appears to be glycosidically linked through ester groups to some of the phenylpropanoid units.

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Gas–Liquid Chromatography of Trimethylsilyl Derivatives of Sugars from Iraqi Dates

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A method for accurate measurement of sugars in date was developed, in which 95% ethanol was used for Soxhlet extraction of sugars from two varieties of Iraqi dates. Sugars wre converted to their trimethylsilyl (Me_3Si) ether derivatives before application to GLC. Arabinose was used as an internal standard, and the separation of the sugar derivatives was carried out on a 3% SE-30 column. Fructose, α- and β-glucose, and sucrose were the major sugars present. Sorbitol and sorbose, which were not previously reported in the literature, were found in small quantities. The identification of sorbose and sorbitol was based on retention data using three different chromatographic columns.

Most of the analyses which have been carried out on date sugars were concerned mostly with the major fractions, fructose, glucose, and sucrose. Sugar determinations are carried out normally by thin-layer chromatography (TLC) (Raadsveld and Klomp, 1971), paper chromatography (PC) (Chan and Cain, 1966), column fractionation (Thompson et al., 1962), and ion-exchange chromatography (Dubois et al., 1956). However, these techniques are time consuming and hard to quantitate.

A more reliable and accurate method is gas chromatography (GLC), which has been used successfully in the analysis of sugars in plant material (Ford, 1974; Phillips and Smith, 1973).

Since sugars comprise about 78% of the total dry weight in dates as reported by Cook and Furr (1953), therefore their detailed analysis would help in better understanding their nutritive value. Sugars can also be used as indicators for ripeness (Ricardo and Rees, 1970).

A detailed study of the individual free sugars present in dates was carried out in our laboratory by using GLC separation of trimethylsilyl (Me_3Si) ether derivatives according to Sweeley et al. (1963) and Richey et al. (1964). This technique was used in the analysis of carbohydrates in food by many workers (Brobest and Lott, 1966; Davison and Young, 1969; Mason and Slover, 1971; Reineccius et al., 1972; Schubert et al., 1973; Kovacs, 1974).

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This paper describes a method for sugar extraction from dates and the GLC analysis of their Me_3Si ether derivatives.

EXPERIMENTAL SECTION

Sample. Two commercial varieties of Iraqi dates were used for this study: Zahdi, which is a semidry date, and Sayer, one of the soft types as described by Dowson and Aten (1972). Dates used in this work were the crop of 1976 harvest from Za'afarania Experimental Station.

The dates used were at two stages of maturation: half-matured dates, characterized by being half yellow and half ripe, and fully matured dates, which are in the final stage in the ripening process. The sugar analysis was carried out immediately after harvest and after 3 weeks of storage at room temperature (25–30 °C).

Procedure for Preparing Date Extracts. Samples of dates were freeze-dried, cooled in liquid nitrogen, and ground to pass through a 1-mm sieve. Two grams of freeze-dried sample was Soxhlet extracted with ether and then with 95% aqueous ethanol. The ether extract was discarded while the ethanol extract was brought up to 100 mL according to Ford (1974).

Arabinose was used as an internal standard. It was chosen because of its absence from date sugars and also because its retention time does not coincide with other date sugar constituents.

Reagents. The sugar standards used in this study were high quality standards obtained from Applied Science Laboratories, Inc., State College, PA. Tenax GC, 60–80 mesh, column packing material was obtained from Chrompack, Middelburg, Holland. All other column packing materials used in this study were obtained from Packard.

Preparation of Trimethylsilyl (Me_3Si) Derivatives. Two milligrams of internal standard was put into 3-mL minivials together with 0.5-mL aliquots from date sugar samples and dried by flushing with dry nitrogen. One milliliter of silylation mixture containing hexamethyldisilazane (HMDS), trimethylchlorosilane (TMCS), and pyridine (3:1:9) (Applied Science Laboratories) was added to each vial. Vials were sealed and shaken vigorously with a vortex mixer while being heated with hot air. Mixtures were left overnight in the refrigerator before they were injected into the gas chromatograph.

