Gas-Chromatographic Determination of Levoglucosan in Corn Syrups and Its Significance in Assessing Their Method of Manufacture

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ABSTRACT

A method for the direct determination of levoglucosan in corn syrups by gas chromatography of their trimethylsilylated derivatives is described. It is postulated that the levoglucosan content of corn syrups is proportional to the degree of acid conversion that the syrups have undergone, and this is substantiated by measuring the levoglusan contents of a range of commercial syrups. It is therefore suggested that the levoglucosan content of corn syrups may serve as a useful index to their method of manufacture.

As a preliminary to assessing the quantities and types of some "rare" sugars in foods, we have developed a gas-chromatographic method for the rapid direct estimation of levoglucosan in trimethylsilylated corn syrups.

The reduction technique of Cayle et al. (1) was judged to be too time-consuming for analytical control purposes, and we have therefore used the direct trimethylsilylation procedure of Brobst and Lott (2) with the addition of *p*-terphenyl as an internal standard. Dextrose and maltose contents were determined on the same column, as these values were rapidly obtained by our method, and, together with dextrose equivalent, DE (reducing power, calculated as dextrose and expressed on a dry weight basis) they provide supplementary information about the method of manufacture of the syrups. Reducing powers were measured according to the standard method of the Corn Industries Research Foundation (3).

The levoglucosan in commercial 41 DE corn syrup was first identified by chromatographic isolation (4) and examination of its infrared spectrum, which was identical with an authentic specimen. Having established its presence and determined the amount of levoglucosan in the 41 DE acid-converted syrup, we

speculated that the substance probably differed from the disaccharide "reversion products" of glucose syrup (5) by originating in the thermal breakdown of starch and its hydrolysis products, in the presence of acid. Significant breakdown of this type occurs at 220° to 230°C. (6). Indeed, the standard method for preparation of levoglucosan is by starch pyrolysis (7). To test our hypothesis we examined six more commercial corn syrups and were able to correlate the levoglucosan content of each with the degree of acid conversion and hence thermal treatment which the product had received. For final confirmation we examined the levoglucosan, dextrose, and maltose contents of nine "unknown" corn syrups by our technique, and eight of the nine samples were correctly identified as to the degree of acid and enzyme treatment they had received.

MATERIALS AND METHODS

Apparatus

The gas chromatograph used was a Pye automatic preparative chromatograph Series 105, fitted with flame ionization detector, attenuator, oven-temperature controller, and Speedomax 'W' recorder.

The peak areas were measured with a Kent planimeter.

A glass column, 5 ft. long and 0.25 in. o.d. packed with 5% silicone (SE 30) coated on J.J. s 60/80 mesh support, (acid washed and dimethylchlorosilane treated) was used throughout. The column ends were plugged with glass wood and conditioning was carried out for 48 hrs., with carrier gas to 300°C. prior to use.

Conditions for operation: Injection port 210°C. (levoglucosan), 230°C. (dextrose), 290°C. (maltose); oven temperature was programmed from 150° to 200°C. for levoglucosan at the rate of 1.5°C. per min. In this temperature range dextrose was completely eluted. The chromatograph was operated isothermally at 180°C. to elute the dextrose and *p*-terphenyl. The temperature was then raised to 250°C. to elute the maltose; gas pressures: Argon-10 lb., 60 ml. per min., air 10 lb., and hydrogen 22 lb., 50 ml. per min.

A Unicam SP 200 spectrophotometer was used to obtain the infrared spectrum of levoglucosan.

PROCEDURE

Reagents

Corn syrup stock solutions: Corn syrups (two, 2.5 g.) were dissolved in pyridine and made up to a total volume of 10 ml. in each case.

Internal standard solutions: Solution A: 10 mg. of *p*-terphenyl in 100 ml. of pyridine solution; solution B; 750 mg. of *p*-terphenyl in 100 ml. of pyridine solution.

Derivatization of samples: Samples were derivatized by the method of Brobst and Lott (2), but including the internal standard solutions as follows: For determination of levoglucosan, each corn syrup stock solution (0.5 ml.) was mixed with pyridine (0.2 ml.), internal standard solution A (0.2 ml.), hexamethyl disilazane (1.0 ml.), and trifluoroacetic acid (0.1 ml.). The mixture was shaken vigorously for 30 sec. and then allowed to stand for 30 min. Five microliters of the solution was then injected.

Similarly standard sugar solutions of levoglucosan, dextrose, and maltose were derivatized with internal standard solutions to calculate the calibration constants.

