Chromatographic Determinations of Sugars and Starch in a Diet Composite Reference Material

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A large quantity of diet composite was collected from a day's menu used in one of the human metabolic studies at Beltsville Human Nutrition Research Center. This material, intended as a reference for the determination of nutrients in complex food mixtures, was processed to provide samples that are being characterized for a variety of nutrients. Our laboratory has determined sugars and starch and, in particular, monitored the stability of the individual sugars. The results from subsamples which were stored either wet or dry under different conditions (e.g., at room temperature, in a refrigerator, or in a freezer) for varying periods of time are reported. Starch was determined by an enzymatic procedure similar to the AACC Method 76-11. Two independent methods based on gas–liquid and high-performance liquid chromatographic techniques were used for the sugar determinations. Comparisons of the two methods and of sample preparation procedures prior to chromatographic analyses are discussed.

INTRODUCTION

An increasing interest and concern in the relationship of sugars to human health has been voiced by nutritionists and food scientists in the last few years (Lineback and Inglett, 1982; Reiser, 1982). In Oct, 1980, a section on the analysis of carbohydrates, sugars in particular, was included in the Workshop on Reference Materials for Organic Nutrient Measurement held at the National Bureau of Standards. At this meeting, evaluation of methodologies, the need for standard reference materials (SRM), and suggested matrices appropriate for SRM's were discussed. (Margolis, 1982). High-performance liquid chromatography (HPLC) and gas–liquid chromatography (GLC) were among those measurement techniques presented for sugar analysis. These methods have been well tested on standards and certain matrices and may be used with confidence for many food samples. (Hurst and Martin, 1979; Dunmire and Otto, 1979; Demaimay, 1978; Prager and Miskiewicz, 1979). However, problems concerning sample matrix and sample storage and preparation must be considered.

Most researchers rely on handbook values for calculating nutrient composition of a food or whole diets used in human metabolic studies. The composition of the food supply is changing constantly and data for many nutrients are unavailable; e.g., there are only scanty data on individual sugars or on starch for many U.S. foods. The carbohydrate value in most handbooks is calculated as the difference between 100 and the sum of the percentage of water, protein, fat, and ash. It is, therefore, important to validate calculated values by chemical analyses (Marshall and Judd, 1982).

For the past several years, we have been using GLC and more recently HPLC for the determination of sugars in foods. Starch has been determined as glucose by GLC after enzymatic hydrolysis according to AACC Method 76-11 (Li et al., 1982). Procedures have been described for sugars in ready-to-eat cereals, fruit juices, and yogurts (Li and Schuhmann, 1981, 1983) and for starch in salty snack foods and fast-food chicken (unpublished data). Each of these food categories represents a different matrix and appropriate sample handling and extraction procedures were developed.

Sugar contents were determined in our laboratory on several subject-collected mixed diets. Nine of the ten randomly selected samples which apparently had not been adequately stored during collection yielded sugars much lower than estimated values. We suspected that decomposition during collection, storage, or processing was a probable cause for the low values and for the complete absence of sucrose in these samples. Such observations led us to conduct a study on the extent of sugar degradation in this type of sample under certain storage conditions.

An appropriate reference material is a key requirement for assessing the accuracy of the measurements and for developing analyte extraction and cleanup methods appropriate to the sample matrix. Such a material has been prepared at Beltsville (Wolf and Ihnat, 1984) from a source of material consisting of 40 identical daily diets. A series of sugar and starch analyses has been carried out to characterize the homogeneity within the different lots of this material, the stability of the sugars under various storage conditions, the adequacy of the extraction procedures, the precision of our methods, and the extent of agreeement between GLC and HPLC values. Our data show that accurate measurement of individual sugars and of starch in a food sample with a complex matrix is feasable and we believe that calculation of carbohydrate by difference is no longer desirable nor necessary.

