DETERMINATION OF SACCHARIDE DISTRIBUTION OF CORN SYRUP BY DIRECT DENSITOMETRY OF THIN-LAYER CHROMATOGRAMS[1]

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ABSTRACT

A rapid method for the determination of saccharide distribution of corn syrup up to nonasaccharides by direct densitometry of thin-layer chromatograms (TLC) is described. The results are of comparable accuracy with those obtained from gravimetric paper chromatography.

Paper chromatographic methods have been applied for determining the saccharide distribution in syrups (1,2,3). This technique is often too time-consuming and laborious for a routine method.

This paper describes a procedure which combines thin-layer chromatography with direct densitometry to give a simple and rapid method for the saccharide distribution of corn syrups. Values obtained by gravimetric paper chromatography were used as a reference.

Materials and Methods

Paper Chromatography. For the paper-chromatographic separation of the oligosaccharides in corn syrup, we used a solvent system of n-butanol-acetic acid-water-ethyl acetate, 6:3:4:6 by volume. This solvent system separated oligosaccharides of different molecular weights but not of different isomeric composition. A 90-mg. sample of syrup at approximately 35% solids was transferred to the Whatman 3MM paper (9 in. by 20 in.).

Descending chromatography at ambient temperature showed satisfactory separation between di- and pentasaccharides after 72 hr. Monosaccharide was not retained on the paper and was determined directly

[1] Manuscript received June 7, 1965. Presented at the 50th annual meeting, Kansas City, Mo., April 1965.

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by the glucose oxidase method (4). Development for 250 hr. was required for a separation between penta- and nonasaccharides, the lower saccharides being drained off the paper. Therefore, two separate paper chromatograms (72 and 250 hr.) were necessary for fractionation up to the nonasaccharides.

An Automatic Fraction Elutor (Cahn Instrument Co., Paramount, Calif.) was used to elute (with water) and collect the fractions for gravimetric determinations to establish standards for the TLC method. The operating theory and procedure for the Elutor have been described by Houk (5). Figure 1 shows the weight and the saccharide distribution of a 43 Dextrose Equivalent (D.E.) regular conversion syrup.

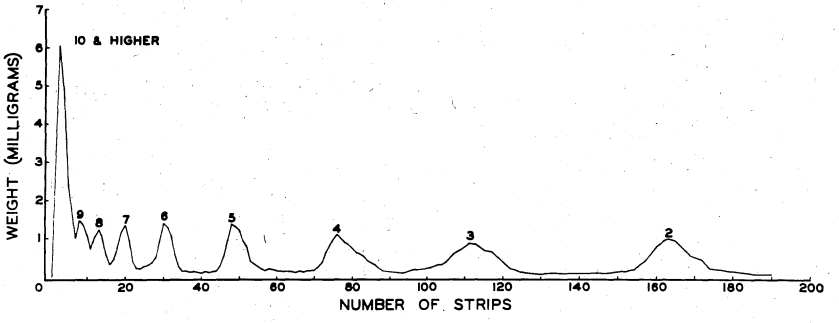

Fig. 1. Saccharide distribution of a 43-dextrose equivalent (D.E.) regular conversion syrup by gravimetric paper chromatography. Peaks 2 to 9: disaccharide to nonasaccharide.

Thin-Layer Chromatography. The thin-layer chromatographic analysis was done on glass plates (20 by 20 cm.) coated with a mixture of Silica Gel G and kieselghur G (Brinkman Instruments, Westburg, N.Y.), 3:1 by weight. The coating mixture was slurried with an equal weight of water, spread on the glass with a Stahl spreader set at 0.5-mm. thickness, and dried at ambient temperature before use.

Syrup samples, with a predetermined dried solids content, were diluted with water to about 1% solids and spotted on the plate with a Hamilton microliter syringe (No. 7001) in amounts from 5 to 30 γ of dried solids. A stream of warm air was directed onto the plate during sample application to keep the size of the spot less than 2.5 mm. in diameter. Each plate could accommodate 12 spots, six of the standard and six of the unknown.

The plate was irrigated, at ambient temperature, in the ascending direction to 12 cm. above the initial spot with a solvent system of ethyl acetate-methanol-water, 52:36:13 by volume. The plate was dried

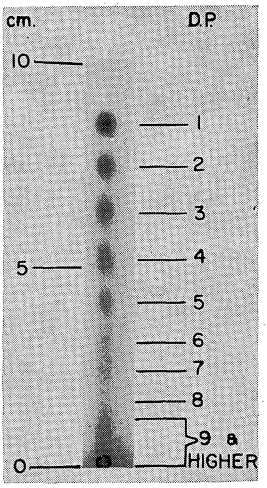

Fig. 2. Thin-layer chromatogram of a 43-D.E. regular conversion syrup. D.P.: degree of polymerization. Peaks 1 to 9: monosaccharide to nonasaccharide. cm.: distance from origin (in cm.).

in a stream of warm air, sprayed with a 50% solution of 36*N* sulfuric acid in water, and charred for 30 min. at 140°C. Figure 2 shows a chromatogram of a 43 D.E. regular conversion syrup. Quantitative measurements were made by transmission densitometry, using a Photovolt Model 520A TLC densitometer equipped with an Integraph Model 49A integrator and a Varicord Model 42B recorder. No filter was used for the light source of the densitometer. Figure 3 is a scan of the same