Gas Chromatography. *Instrumentation.* The apparatus is a Pye 104 fitted with a hydrogen flame ionization detector and a Speedomax W recorder. A hydrogen generator (GE) fitted with an air filter was used to supply hydrogen, and an air compressor (GE) fitted with an air filter was used to supply air to the detector.

Separation of the Me_3Si sugars was carried out on a glass column (150 × 0.6 cm o.d.) packed with 3% SE-30 on 100–120 mesh Diatomite CQ.

Operating Conditions. The column temperature was set at 140 °C, programmed to rise linearly at 4 °C/min up to 250 °C. Injection ports were maintained at 250 °C, and the detector oven was at 270 °C. Nitrogen at a flow rate of 30 mL/min was used as a carrier gas. The hydrogen flow rate was 30 mL/min, and the air flow rate was 300 mL/min. Samples of 1 μL were injected, and the temperature programming was started on the appearance of the solvent peak.

Two other column packings were used in order to confirm the identity of peaks present, 3% OV-17 and Tenax GC, 60–80 mesh. The chromatographic conditions used for the OV-17 column were the same as those used for the SE-30 column. For Tenax GC the column temperature was initially 140 °C, programmed to rise linearly at 5 °C/min up to 260 °C. Injection ports were maintained at 260 °C, and the detector oven was at 280 °C. All other chromatographic conditions were the same as those for the SE-30 column.

Table I. Retention Times, Relative to the Internal Standard, of Date Sugars Compared with Those of Known Compounds[a]

		3% SE-30		3% OV-17		Tenax GC	
no.	name	date sample	standard	date sample	standard	date sample	standard
1	arabinose[b]	1.00	1.00	1.00	1.00	1.00	1.00
2	fructose	1.67	1.65	1.71	1.76	1.26	1.26
3	sorbose	1.77	1.77	2.18	2.18	1.28	1.28
4	α-glucose	1.88	1.87	2.44	2.44	1.32	1.32
5	sorbitol	2.05	2.05	2.44	2.44	1.46	1.46
6	β-glucose	2.16	2.15	2.88	2.91	1.38	1.38
7	sucrose	4.17	4.23	6.47	6.71	2.93	2.93

[a] Operating conditions were as described in the text. [b] Arabinose was used as an internal standard; retention time = 1.0.

Table II. Retention Time and Relative Percentage Area for Trimethylsilyl Derivatives of Pure Sugars Relative to the Internal Standards after Solvent Extraction

name of sugar	peak isomer no.[a]	RRT[b]	% relative area
	1	1.66	42.77
(1) fructose	2	1.67	41.81
	3	1.88	13.51
(2) sorbose		1.77	100.00
(3) α-glucose		1.88	46.15
(4) β-glucose		2.16	53.85
(5) sorbitol		2.05	100.00
(6) sucrose		4.17	100.00
(7) glucose + fructose	1	1.66	20.00
	2	1.67	8.00
	3	1.88	36.20
	4	2.16	35.30

[a] Numbered in order of elution; first to elute = no. 1 for each sugars. [b] Column packing was 3% SE-30; chromatographic conditions were as described in the text. RRT = relative retention time.

RESULTS AND DISCUSSION

Sugars in date samples were fractionated by GLC and then were identified by using standard sugars. Arabinose, which is not one of date sugars, was used as an internal standard to quantitate sugars. Table I represents the GLC retention time of date sugars relative to the internal standard using three different column packings. As we can see, all sugars gave identical retention times with those of the pure standards used.

The identification of sorbitol was confirmed by adding pure sugar to date samples. On the 3% SE-30 column, the addition of excess sorbitol caused an increase in the sorbitol peak, while on 3% OV-17, the addition of sorbitol caused an increase in the α-glucose peak. On the latter, column, sorbitol gave a retention time similar to that of α-glucose. For the Tenax GC column, the retention time of sorbitol was greater than that of β-glucose (Table I).