SAMPLE HANDLING AND STORAGE

Details of preparation and composition of the diet reference material have been previously published (Wolf and Ihnat, 1984). The material consists of commonly eaten foods as shown in Table I. Subsamples from three lots of the material and from 20 combined lots freeze-dried in bulk were used in this study (see Figure 1). Each lot consisted of a composite of two of the 40 individual total daily diets. All lots had been frozen immediately upon collection. Individual lots were partially thawed, blended, and refrozen, and finally combined lots were freeze-dried. Subsamples representing various stages in processing were received by this laboratory and analyzed. As a check on the effect of the bulk drying process, subsamples (70–90 g) from the fresh slurry were quickly frozen and dried in a small-scale freeze dry apparatus (The VirTis Company, Gardiner, NY). Subsamples from each of the lots were stored under different conditions: frozen (−15 °C), refrigerated (4 °C) or at room temperature (20–25 °C). On the day of analysis, samples were thawed (if necessary), weighed into culture tubes (15 × 125 mm), and dried in a vacuum oven at 55 °C to constant weight.

Extraction. Dried samples (350–700 mg) were first extracted with 10 mL of *n*-hexane, then with either 12 mL

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Table I. Menu for Total Daily Diet Material[a,b]

	weight, g
Breakfast	
orange juice, frozen, unsweetened	384
grapefruit segments, canned	160
cereal, LIFE	44
milk, whole	305
muffins, English, with raisins, toasted	62
jelly	27
sugar	11
Lunch	
chicken, breast, roasted	106
noddles, egg, steamed	200
carrots, cooked, without salt	194
asparagus, canned, without salt	152
egg yolk, cooked	6.3
rolls, Brown "n" Serve	65
cookies, shortbread	69
pear nectar, canned	312
Dinner	
fish, haddock, baked	106
lemon juice, bottled	6
tomatoes, canned, stewed	151
sugar	12
potatoes, boiled, without salt	171
parsley, flakes	0.4
bread, rye	62
carrots, shredded	35
cucumbers, chopped	35
brownies, with pecans and coconut	100
milk, whole	305
total	3080.7

[a] Approximately 3200 cal, 19% fat cal. [b] For details see Wolf and Ihnat (1984).

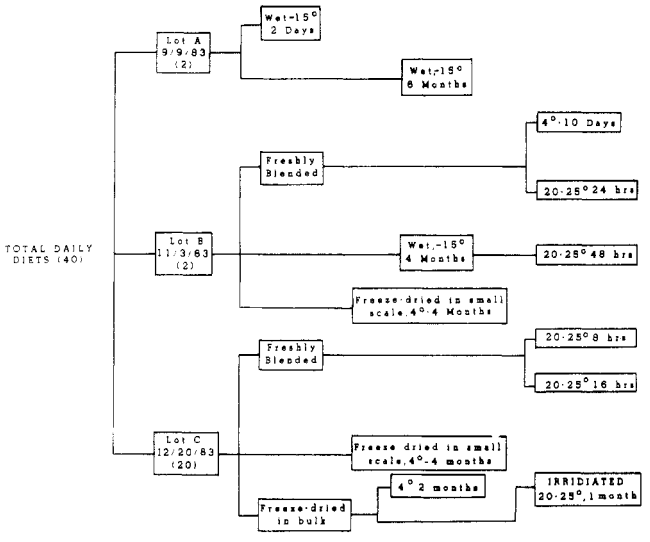

Figure 1. Summary of sample history.

of 80% methanol or 10 mL of deionized water. Sample and extracting solvent were mixed continuously on a Speci-Mix (Thermolyne, Sybron) and periodically in a sonicator. Samples were extracted for 1 h with water and 2 h with methanol. Extracts were centrifuged at 2000 rpm for 10 min and aliquots were removed from the supernatant for chromatographic analyses.

Derivatization for GLC. Aliquots (0.2, 0.5 mL), for determination by gas–liquid chromatography, were placed in 1.5-mL vials and dried on a speed vac concentrator (Savant Instruments, Inc., Hicksville, NY). Pyridine reagent (0.5 mL) containing hydroxylamine hydrochloride and an internal standard, phenyl-β-D-glucopyranoside, was added to the vials. The solution was mixed vigorously, heated at 75 °C for $^1/_2$ h, and then cooled; 0.5 mL of hexamethyldisilazane and 4 drops of trifluoroacetic acid were added. After further mixing and centrifugation, the Me_3Si derivatives of sugars were ready for injection onto a GLC column.

