Four products were analyzed: wheat grain, flour made from the grain, bread made from the flour, and a sample of commercial wheat flakes cereal. The description of these and related wheat products is given elsewhere (Toepfer *et al.*, 1969). The results of the glc determinations are given in Table III. For comparison, data obtained by other investigators for the sugars in wheat and flour, using paper and column chromatography, are included. The major sugars in wheat and flour are the nonreducing sucrose and raffinose, with smaller amounts of glucose and fructose,

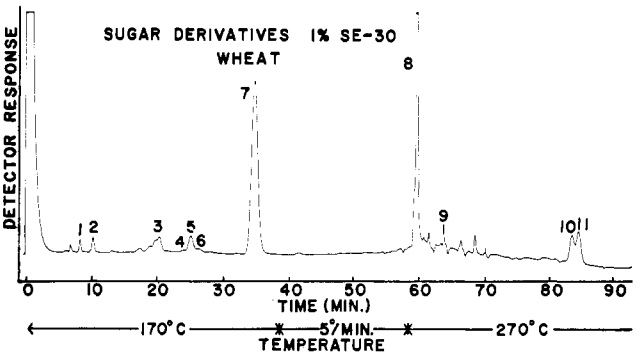

Figure 4. Chromatogram of oxime TMS ethers of reducing sugars and TMS ethers of nonreducing sugars from wheat. The peak identities are: 1, arabinose; 2, unknown; 3, fructose; 4, galactose; 5, glucose; 6, galactose plus glucose; 7, inositol; 8, sucrose; 9, maltose; 10, unknown; 11, raffinose

Table I. Recovery of Sugars Added to Bread Extracts[a,b]

Sugars	Amount in 50 mg Bread mg	Added mg	Total Calcd mg	Total Found mg	Recovery %
Fructose	0.14 ± 0.008	0.25	0.39	0.40 ± 0.033	104
Glucose	1.57 ± 0.014	0.25	1.82	1.79 ± 0.026	88
Sucrose	0.07 ± 0.008	0.25	0.32	0.30 ± 0.034	92
Lactose	0.87 ± 0.087	0.50	1.37	1.37 ± 0.14	100
Maltose	0.41 ± 0.079	0.50	0.91	0.91 ± 0.054	100

[a] Bread made with patent white flour. [b] Averages of triplicate determinations.

Table II. Retention Data for TMS Ethers of Sugars and Sugar Derivatives

Monosaccharides[a]

Derivative	SE-30[b] 170° C[c]	OV-17[b] 130° C[c]	OV-25[b] 130° C[c]
Arabinose Oxime	0.23	0.25	0.27
Ribose Oxime	0.26	0.29	0.31
Fructose Oxime I	0.56	0.65	0.61
Fructose Oxime II	0.59	...	...
Galactose Oxime I	0.69	0.82	0.80
Galactose Oxime II	0.78	0.89	...
Glucose Oxime I	0.73	0.87	0.86
Glucose Oxime II	0.77	...	...
Inositol TMS	1.00	1.00	1.00
Docosane	1.20	1.65	2.12

Di- and Trisaccharides[d]

Derivative	SE-30 270° C[c]	OV-17 220° C[c]	OV-25 220° C[c]
Sucrose	0.31	0.20	0.19
Lactose Oxime	0.46	0.30	0.23
Maltose Oxime	0.54	0.37	0.28
Dotriacontane	1.00	1.00	1.00
Raffinose	2.14	2.39	1.61

[a] Retention time relative to inositol. [b] 1% SE-30, 1% OV-17, 3% OV-25 on 100/120 Gas Chrom Q. [c] Isothermal. [d] Retention time relative to dotriacontane.

Table III. Sugars in Wheat and Wheat Products (Dry Weight Basis)

Component	Wheat glc %	Wheat MacLeod and Preece (1954) %	Wheat Lynch *et al.* (1962) %	Flour glc %	Flour Koch *et al.* (1951) %	Flour MacKenzie (1958) %	Bread glc %	Wheat Flakes Cereal glc %
Arabinose	Trace			Trace				
Fructose	0.11 ± 0.002	0.06	0.06	0.04 ± 0.004	0.02		0.32 ± 0.016	1.30 ± 0.14
Galactose	Trace		0.02	0.01 ± 0.004				
Glucose	0.10 ± 0.010	0.09	0.08	0.03 ± 0.002	0.01	0.04	3.56 ± 0.029	1.20 ± 0.090
Sucrose	0.65 ± 0.025	0.84	0.54	0.16 ± 0.010	0.10	0.27	0.16 ± 0.016	7.08 ± 0.76
Maltose		Trace	0.05	0.05 ± 0.004	0.07	Trace	0.93 ± 0.016	1.19 ± 0.13
Lactose							1.97 ± 0.17	
Raffinose	0.04 ± 0.014	0.33		0.17 ± 0.003	0.07	0.17		Trace
Other sugars		1.44			0.18	0.78		

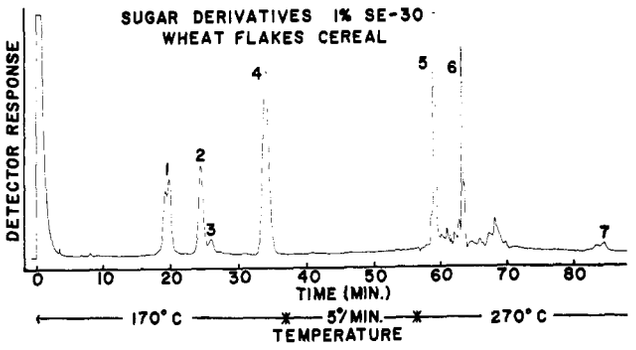

Figure 5. Chromatogram of oxime TMS ethers of reducing sugars and TMS ethers of nonreducing sugars from wheat flakes cereal. The peak identities are: 1, fructose; 2, glucose; 3, glucose; 4, inositol; 5, sucrose; 6, maltose; 7, raffinose

and trace amounts of galactose and arabinose. The values obtained here were comparable to those previously reported. In the chromatogram of the wheat sugar derivatives (Figure 4) there are a number of small unidentified peaks between sucrose and raffinose that may be related to the glucofructans or levosine discussed by Hlynka (1964).

The principal sugars of bread were, as would be expected, glucose and lactose, with smaller amounts of fructose, sucrose, and maltose. No raffinose was found, although it would be expected, since it was present in the flour. Wheat flakes cereal (Figure 5) contained sucrose as the predominant sugar, with smaller amounts of fructose, glucose, and maltose, and trace amounts of raffinose.

The method described is particularly useful for the analysis of complex mixtures of sugars that include both glucose and fructose. Samples containing only a few easily separated sugars may be analyzed with a less complicated program in a shorter time than is required here.

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