Both α and β forms of the glucose were present in date samples. The percentage areas of each were 46.15 and 53.85%, respectively (Table II). The ratio between the α and β forms was constant in both Soxhlet-extracted samples and cold solvent extracted samples. This indicates that the heat used in the Soxhlet extraction had no effect on the ratio between the two forms. The quantities of glucose were calculated by adding both peaks together, and

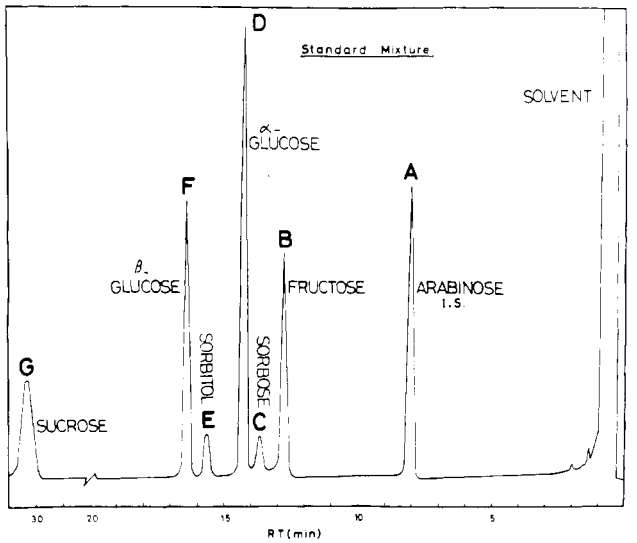

Figure 1. Standard mixture of Me_3Si sugars after recovery from Soxhlet extraction and derivatization.

the net results were the glucose present in our samples.

Fructose was found to give three isomer peaks as shown in Table II. Peak 3 gave a retention time similar to that of α-glucose. The concentration of the α-glucose is then determined by subtracting the calculated area of fructose isomer 3 from the area under peak D according to Phillips and Smith (1973).

Partial silylation is known to occur when insufficient reagent (HMDS) is provided (Kim et al., 1967; Larson et al., 1974; Ellis, 1969; Bedle, 1969; Supina and Rose, 1970). The possibility that the multiple peaks which we found for fructose are due to partial Me_3Si derivatives was tested. Similar chromatographs were found for date samples and for samples azeotroped with benzene before silylation, and also with a sample which had a 10-fold increase in the volume of HMDS, TMCS, and pyridine. This indicates that silylation reagents used in this experiment (1 mL) are adequate and the multiple peaks are due to isomer formation.

Table III. Percentage Recovery of Three Major Sugars in Date[a]

name	quantity added, mg	quantity recovd, mg	% recovery
(1) glucose	2.00	1.90	95
(2) fructose	2.00	1.86	93
(3) sucrose	1.00	0.98	98

[a] After Soxhlet extraction of pure sugars.

Quantities were calculated from standard curves made for individual sugars in which linear responses were found over the concentrations examined for all sugars tested. Calibration curves were made with and without the solvent extraction step in order to carry out the calculations under the same conditions used for date sugars. The results obtained from both curves were the same.

Figure 1 shows a chromatogram for a standard mixture of Me_3Si sugars after the Soxhlet extraction and derivatization steps. The concentrations added in these experiments are within the quantities recovered from dates.

Recovery experiments were carried out in which a 0.4-g sample of each pure sugar was Soxhlet extracted in the same manner as that for our date samples in order to test the efficiency of the Soxhlet extraction. Aliquots (0.5 and 1.0 mL) were derivatized and gas chromatographed as previously described under Experimental Section. Peak areas of the recovered sugars were calculated and compared with the calibration curves. The results of these experiments showed high recovery values for the sugars tested (Table III).