The individual sugars were identified by their retention times compared to known sugars and were quantitated according to the following expression: wt of sugar = (wt of internal standard) × (peak area of sugar)/(response factor) × (peak area of internal standard). The response factor is determined from the slope of a plot of the area ratio vs. the weight ratio of a sugar to the internal standard. The amount of each sugar present is proportional to the amount (known) of internal standard; hence, the exact volume of the final solution is no longer needed for quantitation.

Sample Cleanup for HPLC. Water extracts of the diet material to be analyzed by HPLC were submitted to the following procedure. An aliquot of approximately 5 mL of the supernatant was removed to a test tube and centrifuged for 10 min at 2500 rpm. An accurately measured aliquot of 3 mL or 4 mL was removed from this supernatant to another test tube and mixed with 0.2 mL of a xylose solution (50 mg/mL) as an internal standard. The extract containing xylose was then evaporated under N_2 at 60 °C to about one-half the volume. An equal volume of acetonitrile was then added and the tube was shaken and allowed to stand for at least 30 min for complete precipitation. The tube was again centrifuged and the supernatant was then processed through a SEP-PAK C_{18} cartridge (Waters Associates, Inc. Milford, MA) and 0.45-μm PTFE filter before injecting onto a HPLC column. Methanol extracts were treated similarly after an aliquot was first evaporated to dryness under N_2 and then redissolved in deionized water.

The individual sugars were identified and quantitated with standard solutions of sugars and an internal standard. The peak heights instead of areas were used in the calculation as described for GLC.

Chromatography Equipment. Gas–liquid chromatograph: a Hewlett-Packard 5840A was equipped with flame ionization detector and automatic sampler. Column: 6 ft × $^1/_8$ in. stainless steel packed with 3% SP2250 on 80/100 mesh Supelcoport (Supelco, Inc. Bellefonte, PA). Operating conditions: injection port, 200° C; detector, 325 °C. Column, 170–300 °C programmed at 10 °C/min; helium carrier flow, 30 mL/min; hydrogen flow, 40 mL/min; air flow, 300 mL/min. Sample volume, 1 μL.

High-performance liquid chromatograph: a modular system consisting of a Model 110A solvent metering pump, Model 156 refractive index detector, Model C-R1A recording integrator with built-in printer/plotter (Beckman Instruments, Inc. Irvine, CA), Model 7126 injector valve (Rheodyne Inc., Cotati, CA), radial compression Z-Module with Radial-PAK μ-Bondapak NH_2 cartridge (Waters Associates, Inc. Milford, MA). Operating conditions: mobile phase, 75/25 acetonitrile–water recycled into a 800-mL reservior; flow rate, 1.5–2.5 mL/min at ambient temperature. Sample volume, 20 μL.

Starch Hydrolysis. The residues (125–250 mg), after 80% methanol extraction of sugars, were suspended in 30 mL of H_2O and gelatinized in an autoclave at 130 °C for 1 h. A mixture of 1.5 mL of acetate buffer (pH 4.5), 1.5 mL of H_2O, and 3.0 mL of enzyme solution (Sigma No. A-7255 amyloglucosidase, 30 I.U./mL) was added to the cooled 55 °C suspension. The culture tubes were tightly capped and were maintained at 55 °C in an incubator for 2 h with occasional shaking. The hydrolyzate was centrifuged at 2000 rpm for 10 min. Aliquots (0.1 or 0.2 mL)

Table II. Homogeneity of Diet Composites (within Lots and between Lots)

lot	no. of samples	g/100 g of dry wt fructose	glucose	sucrose	lactose	maltose	total sugar[a]	starch
A[b]	2	5.93 (1.18)[e]	6.19 (1.29)	11.6 (1.83)	3.52 (0.57)	1.52 (3.95)	28.7 (1.05)	25.4 (0.83)
B[c]	8	6.29 (2.07)	6.14 (1.79)	12.6 (2.06)	3.64 (3.02)	1.50 (4.00)	30.2 (1.95)	23.9 (1.21)
C[c]	4	5.97 (0.50)	6.29 (0.64)	11.8 (1.10)	3.50 (1.14)	1.67 (2.99)	29.2 (0.62)	24.0 (1.04)
C[d]	6	6.03 (1.76)	6.27 (3.09)	11.4 (1.72)	3.43 (1.00)	1.42 (3.70)	28.5 (1.58)	24.2 (1.53)
av	20	6.06	6.22	11.8	3.52	1.53	29.2	24.4
RSD[e]		2.67	1.12	4.46	2.48	6.82	2.60	2.85

[a] Total sugar = the sum of individual sugars. [b] Frozen wet composite. [c] Freshly blended composite. [d] Freeze-dried composite. [e] Relative standard deviation.