Calibration and Calculations

p-Terphenyl was chosen as an internal standard (8) because of its desirable retention time with respect to the sugars under study. It is an inert material under the conditions of the trimethylsilylation reaction and gives a single peak.

Calibration constants (a_x/a_y) were calculated from the ratio of the amount of sugar (m_x) and its peak area (A_x) divided by the ratio of the amount of internal standard (m_y) and its peak area (A_y). The amount of unknown sugar in the sample was then calculated from the formula:

$$m_x = \frac{a_x}{a_y} \frac{A_x m_y}{A_y}$$

Values of a_x/a_y determined for use in this way were: 1.00 (levoglucosan), 0.63 (dextrose), and 0.80 (maltose).

RESULTS AND DISCUSSION

With *p*-terphenyl as an internal standard, there was a linear relation between peak area and concentration of the standard and also between the retention distance of the standard and oven temperature. Figure 1 shows the calibration curves for dextrose, maltose, and levoglucosan, based on the ratio of their peak areas to those of the standard.

Figure 2 shows a typical chromatogram for trimethylsilylated corn syrup, and Table I shows the results obtained with seven "known" corn syrups in which the levoglucosan contents vary between 0 and 0.09%. The history of these samples was established by preconsultation with the manufacturers, and their dextrose and maltose contents also reflect the treatment which they had received. For example, syrup K with 34.6% maltose has clearly been manufactured with malt enzyme after an initial acid treatment up to 19 to 21 DE. Syrup N has a dextrose value far lower than published figures for acid-converted corn syrups of 60 DE (9), indicating enzyme treatment, and its levoglucosan content (0.07%) results from acid conversion to 40 DE.

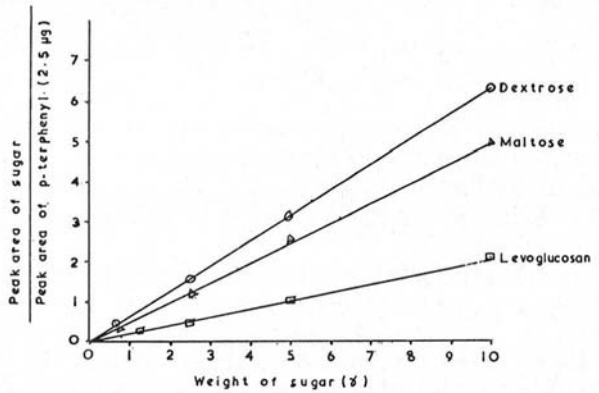

Fig.1. CALIBRATION CURVES OF DEXTROSE, MALTOSE AND LEVOGLUCOSAN SHOWING WEIGHT OF SUGARS VERSUS SUGAR / INTERNAL STANDARD CHART AREAS.

Fig. 1. Calibration curves of dextrose, maltose, and levoglucosan, showing weight of sugars versus sugar internal standard chart areas.

