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AUTOMATED DETERMINATION OF THE DEXTROSE EQUIVALENT OF CORN STARCH HYDROLYSATES[1]

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ABSTRACT

Dextrose equivalent (DE) is the criterion employed by the corn wet-milling industry to indicate the extent of hydrolysis of starch in the manufacture of corn syrups and sugars. It is defined as the percentage of reducing sugars calculated as dextrose and expressed on a dry-substance basis. Reducing sugars are determined by the method of Lane and Eynon, which specifies an alkaline copper tartrate reagent. The present work shows how DE can be determined by an automated procedure with the Technicon Auto-Analyzer. In this procedure, consecutive reducing sugar determinations are made on the sample before and after hydrolysis with hydrochloric acid. Reducing sugars are determined with reagents made with potassium ferricyanide, potassium cyanide, and sodium hydroxide. The quantity found before hydrolysis (x) is related to reducing sugar content, and that after hydrolysis (y) is a function of the carbohydrate dry substance. The DE is related to the two determinations as follows: $DE = (ax)/(y+bx)$, where a and b are constants. The procedure was applied to corn starch hydrolysates and to various sugars and related compounds.

Dextrose equivalent (DE) is the criterion employed by the corn wet-milling industry to indicate the extent of hydrolysis of starch in the manufacture of corn syrups and sugars. The term was introduced by Evans and Fetzer (1) about 25 years ago, to replace the term "Purity" (2). DE is defined as the percentage of reducing sugars calculated as dextrose and expressed on a dry-substance basis. Quantitative paper chromatographic analysis (3) of corn syrups prepared by acid hydrolysis has shown a definite relationship between DE and saccharide composition. No such definite relationship exists for syrups prepared by enzymatic hydrolysis or by combinations of acid and enzymatic hydrolysis. Nevertheless, for any given sequence of hydrolytic steps employing enzymes, or acid plus enzymes, DE may be used as a process control criterion to obtain hydrolysates of constant saccharide composition.

[1]Manuscript received May 21, 1964. Contribution from Clinton Corn Processing Co., a division of Standard Brands Inc., Clinton, Iowa.

DE, according to definition, requires a determination for reducing sugars and one for dry substance. Reducing sugars are presently determined by a modified Lane and Eynon procedure (4,5) with an alkaline copper tartrate reagent. Adherence to strict experimental procedure is required to obtain a satisfactory result. The preferred dry-substance method is the filter cel method of Cleland and Fetzer (6).

Hodge and Davis (5) have compiled selected methods for the determination of reducing sugars. These involve the reduction of copper, ferricyanide, or iodometric reagents.

Wood (7) investigated potentiometrically the effect of several variables on the reduction of ferricyanide by dextrose. Summarized briefly, his work showed that all variables must be carefully controlled to assure reproducible results. His study pointed out the similarity between alkaline ferricyanide and copper methods for the determination of dextrose. It also showed the effect of the presence of sodium cyanide on the reducing power of dextrose. The reducing power was found to increase markedly as the concentration of sodium cyanide was increased.

Nussenbaum and Hassid (8) developed a procedure for determining the reducing end groups of starch polysaccharides, using reagents containing potassium ferricyanide, sodium carbonate, and sodium cyanide. They demonstrated that the reducing powers of dextrose, maltose, heptaose, and amylodextrins containing 23 and 42 anhydroglucose units were independent of chain length and dependent only on the number of moles of reducing end groups; i.e., all had equivalent molar reducing power.

Hill and Kessler (9) have reported an automated method for total reducing substances, using the Technicon AutoAnalyzer and employing reagents similar to those described by Nussenbaum and Hassid. In the authors' laboratory, the method gave results with corn syrups that were considerably lower than those obtained by the Lane and Eynon procedure.

