

SUGARS

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Nutritionally speaking, sugars belong to the class of macronutrients known as Carbohydrates. Dietary carbohydrates are sometimes classified into groups, such as:

Monosaccharides - e.g. fructose, galactose, glucose
Oligosaccharides - e.g. lactose, maltose, sucrose, malto-oligomers
Polysaccharides - e.g. starch, cellulose, hemicelluloses, pectins

Mono- and oligosaccharides are usually referred to as sugars. For this presentation, I shall restrict my discussion to sugar analysis in foods.

There are numerous methods for determining sugars, however, only a few that are accurate, fast and applicable for sugar mixtures in complex matrices. Methods that are based on gas-liquid chromatography (GLC) and high-performance liquid chromatography (HPLC) are by far the most popular and useful. The following is a list of various topics related to these two chromatographic techniques.

GAS-LIQUID CHROMATOGRAPHY

Derivatives

For lack of volatility, sugars must be converted to their derivatives in order to be analyzed by GLC. The most widely used derivatives are:
1) trimethylsilyl ethers - the formation of anomers and thus the resultant multiple peaks produced for each sugar make quantitation difficult for mixtures containing more than two sugars. 2) trimethylsilylated oximes - on certain columns, the oximes of each sugar yield only one single peak. 3) alditol acetates - sugars are first reduced to their corresponding alcohols and then reacted to form acetates. Fructose and glucose, for example, will both give rise to sorbitol and become indistinguishable. 4) aldonitrile acetates - these derivatives can be formed only from aldoses and not ketoses, such as fructose.

Columns

There are three general types of column: 1) packed - metal or glass with OD (outer diameter) between 1/8" and 1/4" and ID (inner diameter) of 2 mm and 4 mm. 2) widebore - fused silica with ID of 0.53 mm, length of 10 and 30 m. 3) capillary - fused silica or glass with ID of 0.2 mm and 0.32 mm, length of 12, 25 and 50 m. Choice of a column depends on the resolution, sample capacity, and sensitivity needed for a specific separation.

Packings

A wide variety of stationary phases is available for packed columns, but relatively few for fused silica capillary columns. Those that are found to be suitable for separating sugar derivatives are: 1) 50% phenylmethyl silicone, 2) 5% phenylmethyl silicone, and 3) methyl silicone gum. The polarity of a column usually determines how well a particular mixture of sugar derivatives can be separated.

Detectors

The three most commonly used detector in GLC are: 1) thermal conductivity - a universal detector for all compounds. It is simple and inexpensive but requires good temperature and flow control. 2) flame ionization - responds to all organic compounds and has excellent stability. 3) electron capture - high specificity for halogenated compounds. These detectors all provide a wide dynamic range of responses.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Columns

Most HPLC columns are made of stainless steel and they are available in various sizes. 1) analytical - ID, 2 - 6 mm; length, 10 - 30 cm and 50 - 100 cm. 2) capillary - flexible stainless steel with 0.2 mm ID, 0.5 mm OD and length of 25 cm. 3) microbore - ID, 1 mm and 2 mm with length of 25 cm and 50 cm. 4) cartridge - glass-coated stainless steel or radial compressed flexible tube, 8mm ID and 10 cm in length.

Packings

The ability to manufacture small particle size silica with uniform pore sizes and other synthetic spherical polymers is mainly responsible for the rapid advancement of HPLC column technology. For sugar analysis, four types of packings are available. 1) amine bonded silica - utilizes as mobile phase a mixture of acetonitrile and water. 2) silica modified with amine - also uses acetonitrile and water but with a small amount of amine added. 3) cation exchange - Ca, Pb or Ag loaded sulfonated polystyrene divinylbenzene resin, uses water as mobile phase. 4) anion exchange - quaternary ammonium functional groups attached to polystyrene divinylbenzene resin, uses dilute base as mobile phase.

Detectors

Refractive index measurement is often used for detecting sugars in HPLC, however, it lacks high sensitivity and is limited to isocratic elution. Sugars do not absorb light in the ultraviolet region, but they do in the near-ultraviolet region of 180 - 220 nm, where very high purity solvents are required. Recently, a few methods have been developed to react reducing

sugars with aliphatic amines forming fluorogens, which can be quantified with a fluorescence detector. Sugars that have been separated on an anion exchange column with a sodium hydroxide eluant can be detected by oxidation at a gold electrode with triple pulse amperometry.

ANALYSIS OF A DIET COMPOSITE

I shall describe the procedures we used to determine sugar contents of a diet reference material, which is a composite of a day's menu collected from a Human Study conducted at the Beltsville Human Nutrition Research Center.

The freeze-dried and well-blended diet composite was extracted with n-hexane to remove excess lipids and the residue was dried and extracted with 80% methanol. Sugars that were extracted into dilute alcohol were separated and quantified by either GLC or HPLC.