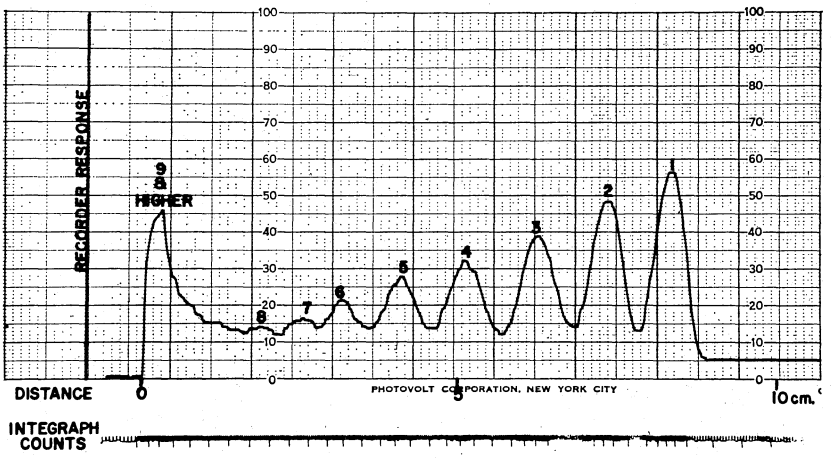

Fig. 3. Densitometer scan of the thin-layer chromatogram from Fig. 2. Peaks 1 to 9: monosaccharide to nonasaccharide.

syrup as shown in Fig. 2. The amount of each saccharide was determined by comparing the peak area with that of the corresponding saccharide of the known sample.

Results and Discussion

Three types of corn syrups were selected to illustrate the TLC method for saccharide distribution analysis: 43 D.E. regular conversion syrup, 43 D.E. low dextrose-high maltose syrup, and 70 D.E. high dextrose-high maltose syrup. Their saccharide distributions were first determined by gravimetric paper chromatography. There were two reasons for this: 1) no chemical analysis was required, and 2) accuracy could be checked by the percent recovery; i.e., the total weight of the eluted fractions against the weight of the sample applied on the paper. The results (Table I) indicate 99.1 to 100.7% recovery.

In the TLC measurements, transmission densitometric determinations have shown a straight-line relationship between the peak area and concentration, although different saccharides gave lines of different slopes. This straight-line relationship made quantitative analysis possible by comparison of peak areas of the unknown with those from the standard. For better accuracy, since saccharide distribution varied widely in different types of syrup, we used different standards for each type rather than a single one. Table I summarizes the results.

TABLE I
SACCHARIDE DISTRIBUTION OF CORN SYRUP

	Saccharide (by weight)								
	Mono	Di	Tri	Tetra	Penta	Hexa	Hepta	Octa	Nona and Higher
	%	%	%	%	%	%	%	%	%
	A. Regular conversion: 43 D.E.								
Grav.[a]	20.5	15.4	11.3	9.8	7.7	6.0	4.7	3.9	21.5
TLC[b]	20.1	14.6	11.5	8.6	7.2	5.4	4.9	3.4	23.9
S for TLC[c]	0.70	0.51	0.40	0.30	0.37	0.35	0.11	0.20	0.41
	B. Low-dextrose, high-maltose: 43 D.E.								
Grav.	6.8	34.4	17.2	9.4	2.3	2.6	2.4	4.0	20.0
TLC	7.3	35.1	17.5	8.5	2.5	2.4	2.0	2.8	21.6
S for TLC	1.25	4.36	3.10	0.66	0.70	1.10	0.77	0.65	1.33
	C. High-dextrose, high-maltose: 70 D.E.								
Grav.	41.1	41.4	3.2	4.8	3.8	1.8	2.4[d]		1.6
TLC	40.8	42.1	3.5	3.1	2.7	1.5	2.4		3.1
S for TLC	1.25	1.62	1.28	0.65	0.26	0.37	0.78		0.32

[a] As determined by gravimetric paper chromatography.
[b] As determined by thin-layer chromatography (average of three determinations).
[c] Standard deviation for b.
[d] Combining hepta- and octasaccharides.

The standard deviations are lower for the regular conversion syrup than for the high dextrose-high maltose and the low dextrose-high maltose syrup. This is probably due to the necessity of increasing the sample weight on the TLC plate so as to adequately detect the small amount of the low-percent saccharides. This larger sample size increases the absorbance of the higher saccharide spots to near the useful detection limit of the densitometer.

In conclusion, we have found that the TLC method for determining saccharide distribution in corn syrup is simple and rapid, with acceptable accuracy and precision. The time required for a complete analysis is generally less than 7 hr. We are continuing the investigation, particularly to improve separations beyond nonasaccharide, and are currently investigating development of chromatograms at higher than ambient temperatures.

Acknowledgment

The authors thank E. E. Fisher for his interest and guidance during this work, also A. W. Turner and R. F. Larson for supplying syrup samples, and K. M. Brobst for the glucose analysis.

Literature Cited

1. Dimler, R. J., Schaefer, W. C., Wise, C. S., and Rist, C. E. Quantitative paper chromatography of D-glucose and its oligosaccharides. Anal. Chem. **24**: 1411–1414 (1952).
2. Ough, L. D. Paper chromatography analysis of the disaccharide fraction of 60 Dextrose Equivalent acid-hydrolyzed corn syrup. Anal. Chem. **34**: 660–664 (1962).
3. Whistler, R. L., and Hickson, J. L. Determination of some components in corn syrups by quantitative paper chromatography. Anal. Chem. **27**: 1514–1517 (1955).
4. Taufel, K., Ruttloff, H., and Friese, R. Zur Bestimmung der Glucose in Stärkesirup mittels Glucose-Oxydase. Die Stärke **14**: 309–318 (1962).
5. Houk, A. E. H. Gravimetric evaluation of paper chromatograms. J. Pharm. Sci. **52** (8): 738–743 (1963).