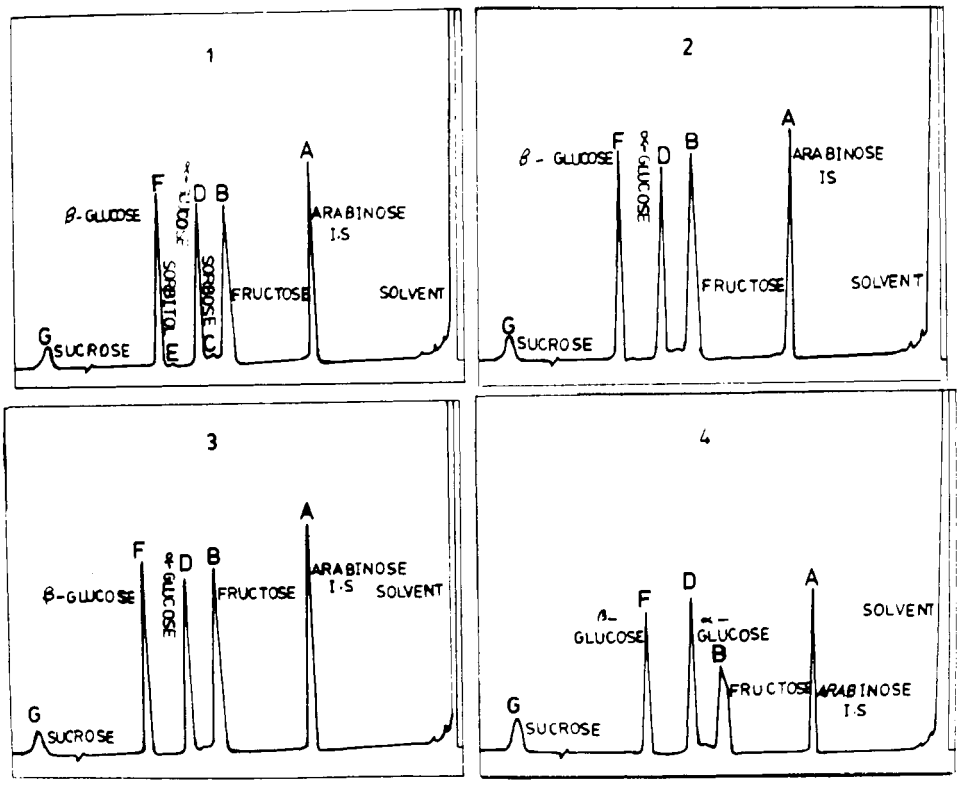

Figure 2. Chromatograms of Me_3Si sugars from Zahdi dates. The sugars are (A) arabinose (I.S.), (B) fructose 1 and 2, (C) sorbose, (D) α-glucose, (E) sorbitol, (F) β-glucose, and (G) sucrose. (Chromatogram 1) Fully mature Zahdi at day 1; (2) fully mature after storage; (3) half-mature at day 1; (4) half-mature after storage.

Table IV. Quantities[a] of Each Sugar Present in Two Varieties of Iraqi Dates

date sample	no.	treatments	fructose	sorbose	glucose	sorbitol	sucrose	total sugars
Sayer	1	fully mature day 1	0.314	Tr[b]	0.390	0.015		0.718
	2	fully mature after storage	0.355	0.014	0.359	0.017		0.745
	3	half-mature day 1	0.292	0.008	0.382	0.017	0.058	0.757
	4	half-mature after storage	0.300	0.017	0.363	0.011		0.699
Zahdi	1	fully mature day 1	0.361	0.006	0.351	Tr	0.088	0.806
	2	fully mature after storage	0.341	0.008	0.308	0.015	0.068	0.739
	3	half-mature day 1	0.335	0.004	0.355	0.014	0.072	0.780
	4	half-mature after storage	0.326	0.005	0.332	Tr	0.111	0.774

[a] Grams of sugar per gram of dry sample. [b] Tr = trace.