To be analyzed by GLC, extract containing sugars was dried and derivatized as shown in Figure 1. Chromatographic conditions are given in Figure 2. Starting with dried sugar extract and ending with a GLC chromatogram, the entire procedure can be accomplished within 1-1/2 hours. For the analysis by HPLC, the same sugar extract was treated as shown in Figure 3 and analyzed under the conditions given in Figure 4. This particular procedure requires less than 1 hour of preparation and analysis time. Using these two methods, a good comparison was obtained between the values for individual sugars of the diet composite (see Figure 5).

There are certain precautions that should be taken at various stages of sugar analysis.

1. Any sample containing sugars should be dried in a vacuum oven with temperature set at no greater than 60°C and under pressure of no less than 10 mm mercury.
2. Samples containing more than 10% sugars should always be kept frozen in order to prevent sucrose hydrolysis or microbial degradation.
(see Table 1).
3. Use of water for sugar extraction sometime leads to sucrose hydrolysis.
(see Table 2).
4. For sample cleanup of sugar extract prior to HPLC analysis, strong anion exchange resins, e.g. Dowex AG1-X8 (hydroxyl form), should be avoided. The use of cationic exchangers or anion exchangers (chloride form) will not affect recovery of sugars.

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SUGAR DERIVATIZATION PROCEDURES
TMS OXIMES

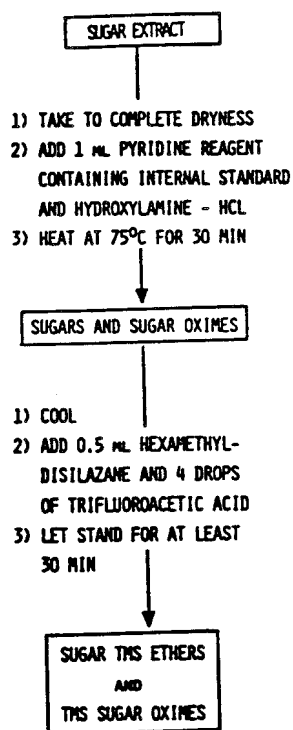


Figure 1

GAS-LIQUID CHROMATOGRAPHIC CONDITIONS:

CHROMATOGRAPH: HEWLETT-PACKARD 5840A EQUIPPED WITH AN AUTOMATIC SAMPLER AND A FID

COLUMN: 6' x 1/8" STAINLESS STEEL COLUMN PACKED WITH 3% SP2250 OR 3% OV-17 ON 80/100 MESH SUPELCOPORT

COLUMN TEMPERATURE: 170°- 300° PROGRAMMED AT 10°/MIN

DETECTOR TEMPERATURE: 325°C

INJECTION PORT TEMPERATURE: 200°C

CARRIER GAS AND FLOW RATE: HELIUM, 30 ML/MIN

Figure 2

SAMPLE PRETREATMENT
FOR HPLC

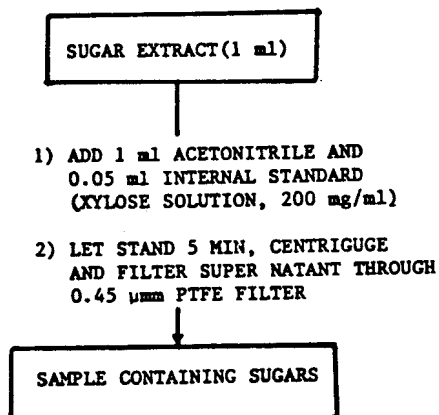


Figure 3

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC CONDITIONS:

CHROMATOGRAPH: BECKMAN MODEL 110A PUMP, MODEL 156 RI DETECTOR, MODEL C-RIA RECORDING INTEGRATOR/PRINTER/PLOTTER

COLUMN: WATERS ASSOCIATES RADIAL COMPRESSION Z-MODULE WITH RADIAL-PAK u-BONDAPAK NH₂ CARTRIDGE

TEMPERATURE: AMBIENT

MOBILE PHASE: 75/25 ACETONITRILE/WATER

FLOW RATE: 2.0 ML/MIN.