Table III. Effect of Moisture Content and Storage Conditions on Diet Composites

lot	% moisture	g/100 g of dry wt fructose	glucose	sucrose	lactose	maltose	total sugar
A[a]	75.6	6.39	6.48	10.9	3.48	1.46	28.7
B[b]	76.2	6.34	6.40	11.9	3.59	1.54	29.8
B[c]	23.6	6.79	6.04	12.2	3.79	1.54	30.3
C[c]	7.1	6.50	5.90	12.9	3.79	1.46	30.6
C[d]	2.2	6.65	6.00	12.0	3.71	1.44	29.8
av		6.53	6.16	12.0	3.67	1.49	29.8
RSD[e]		2.85	4.20	6.0	3.68	3.24	2.42

[a] Frozen for 6 months. [b] Frozen for 4 months. [c] Freeze-dried in small-scale apparatus, then refrigerated for 4 months. [d] Freeze-dried in bulk then left at room temperature for 1 month. [e] Relative standard deviation.

Table IV. Effect of Storage Temperature and Storage Time on Wet Diet Composition

storage temp, °C	time[a]	g/100 g of dry wt mannitol	fructose	glucose	sucrose	lactose	maltose	total sugar	starch
−15	5 months	*b*	6.38	6.19	12.6	3.67	1.62	30.4	24.7
4	none	*b*	6.29	6.14	12.6	3.64	1.50	30.2	23.8
4	10 days	*b*	7.66	7.50	9.50	3.66	2.32	30.6	24.0
20–25	8 h	*b*	6.39	6.73	11.2	3.58	2.15	30.0	24.0
20–25	16 h	*b*	6.63	6.91	10.6	3.58	2.44	30.2	21.5
20–25	24 h	4.51	7.42	6.10	*b*	3.30	1.20	18.0	23.1
20–25	48 h	8.95	1.92	3.55	*b*	3.02	0.82	9.31	24.2

[a] Storage time after blending. [b] Not detectable.

of the supernatant were removed, dried under a stream of N_2 in a water bath (60 °C), and further dried in a vacuum desiccator. The dried residues were derivatized as described above and analyzed for glucose by gas–liquid chromatography. The amount of starch present is equal to the percent glucose × 0.9. This corrects for the addition of a molecule of H_2O for each glycosidic linkage broken during hydrolysis.

RESULTS AND DISCUSSION

Samples from three separately blended and mixed lots of the diet composite (as described under sample handling and storage) were processed under similar conditions and analyzed by GLC over a period of 5 months. Individual sugars and starch contents of these subsamples (Table II) have relative standard deviations (RSD) between 1–7%, which are similar to those of standard sugar mixtures (1–2%). This is indicative of (1) the homogeneity of the separate lots, (2) the repeatability of the initial blending and processing of the partially thawed diet mixtures, and (3) the precision of the analytical method. The samples (5 subsamples from each of 3 lots) were stored for 1–5 months following the initial analyses. The wet material was kept in a freezer, the freeze-dried material was stored either in a refrigerator or at room temperature. Then, 1 month after the analysis of the last lot, all the subsamples were reanalyzed on the same day. Comparison of the average values of individual sugars and their RSD in Table III with those in Table II led to the conclusion that the integrity of sugars could be maintained in this particular diet mixture under the conditions mentioned above.