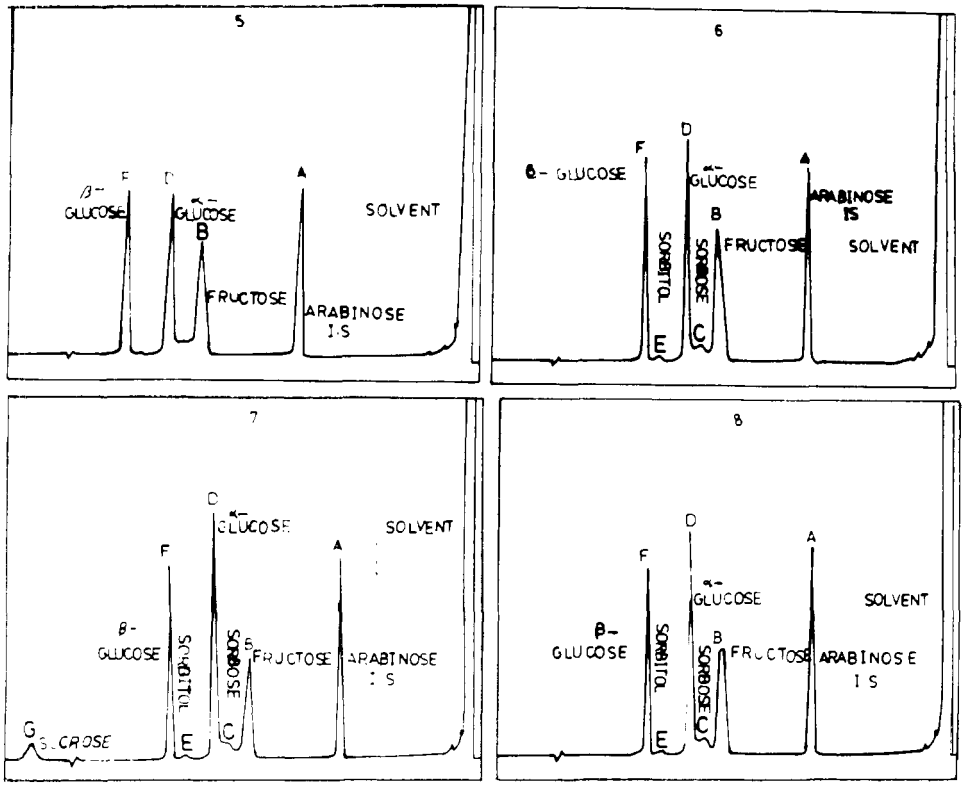

Figure 3. Chromatograms of Me_3Si sugars from Sayer dates. Sugars are the same as in Figure 1. (Chromatogram 5) Fully mature Sayer at day 1; (6) fully mature after storage; (7) half-mature at day 1; (8) half-mature after storage.

Table IV represents the quantities of each sugar present in the two varieties of Iraqi dates under study. Results are given in grams of sugar per gram of dry sample. Figure 2 and 3 are Zahdi and Sayer date chromatograms, respectively.

The quantities of sugar recovered from both Zahdi and Sayer dates were in agreement with those found by Cook and Furr (1953), who calculated the total sugars, after hydrolysis, and the reducing sugars by a chemical method. The quantities of sucrose were calculated from the difference between the two. Recent work was reported by Benjamin et al. (1975) which confirms the above results, using the same technique that was used by Cook and Furr (1953).

A qualitative paper chromatographic separation of sugars present in Zahdi date was reported by Al-Dawody et al. (1967). However, this work dealt only with the major fractions present in dates.

The sugar analysis of half-matured Zahdi dates immediately after harvesting and after 3 weeks of storage showed slight differences in sucrose content and the fruit turned to the fully matured stage after the end of the storage period. As for the fully matured Zahdi dates, a reduction in sucrose content was observed at the end of the storage period from that of the day 1 analysis.