Nath and Singh (10) investigated the oxidation of dextrose and lactose with potassium ferricyanide and sodium hydroxide reagents. They found that the reaction rate was independent of the ferricyanide concentration, but that it increased with an increase in the concentration of both sugar and hydroxyl ion and also with an increase in temperature.

The automated method of DE determination presented herewith is based on two consecutive analyses of identical samples. In the first analysis, the sample is mixed with hydrochloric acid solution and hydrolyzed to dextrose, and the amount of dextrose is determined by reduction of ferricyanide reagent. In the second analysis, the hydroly-

sis step is omitted to obtain a determination of reducing sugars. The ratio of the reducing powers before and after hydrolysis of corn syrup, essentially a mixture of glucosidically linked polymers, is a function of the DE of the sample.

Materials and Methods

Reagents. The reagents used in the automated procedure were: 1) hydrochloric acid solution, 4.0*M*; 2) buffered sodium chloride solution, 4.0*M* in NaCl, 0.0039*M* in NaH_2PO_4, and 0.0061*M* in Na_2HPO_4; 3) sodium hydroxide-potassium cyanide solution, 2.0*M* in NaOH and 0.233*M* in KCN; and 4) potassium ferricyanide solution, 0.00486*M*.

Dextrose Standards. These were prepared from a stock solution containing 20.00 g. of redried National Bureau of Standards standard dextrose per liter. The dextrose was dissolved in a sufficient volume of saturated benzoic acid solution to make 1 liter. As required, volumes of the stock solution were diluted with saturated benzoic acid solution to furnish solutions containing 50–600 mg. dextrose per 100 ml. The saturated benzoic acid solution serves as a preservative (9).

Corn Syrup Standards. These were prepared with anhydrous dextrose and two acid-converted corn starch hydrolysates: a 42 DE corn syrup and a dried 15 DE corn starch hydrolysate. The anhydrous dextrose and 15 DE hydrolysate formed two of the standards. The others were combinations of the three. Determined by the Lane and Eynon and filter cel dry-substance procedures, the DE's of the standards were: 15.0, 31.9, 48.7, 66.2, 82.9, and 100.0. These were found sufficient for the establishment of calibration curves. Stock solutions of the syrups were prepared with saturated benzoic acid solution to contain 20.00 g. of dry substance per liter. These were diluted with saturated benzoic acid solution to furnish 500 mg. dry substance in 100 ml. for use in calibrating the AutoAnalyzer.

Flow Diagram. Figure 1 is a flow diagram indicating manifold and reagent system. Figure 2 shows the assembled apparatus.

For the determination, two identical samples are required, that is, two consecutive cups in the sample holder are filled with the same sample. With the sampler set to deliver 20 samples per hr., ten complete DE determinations can be made in that time.

The flow diagram indicates that the sample is united at point "m" with either the hydrochloric acid or buffered sodium chloride solution, and segmented with air. Metering of either hydrochloric acid or buffered salt solution to point "m" is controlled by an automatic valve assembly synchronized with the sampler module. After passing a mixing coil, the segmented stream enters the 100°C. heating bath where it