However, as shown in Table IV, if a wet sample was left at room temperature, we were able to measure loss of sucrose after 8 h. The total sugar value remained constant up to 16 h at room temperature but inversion of sucrose was continuing. Inversion of sucrose was also observed in wet samples stored for 10 days at 4 °C. After 24 h at room temperature, the total loss of sucrose and varying changes in all others sugars were accompanied by the appearance of mannitol. On previous occasions when analyzing mixed food samples composited from inadequately stored subject-collected diets, we have encountered a large peak on GLC at a retention time slightly shorter than sorbitol. Sorbitol, present in many fruits, elutes just before fructose. The identity of mannitol was confirmed by GC/MS and by comparison of retention times of authentic mannitol in several GLC programs. Mannitol is not commonly found in foods at a level detectable by the GLC method described above (<0.2%), but it has long been known as a fermentation product of solutions containing sugars. (Birkinshaw et al., 1931; Onishi and Suzuki, 1968). We have found it to be a sure indicator of sample degradation. The presence of carbon dioxide, ethanol, acetic acid, and lactic acid could be considered indicative of carbohydrate metabolism by microorganisms present in a food mixture, but the more volatile of these would be lost during drying and the rest would elute with the solvent peak.

A portion of the freeze-dried diet composite was sterilized with cobalt-60 γ radiation (5–7 Mrad). Sugars and starch were determined in several irradiated subsamples and values did not differ from those in the nonirradiated freeze-dried diet composite. A study was conducted to

Table V. Effect of Room Temperature Storage of Reconstituted Diet Composite

	storage (day)	g/100 g of dry wt							
		mannitol	fructose	glucose	sucrose	lactose	maltose	total sugar	starch
nonirradiated	0	a	6.51	6.81	12.4	3.82	1.58	31.1	24.4
	1	a	7.86	7.21	8.07	3.61	1.54	28.3	23.4
	2	8.23	4.04	4.57	a	3.53	0.86	13.0	24.1
	6	10.8	1.17	3.39	a	3.49	1.39	9.44	25.7
	7	9.70	1.20	3.08	a	3.19	1.38	8.85	24.7
	14	9.57	a	0.2	a	3.32	1.92	5.44	23.9
irradiated	0	a	6.26	6.60	11.7	3.69	1.51	29.8	24.1
	1	a	6.92	7.17	11.1	3.74	1.81	30.7	22.9
	2	a	7.40	7.67	10.2	3.77	2.05	31.1	23.7
	6	a	8.95	9.18	6.60	3.71	2.65	31.1	22.5
	7	a	9.36	9.68	5.84	3.72	2.71	31.3	22.5
	14	a	10.9	11.8	2.23	3.52	3.19	31.6	22.9

[a] Not detectable.

Table VI. Water vs. 80% Methanol Extraction

sample	solvent	g/100 g of dry wt					
		fructose	glucose	sucrose	lactose	maltose	total sugar
A	80% methanol (D)[a]	6.07	6.37	12.0	3.47	1.42	29.3
	80% methanol (P)[b]	6.67	7.00	12.8	3.70	1.50	31.7
	water	6.69	7.03	12.6	3.85	1.64	31.8
B	80% methanol (D)	6.10	6.41	12.0	3.47	1.42	29.4
	80% methanol (P)	6.48	6.84	13.0	3.71	1.54	31.6
	water	6.65	6.99	12.4	3.74	1.57	31.4
C	80% methanol (D)	6.43	6.84	10.9	3.37	1.42	29.0
	80% methanol (P)	6.89	7.35	11.8	3.66	1.57	31.3
	water	7.00	7.40	11.4	3.78	1.65	31.2

[a] Aliquot ($^1/_2$ mL) withdrawn with dry automatic pipet tip. [b] Same as above but with prewetted tip.

determine the comparative stability of sugars and starch in irradiated vs. nonirradiated diet composites. Dried samples (6–7 g) were reconstituted to slurries containing 75% moisture by the addition of deionized water. The wet samples were left at room temperature (20–25 °C); subsamples were removed at intervals of 1, 2, 6, 7, and 14 days and analyzed according to the procedures described earlier. For the nonirradiated material, all the subsamples taken after 2 days showed large amounts of mannitol, complete absence of sucrose, progressive loss of fructose and glucose, but little change in lactose, maltose, and starch content. On the other hand, mannitol was not found in any of the subsamples from the irradiated material even after 14 days. No significant change was observed for lactose, maltose, starch, or even total sugar. However, inversion of sucrose to fructose and glucose was noticeable after 2 days (Table V). This is to be expected since the reconstituted material had a pH of 5.1.