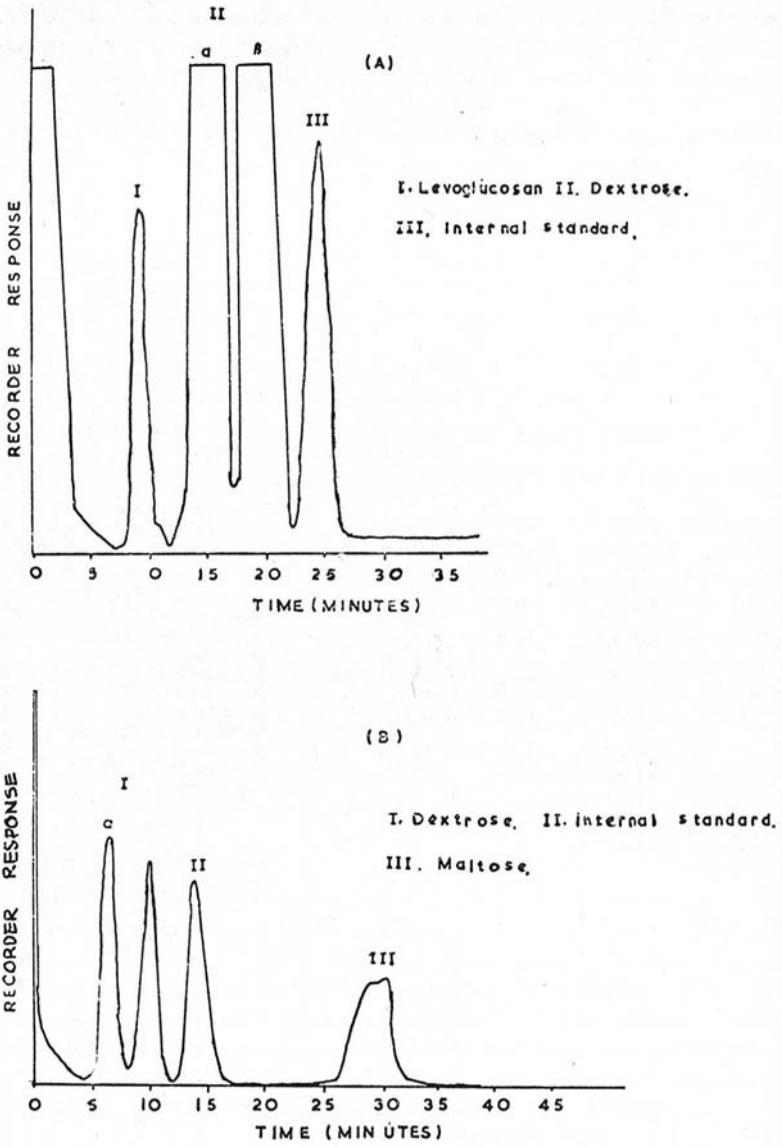

Fig. 2. Typical gas chromatogram of trimethylsilylated corn syrup.

Table II shows our allocation of syrups A to I into acid or acid-enzyme-converted types, according to their levoglucosan contents and DE values, as compared with their actual histories as subsequently disclosed by the manufacturer. The levoglucosan content in this series of syrups varies from 0.015 to 0.121%, and the only one that appears to be incorrectly allocated is sample E. This odd result does not of itself indicate the inapplicability of our method, although we

cannot exclude the possibility that the levoglucosan concentration might never exceed 0.2% in the final product and thus confine the usefulness of the method to syrups of DE lower than 50 to 55. Alternatively, variations in the corn starches used for manufacture might affect the final levoglucosan content.

TABLE I. GAS-CHROMATOGRAPHIC ANALYTICAL COMPOSITION OF "KNOWN" CORN SYRUPS

Syrup	Levoglucosan %	Dextrose %	Maltose %	DE	Conversion
K	0.044	8.0	34.6	41.0	Acid to 21 DE then enzyme
L	0.083	13.9	11.1	36.0	Acid only
M	0.090	21.2	18.6	41.0	Acid only
N	0.074	22.6	21.8	60.0	Acid to 41 DE then enzyme
R	0.074	66.1	7.3	91.0	Acid to 41 DE then enzyme
S	0.013	4.0	6.1	15.0	Acid only
Y	0.000	2.5	50.9	38.6	Enzyme only

TABLE II. GAS-CHROMATOGRAPHIC ANALYTICAL COMPOSITION OF "UNKNOWN" CORN SYRUPS

Syrup	Levo-glucosan %	Dextrose %	Maltose %	DE	Conversion allocated by levoglucosan content	Actual conversion
A	0.026	3.8	8.2	21.3	Acid to 10-12 DE then enzyme	Acid-enzyme
B	0.015	5.3	28.2	39.8	Acid to 10-12 DE then enzyme	Acid-enzyme
C	0.110	18.2	12.7	39.2	Acid only	Acid only
D	0.121	18.8	15.4	37.8	Acid only	Acidonly
E	0.103	34.3	21.0	55.8	Acid to 40 DE then enzyme	Acid only
F	0.087	20.7	16.6	41.3	Acid only	Acid only
G	0.084	32.5	26.4	62.6	Acid to 36-40 DE then enzyme	Acid-enzyme
H	0.037	74.5	6.3	85.2	Acid to 12-15 DE then enzyme	Acid-enzyme
I	0.083	65.2	8.3	78.4	Acid to 36-40 DE then enzyme	Acid-enzyme

With the wide range of enzyme- and acid-converted syrups now available, DE no longer serves as a useful means for characterizing the syrups. Dextrose and maltose determined individually or the dextrose-maltose ratio has been used to characterize syrups now available for technological purposes, but even this type of analysis or a complete chromatographic determination of all the major carbohydrate components may not serve as an index of sample history (10). Our results show that the quantity of a minor constituent such as levoglucosan may be useful in assessing the method of corn syrup manufacture.

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