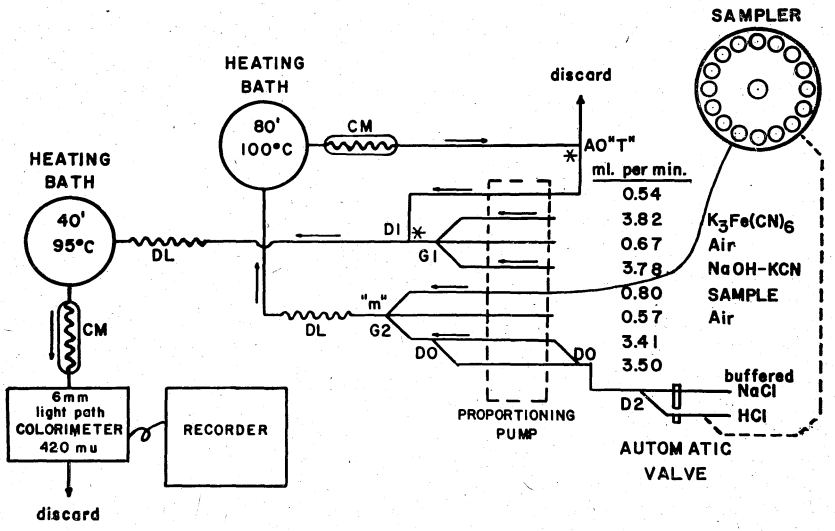

Fig. 1. Flow diagram for automated DE determination. D0, D1, D2, G1, G2, AO"T": glass connectors; DL: double-length mixing coil; CM: cooling and mixing coil.

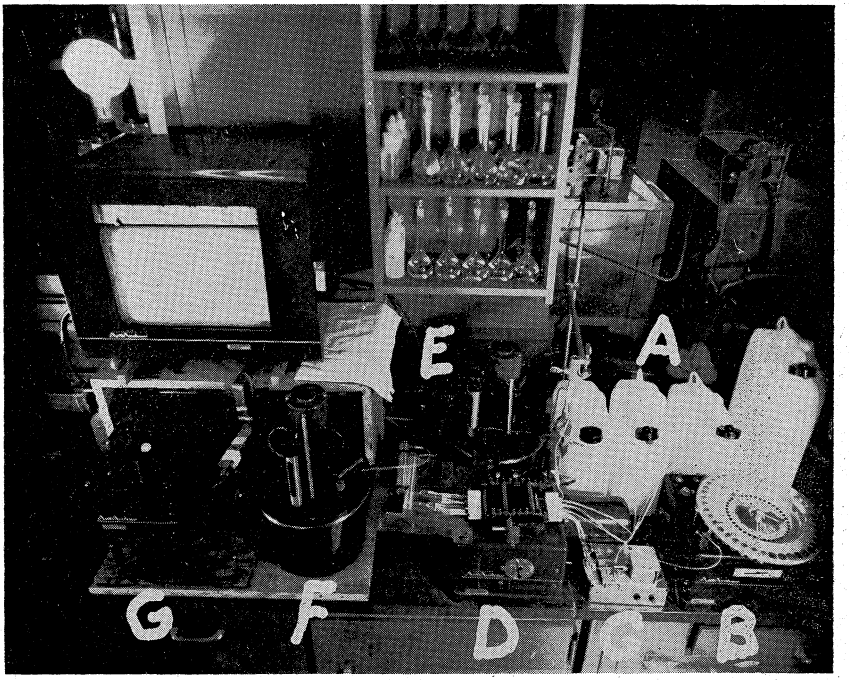

Fig. 2. AutoAnalyzer assembled. A, reagent supply; B, sampler; C, automatic valve; D, proportioning pump; E, 100°C. heating bath; F, 95°C. heating bath; G, flow colorimeter. Recorder is at upper left.

passes through 80 ft. of glass coil. When mixed with acid, the sample is hydrolyzed in the heating bath; when mixed with salt solution, it passes through unchanged. The buffered salt solution is used to elevate the boiling point of the liquid as it passes through the 100°C. bath. It is buffered to minimize the effect of any small amount of acid which may adhere to the walls of the tubing. On emerging from the 100°C. bath, the stream is cooled by passing through a water-jacketed coil.

The emergent stream is divided into deaerated and air-segmented streams at AO"T." The distance between the points indicated by asterisks on the diagram is as short as possible. The air-segmented stream is discarded. The deaerated stream is pumped through the proportioning pump, united with potassium ferricyanide and sodium hydroxide-potassium cyanide reagents, and segmented with air. The segmented stream passes through another mixing coil and enters the 95°C. heating bath, where reduction of the ferricyanide occurs. The emergent stream passes through another water-jacketed coil into the colorimeter flow cell and is eventually discarded.[2] Absorbance is measured at 420 mμ in a 6-mm. flow cell and recorded.