In previous experiments, we found that 80% methanol was adequate and necessary for the extraction of sugars from certain foods since the use of water led to sucrose hydrolysis. However, for samples which have in excess of 5% of lactose and maltose, methanol extractions may not be quantitative. Therefore, parallel extractions were carried out with 80% methanol, cold water (20 °C) or hot water (80 °C) on one set of freeze-dried material. Very little difference was found between the cold water and hot water extracts. Table V shows that the values for individual sugars in 80% methanol extracts were comparable to those obtained from water extracts provided that the automatic pipet tips used for withdrawing the aliquots from methanol extracts were first prewetted with the extract. With water extracts, the tips may be used either dry or prewetted. This calls to our attention the importance of calibrating the various measuring devices used in the laboratory. Having established that for this particular diet composite, one may use either methanol or water for sugar extraction, the choice depends on the chromatographic technique to be used later on. For GLC and subsequent starch determination, methanol is preferred. For HPLC, water extraction is more convenient. Methanol must be removed before HPLC analysis because it is retained on the column longer than water and causes a large negative peak, which interferes with the measurement of the internal standard, xylose.

One important aspect for the characterization of this diet material as a reference for determination of nutrients is the confirmation of sugars and starch content by different analytical techniques. Since the report by Palmer and Brandes in 1974, HPLC has become popular with many researchers for rapid quantitative analysis of sugars in foods (De Vries et al., 1979; Folkes and Taylor, 1982; Zygmunt, 1982). GLC had previously been the leading technique (Sweeley et al., 1963; Mason and Slover, 1971). Recently, comparison of GLC and HPLC methods has been the subject of several publications (Ugrinovits, 1980; Iverson and Bueno, 1981; Reyes et al., 1982). The main advantage of HPLC is in the ease of sample preparation; there is no need to dry the extract or to prepare a derivative. Since we have more experience with the GLC technique we have chosen it as our principal method. One advantage of GLC is the stability of the SP2250 column which we used relative to that of any HPLC columns recommended for carbohydrate analysis (Lee et al., 1983).

After thorough characterization of the diet composite using a GLC method, we analyzed several sets of samples by using both methods. This is a direct check on the completeness of the formation of the Me_3Si oximes for GLC analysis. We compared data from aliquots processed independently for each of the methods with data obtained by using a portion of the HPLC sample for derivatization and GLC analysis. Individual sugar values thus obtained on four separate extracts are presented in Table VII. Chromatograms of one pair of these are given in Figure 1. The retention times and order of elution are similar for the two methods, except for the reversal of lactose and maltose. The HPLC quantitation by peak height provides RSD between 1 and 2% for all sugar standards, except

Table VII. Comparison of GLC and HPLC Data

extract	method	g/100 g of dry wt fructose	glucose	sucrose	lactose	maltose	total sugar
1	GLC[a]	5.77	5.89	11.7	3.15	1.27	27.8
	HPLC	5.33	5.88	11.4	2.93	1.16	26.7
2	GLC[b]	6.13	6.22	12.5	3.85	1.13	30.5
	HPLC	6.29	6.79	12.5	3.32	1.34	30.2
3	GLC[b]	6.13	6.22	12.0	3.51	1.53	29.4
	HPLC	6.01	6.68	12.0	3.18	1.40	29.2
4	GLC[b]	5.68	5.97	10.8	3.29	1.32	27.1
	HPLC	5.99	6.82	11.4	3.13	1.28	28.7
av	GLC	5.98	6.18	11.8	3.45	1.31	28.7
		(5.18)[c]	(5.34)	(6.02)	(8.70)	(12.2)	(5.33)
	HPLC	5.90	6.54	11.8	3.14	1.30	28.7
		(6.95)	(6.73)	(4.49)	(5.10)	(7.69)	(5.12)

[a] Aliquot from a solution processed for HPLC. [b] Aliquot from the original extract. [c] Relative standard deviation.