Figure 4

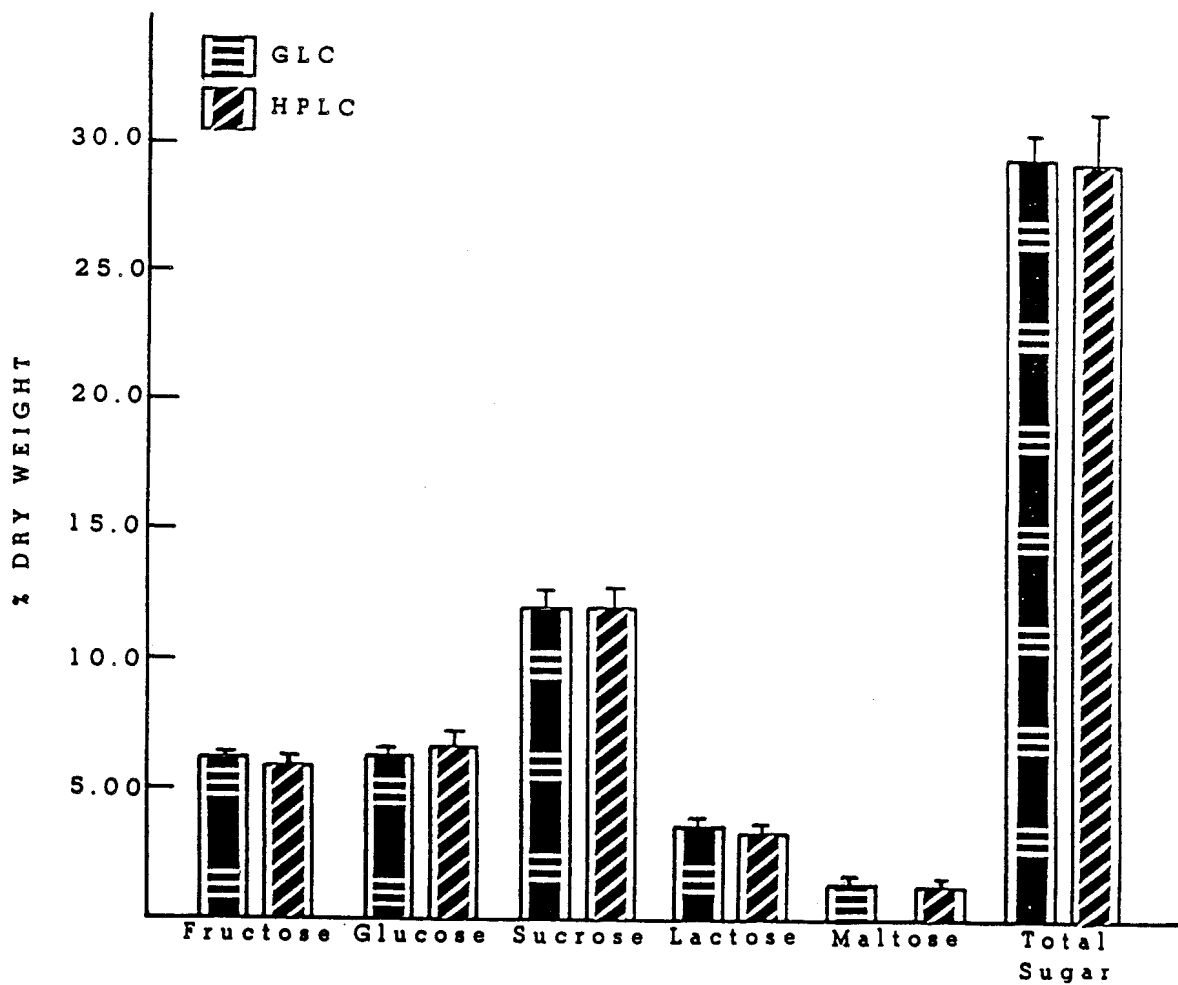


Figure 5 Sugar Contents of A Diet Composite, analyzed by Gas-liquid Chromatography and High-performance Liquid Chromatography.

Table 1. Effect of Improper Storage of Wet Diet Composites

storage temp. °C	time ¹	g/100 g dry wt						total sugar
		mannitol	fructose	glucose	sucrose	lactose	maltose	
-15°	5 mos.	N.D. ²	6.38	6.19	12.6	3.67	1.62	30.4
4°	none	N.D.	6.29	6.14	12.6	3.64	1.50	30.2
4°	10 days	N.D.	7.66	7.50	9.50	3.66	2.32	30.6
20-25°	8 hrs.	N.D.	6.39	6.73	11.2	3.58	2.15	30.0
20-25°	16 hrs.	N.D.	6.63	6.91	10.6	3.58	2.44	30.2
20-25°	24 hrs.	4.51	7.42	6.10	N.D.	3.30	1.20	18.0
20-25°	48 hrs.	8.95	1.92	3.55	N.D.	3.02	0.82	9.31

1 Storage time after blending

2 N.D. - Not Detectable

Table 2. Sugar Contents of Granola Cereals Containing Raisins

Cereal	Extracting Solvent	g/100g dry wt				
		fructose	glucose	sucrose	lactose	total sugar
Quaker, 100% Natural	water	6.80	8.08	9.89	2.55	27.3
		6.85	8.06	9.71	2.26	26.9
	80% methanol	3.87	5.08	15.5	2.08	26.5
		3.85	5.24	15.4	2.43	26.9
Vita Crunch	water	1.90	1.95	21.2	¹	25.0
		1.92	1.98	21.3	-	25.2
	80% methanol	1.86	1.83	21.5	-	25.2
		1.89	1.88	21.1	-	24.9

¹ not detectable