Mixing of sample with acid or with buffered salt solution at point

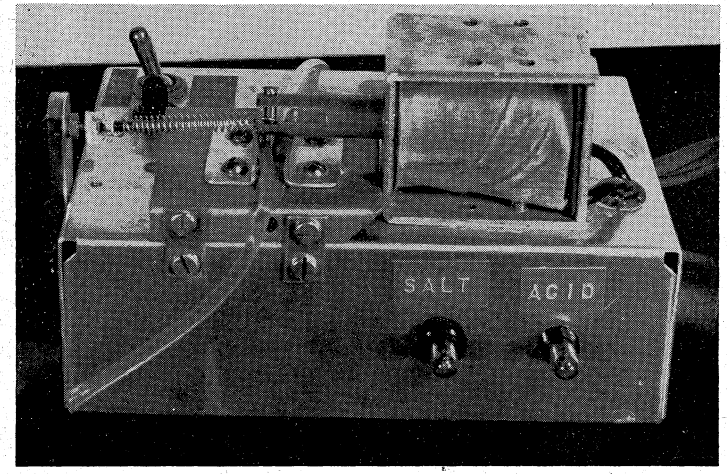

Fig. 3. Automatic valve. View shows only one reagent supply tube in place to expose the pin compressing it. Normally two tubes (one for acid solution, the other for buffered salt solution) are placed in the valve assembly so that the pin closes one tube while simultaneously opening the other.

[2] Attention must be called to the fact that two streams are discarded. One is acidic and the other is basic and, in addition, contains potassium cyanide. Our practice has been to sewer the acidic stream and to collect the basic stream in a large bottle. The bottle is emptied accompanied with a copious flow of water at a time when the apparatus is shut down and not sewering the acidic stream, which contains more than enough acid to liberate hydrogen cyanide from the basic stream.

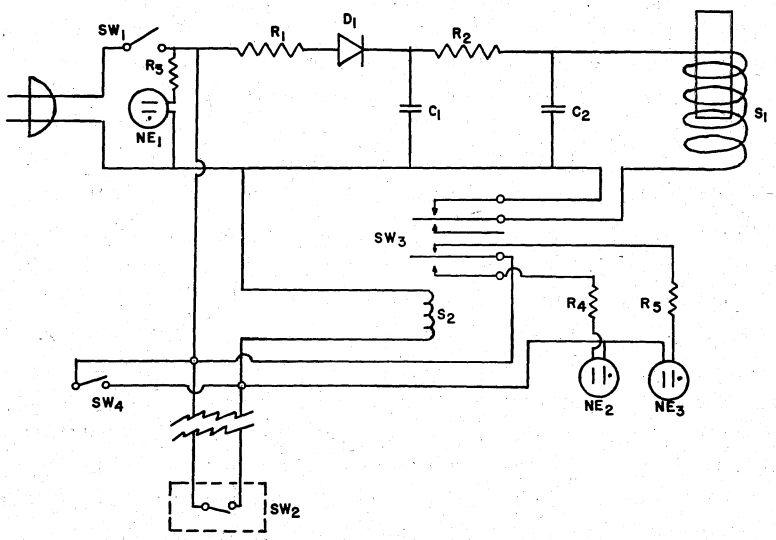

Fig. 4. Wiring diagram of power supply and solenoid for automatic valve. R_1, 22 ohm, 2 w.; R_2, 175 ohm, 10 w.; R_3, 100k ohm, 0.5 w.; R_4, 100k ohm, 0.5 w.; R_5, 100k ohm, 0.5 w.; G_1, 50 μf, 150 VDC; G_2, 50 μf, 150 VDC; D_1, silicon diode, 750 ma.; SW_1, line switch; SW_2, remote microswitch in the sampler; SW_3, DPDT ratchet relay; SW_4, manual trip switch; S_1, solenoid; S_2, relay coil; and N_1, N_2, N_3, neon bulbs.