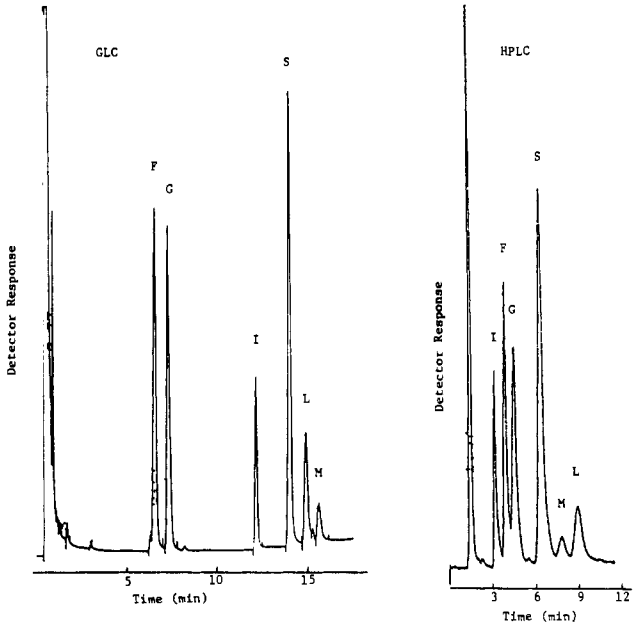

Figure 2. GLC of Me_3Si derivatives of sugars in diet composite. Column, SP2250 on 80–100 mesh Supelcoport, 6 ft × 1/8 in. stainless steel; flow rate, 30 mL/min, He; detector, FID; temperature, 170–300 °C at 10 °C/min; (F) fructose; (G) glucose; (I) internal standard, phenyl-β-D-glucopyranoside; (S) sucrose; (L) lactose; (M) maltose. HPLC of sugars in diet composite. Column, radial compression Z module with radial-Pak μ-Bondapak NH_2 cartridge; temperature, ambient; detector, RI; mobile phase, 75/25 acetonitrile–water; flow rate, 2.0 mL/min. (I) internal standard, xylose; (F) through (L) same as for GLC.

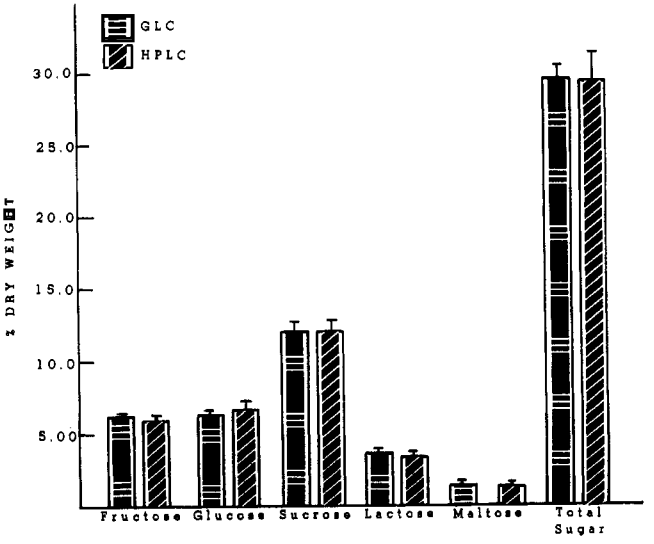

Figure 3. Average values for sugars as determined by GLC and HPLC on 20 extracts of a diet composite.

lactose which gives a RSD of 5–10%. For GLC, the RSD for all sugar standards are between 0.5 and 1.3%. The average value and its standard deviation for each sugar in 20 different extracts are shown in Figure 2. Confining ourselves to the trimethylsilylated oxime derivatives for GLC and an amine-bonded silica column for HPLC we have shown that comparable results can be obtained with these two techniques on a complex food matrix such as the diet composite we have used in this study.

CONCLUSION

A diet composite which may potentially be used as a reference material for the determination of sugars and starch in complex food mixtures has been prepared in quantity. We found the following: (1) The diet composite was homogeneous, within analytical error of ±5–10% from lot to lot with respect to sugar and starch contents. (2) Values for individual sugars were determined by both GLC and HPLC with comparable precision and accuracy. (3) Freeze-dried samples of this material may be kept either refrigerated or at room temperature for up to 5 months. Wet samples must be kept frozen (−15 °C) until analysis time. (4) Reconstituted irradiated material is much more resistant to fermentation than the reconstituted nonirradiated material. (5) The bulk freeze-dried material did not differ from the freshly blended material dried in a vacuum oven or from samples processed in a small-scale laboratory apparatus. All samples were dried to constant weight before extraction. (6) Either water or 80% methanol may be used to extract sugars.