The enzyme invertases are fully active in the fully matured stage (Hasegawa and Smolensky, 1970), and this would explain the reduction in sucrose content during the storage period of fully matured Zahdi dates. Half-matured dates contain less active invertases than those of fully matured dates (Kanner et al., 1978), and this is in agreement with our results which showed stability of the sucrose during the storage period of half-matured Zahdi dates.

Sayer dates contain less sucrose, and they normally contain no sucrose during the late stages of maturation (Dowson and Aten, 1972). From our results, we also found that during the first few days of storage, the half-matured Sayer dates became fully matured and the sucrose content dropped rapidly.

From this study, it was concluded that the accurate measurements of sugars in date can be achieved by using gas–liquid chromatography after derivatization. The method is fast, quantitative, and suitable for dealing with large numbers of samples. Fructose, α- and β-glucose, and sucrose were the major sugars present, while sorbitol and sorbose, which were not reported in the literature, were found in small quantities. The identification of sorbose and sorbitol is based only on retention data using different chromatographic columns.

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Expression of Alfalfa Juice

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A double-roll laboratory-scale press was developed for the expression of juice from alfalfa or other vegetation. It has a wet feed capacity of approximately 3 kg/h. Some preliminary studies were performed with the press. Cooling the forage before processing had no effect on the expression of alfalfa juice while heating generally had detrimental effects. Maceration before pressing proved advantageous, but the importance of the addition of water to forage before pressing was not conclusively shown.

The fractionation of green crops is theoretically attractive. Although high yields per hectare of crude protein and dry matter can be obtained from green crops, the crude protein is used inefficiently by ruminant animals. In addition to this, the whole crop cannot be used efficiently by nonruminants and humans (Jones, 1977).

It is possible to mechanically extract protein for nonruminant animal and human consumption with the remaining residue being used by ruminants. The processes range from the extraction of a low protein with the pressed crop still being the final product to exhaustive extraction with leaf protein concentrate for human consumption being the final product (Jones, 1977).

In order for green crop fractionation to be widely adopted, it must be possible to inexpensively express plant juice at reasonably high rates. This requires a knowledge of the concepts of cell rupture and juice expression. The nature and requirements of these processes have been investigated. Jones (1977) has compiled information on the principles of green crop fractionation and has identified some areas where additional research is needed.

While the largest differences in protein yield can be attributed to varietal differences for the leaves involved, substantial yield differences appear to be due to differences in growth and harvesting conditions, processing conditions prior to expression, and expression conditions. Juice protein yield has been empirically correlated with various processing-, harvest-, and growth-related factors.

A wide variety of factors influence juice and protein recovery during juice expression from macerated alfalfa leaves. These include (1) cell rupture, (2) fragmentation of protein-containing cell organelles, particularly chloroplasts, (3) pressing rates during expression, (4) press cake mass per unit of outflow area, (5) juice viscosity, (6) blinding of the expression outflow medium, (7) organelle and juice entrapment within pores which are blocked during the compaction that accompanies expression, and (8) soluble protein coagulation and chloroplast flocculation prior to and during expression. Schwartzberg et al. (1977) indicated that all of the above factors are important.

Roughly 88–95% of the protein-containing cells in alfalfa are opened by vigorous maceration, but, because of press cake blinding, only 65–75% of the mobile protein content (46–53% of the total protein content for alfalfa, 80% of whose protein is mobile) can be recovered by simple, single-stage pressing. However, almost all of the remaining mobile protein in the open cells can be recovered by multistage addition of water and expression following the first pressing. The protein concentration reductions caused by such rewetting and expression can be minimized by carrying out the process in a countercurrent fashion. Approximately 20% of the protein in the alfalfa appeared to be immobile and susceptible to recovery only by chem-

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