"m" is controlled by an automatic valve assembly (Fig. 3). A wiring diagram of the automatic valve is shown in Fig. 4. A cam is mounted inside the sampler module, Fig. 5, on a spur gear which makes one complete revolution per sample delivered. For each sample delivered, the cam momentarily closes a microswitch, SW_2, Fig. 4, to operate a ratchet relay which in turn energizes the solenoid circuit powered by a direct current supply. A pin is attached to the solenoid plunger, Fig. 3, to close the salt reagent delivery tube, simultaneously opening the acid delivery tube. On the second rotation of the cam and delivery of the second sample (identical to the first), the solenoid is de-energized to switch from delivery of acid solution to delivery of salt solution. With the sampler set to deliver 20 samples per hr., sample is pumped for 2-min. periods followed by 1-min. periods during which air is pumped. The acid and salt solutions are pumped for 3-min. periods per sample. Operation of the valve is synchronized by proper positioning of the cam so that one or the other of the reagents (hydrochloric acid or buffered sodium chloride) reaches the mixing point "m" before the sample does and continues until after the sample passes "m."

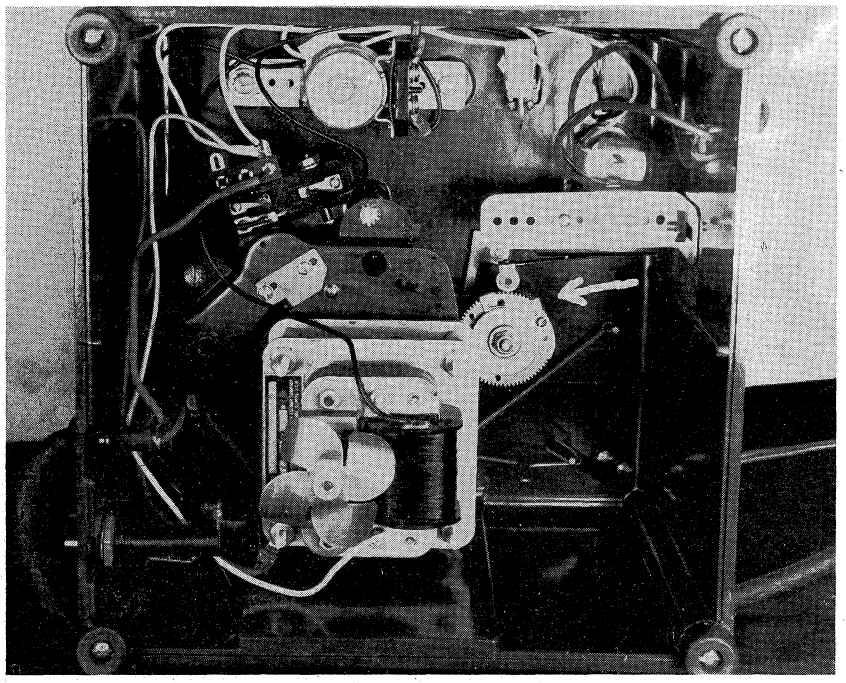

Fig. 5. Cam (arrow) attached to spur gear of sampler module.

Results and Discussion

Theoretical Considerations. DE is defined as:

$$DE = \frac{100\,x}{d} \tag{1}$$

where x is the amount of reducing sugar expressed as dextrose and d is the dry substance. For substantially pure carbohydrate systems composed of dextrose and its glucosidically linked polymers (a corn syrup, for example), a quantity d of carbohydrate yields on complete hydrolysis an amount y of dextrose. The dry substance d is related to x and y as follows:

$$d = \frac{M_a}{M_g}y + \frac{M_w}{M_g}x \tag{2}$$

where M_a, M_g, and M_w are the molecular weights of anhydroglucose, dextrose, and water, respectively.