Regardless of the method, the accuracy of the analysis depends upon the use of pure sugar standards and the precision is improved by the use of an internal standard. The use of a dry and well-characterized reference material is essential for quality assurance of the analytical measurements, but the accuracy and usefulness of the data ultimately depends on the integrity of the material sampled. The fermentability of sugars must be considered at all times.

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Registry No. Starch, 9005-25-8; fructose, 57-48-7; glucose, 50-99-7; sucrose, 57-50-1; lactose, 63-42-3; maltose, 69-79-4; mannitol, 69-65-8.

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Photohydrolysis of Ethylene Dibromide

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Ethylene dibromide in aqueous solution undergoes a rapid photohydrolysis upon irradiation with a 450-W medium-pressure mercury lamp. The photoreaction proceeds in two steps: (i) conversion of ethylene dibromide to bromoethanol ($t_{1/2}$ 7.6 min); (ii) cyclization of bromoethanol to ethylene oxide ($t_{1/2}$ 64 min). The rate of hydrolysis of ethylene oxide to ethylene glycol is not enhanced by light ($t_{1/2}$ ~10 days). The rates of reactions i and ii are respectively 32 and 3.8 times faster than the reduction of ferrioxalate ion under identical conditions. For ethylene dibromide the photo process represents a rate enhancement of the order of 10^5 over the nonphotolytic pathway.

INTRODUCTION

Ethylene dibromide (EDB) has been used as a soil fumigant, a sterilant for grains and wheat products as well as an antiknock additive to gasoline. The substance is a known carcinogen (U.S. Dept. of Health, Education & Welfare, 1979). Trace amounts of EDB have been detected in well water, and ppb levels have also been found in processed food products (Sheneman, personal communication). The presence of even small quantities of EDB in the environment has been the basis for serious concern.

In soil the substance can be dehalogenated by bacteria (eq 1) (Castro and Belser, 1968). The same transformation to ethylene has been observed to occur with the nematode *Aphelenchus avenae*. The main low level conversion of EDB by these animals however results in *o*-acetylserine (eq 2) (Castro and Belser, 1978). The ethylene dibromide carbon atoms were those of the acetyl moiety.

$$BrCH_2CH_2Br \rightarrow CH_2{=}CH_2 + 2Br^- + 2H^+ \quad (1)$$

$$BrCH_2CH_2Br \rightarrow CH_3C(=O)OCH_2CHNH_2CO_2H \quad (2)$$

Nonbiological conversion in the environment might be expected to be slow. For example, the rate of hydrolysis of EDB in neutral water is quite slow ($t_{1/2}$ ~16 years).

We report here that ethylene dibromide will undergo rapid photohydrolysis. The process could be a means of environmental detoxification.

EXPERIMENTAL SECTION

Materials. Ethylene dibromide, Matheson, Coleman and Bell, mp 9–10 °C, ethylene oxide, Matheson, and ethylene glycol, Mallenckrodt analytical reagent, were employed without purification. Bromoethanol, Eastman Kodak white label, was distilled (bp 55–56 °C (20 mm)) before use. All substances exhibited a single peak upon gas chromatography and showed correct mass spectra. Potassium ferrioxalate was prepared according to the procedure of Parker (1953). Water was deionized and glass distilled.

Methods. Product Identification and Analysis. Bromide ion was determined potentiometrically from 3-mL aliquots of the reaction in the manner previously described (Castro and Belser, 1968). Ethylene dibromide, (138 °C, 8.0 min), bromoethanol (138 °C, 5.4 min), ethylene glycol (138 °C, 4.0 min), and ethylene oxide (90 °C, 3.0 min) were determined from 1-μL reaction aliquots by direct flame ionization gas chromatography on a 3.5 ft, $^1/_8$ in. Poropak P column containing 3% DEGS and 6% DC-710. Ethylene oxide was monitored on a 2 ft, $^1/_8$ in. Poropak R column (90 °C, 3 min). The temperature and emergence

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