From equations 1 and 2, the following is derived:

$$DE = \frac{ax}{y + bx} \tag{3}$$

where $a = \frac{100\ M_g}{M_a} = 111.1$, and $b = \frac{M_w}{M_a} = 0.1111$.

In the development of the automated method, the problem resolved to finding conditions such that the reducing value, x, relative to dextrose as determined by the ferricyanide reagent, would approach closely that given by the Lane and Eynon method; and further, to obtain substantially complete hydrolysis of carbohydrate to dextrose so that the quantity y could be reliably determined.

Preliminary experimentation showed that substantially complete conversion of dilute corn syrups to dextrose was reached within 4 min. in 3.5*M* hydrochloric acid at 108°C. In the automated method, the conditions specified for hydrolysis approach these requirements closely.

Effects of Sodium Hydroxide and Potassium Cyanide Concentrations on the Reduction of Ferricyanide by Corn Starch Hydrolysates. To determine quantities x and y, the AutoAnalyzer was first calibrated with the dextrose standards. Figure 6 shows a recorder chart resulting from such a standardization. Minimum absorbances obtained from the

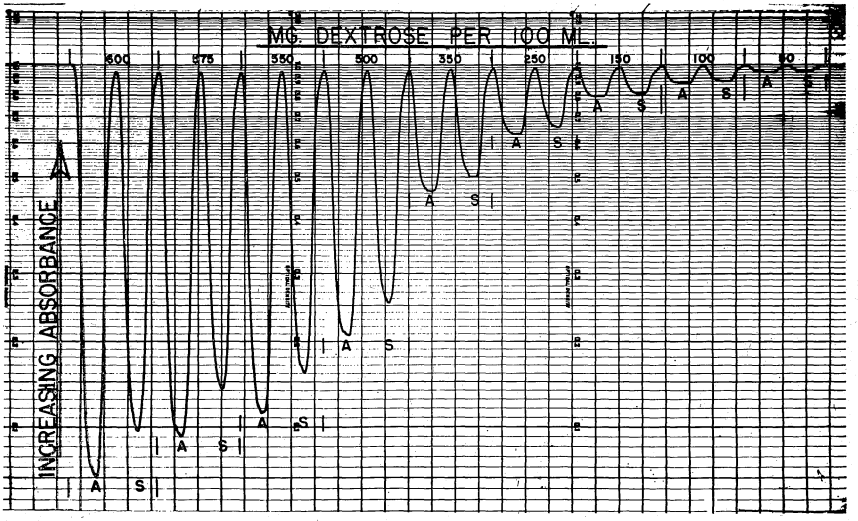

Fig. 6. Recorder chart from standardization with dextrose standards. A, response obtained when dextrose is mixed with hydrochloric acid solution. S, response when dextrose is mixed with buffered salt solution.

chart were plotted against concentrations of dextrose, to prepare separate calibration curves for the computation of x and y values as defined for equation 3.

DE's calculated by equation 3 (when $a = 111.1$ and $b = 0.1111$) varied with changes in concentration of both sodium hydroxide and potassium cyanide. The cyanide concentration had by far the greater effect. Figure 7 shows the resultant effect for the corn syrup standards

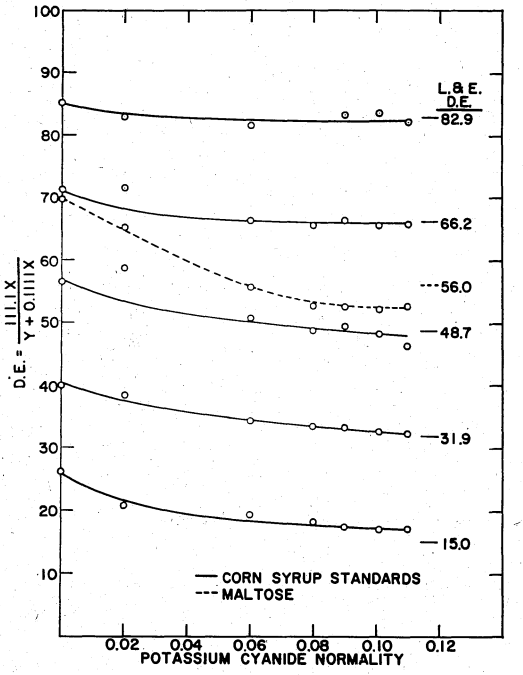

Fig. 7. Effect of concentration of potassium cyanide on automated DE of corn syrup standards and a sample of maltose. Paper chromatographic analysis (3) showed the sample of maltose to be composed of 2% monosaccharides, 89% disaccharides, and 9% higher saccharides on a total carbohydrate basis as dextrose.

and for a maltose sample under the following conditions. The pumped hydrochloric acid was 6.05*M*. The basicities of the stream entering the 95°C. reduction bath for samples mixed with buffered salt and acid solution were 0.96 and 0.61*M*, respectively. Concentrations of potassium cyanide were varied from zero to 0.11*M* in the stream entering the 95°C. reduction bath.

As observed in Fig 7, the calculated DE's of the corn syrup standards decreased to values approaching the Lane and Eynon values tabulated in the right margin. The effect on the maltose sample was

outstanding. Its DE changed from 70 to 52.5, which is about the theoretical value pure maltose should have.

Effects of Various Sugars and Related Compounds. The response of several sugars and related compounds in the automated procedure was determined to explore the possible scope of the method. For this work, concentrations of reagents were used as discussed in the section "Materials and Methods." Results are compared in Table I with DE's

TABLE I

RESPONSE OF SEVERAL SUGARS AND RELATED COMPOUNDS IN THE AUTOMATED DE PROCEDURE

COMPOUND	CONCENTRATION	x	y	x/y	AUTOMATED[a] DE	LANE-EYNON DE
	mg./100 ml.	*mg./100 ml.*	*mg./100 ml.*			
HMF[b]	100	145	104	1.39	136	85.7
	250	350	260	1.35	130	
Levoglucosan	500	0	540	0.00	0	0.0
Fructose	200	210	179	1.20	115	93.7
	350	366	318	1.15	113	
	500	526	451	1.17	115	
Mannose	500	491	486	1.01	100.6	98.4
Galactose	500	440	446	0.99	98.7	91.5
Maltose[c]	500	255	520	0.49	51.5	57.2
α,α-Trehalose	475	0	269	0.00	0.0	0.0
Cellobiose	575	338	568	0.60	62.0	76.3
Lactose	475	268	449	0.60	62.2	70.1
Sucrose[d]	500	539	502	1.07	106.6	0.0
		(0)	(502)	(0.00)	(0.0)	

[a] Calculated according to equation 3 where a = 111.1 and b = 0.1111.
[b] 5-hydroxymethyl-2-furaldehyde.
[c] Pure maltose derived from the twice-recrystallized octaacetate.
[d] Results in parentheses were obtained after the composition of the buffered salt solution was changed to 0.1*M* in Na_2HPO_4 and 4.0*M* in NaCl.

determined by the Lane and Eynon procedure. The table shows the concentrations of the samples analyzed, x and y values as defined for equation 3, and the ratio of x to y values, as well as DE by the automated and the Lane and Eynon procedures. Because 5-hydroxymethyl-2-furaldehyde (HMF) (11) and levoglucosan (12) as well as many glucodisaccharides (13) are known constituents of starch hydrolysates manufactured by acid hydrolysis, their effects on the automated DE procedure are of interest.

The significance of the x/y ratio in Table I is as follows: For nonreducing substances, the ratio should be zero. For monosaccharides, the ratio should be unity, provided that any destruction which takes place during the hot acid treatment to which the sugar is subjected in the determination of the y value is equivalent to that undergone by the dextrose standards. Destruction of dextrose under conditions of the hot

acid treatment (in this case, 3.5 min. at 100°C., in 3.66*M* HCl) was determined to be no greater than 2%.[3] Ratios of greater than 1 indicate that the destruction of monosaccharide is greater than that encountered for dextrose. Thus, considerable destruction of HMF and fructose was encountered, whereas the effect of hot acid treatment on mannose and galactose was minimal.

For reducing disaccharides, the ratio should theoretically be 0.50, provided: 1) that complete hydrolysis to monosaccharide is achieved, 2) that the molar reducing powers of the disaccharide and its constituent monosaccharides are equal, and 3) that no destruction of sugar occurs during acid hydrolysis. For maltose, these conditions are substantially met. The disaccharide α,α-trehalose does not reduce the ferricyanide reagent as expected. Moreover, it is not completely hydrolyzed to dextrose as indicated by the low y value obtained. A low hydrolysis rate of α,α-trehalose has been reported previously (14).

The high x/y ratios for cellobiose and lactose are due chiefly to a combination of high molar reducing values as compared to glucose, and lack of complete hydrolysis to monosaccharides. Wolfrom, Thompson, and Timberlake (15) have shown that cellobiose is hydrolyzed with more difficulty in acid than is maltose.

The anomalous results obtained with the nonreducing disaccharide sucrose were due to cross-contamination of buffered salt solution with acid when the sample was mixed with the former for determination of its x value. The lowering of the pH of the salt solution by cross-contamination with acid was sufficient to establish conditions favorable for the hydrolysis of the sucrose to monosaccharide. Sucrose is much more easily hydrolyzed in acid than are the glucodisaccharides (14). This situation was rectified by changing the composition of the buffered salt solution to raise its buffering capacity.

Inspection of the results in column 6, Table I, shows that the automated procedure is applicable to pure carbohydrate systems composed of glucose and its polymers, linked principally by $\alpha,1\rightarrow4$ glucosidic bonds. The presence of appreciable quantities of HMF or of difficultly hydrolyzed disaccharides (as for example α,α-trehalose) in such systems would lead to erroneously high results.

Applications. To apply the automated method to the determination of the DE of starch hydrolysates, the corn syrup standards, instead of the dextrose standards, were used to calibrate the AutoAnalyzer. The corn syrup standards and reagent system are described under "Materials and Methods." Figure 8 shows a recorder chart resulting

[3] Determined by measuring dextrose recovery after acid treatment by means of an automated glucose oxidase-peroxidase procedure (9).

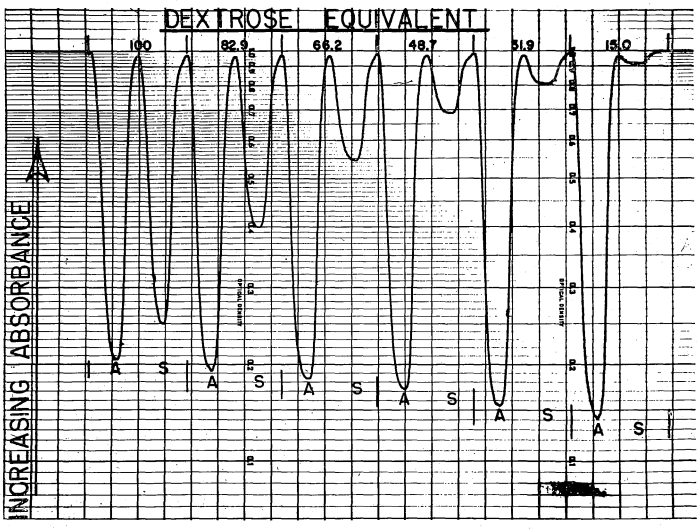

Fig. 8. Recorder chart from standardization with corn syrup standards. A, response obtained when standard is mixed with hydrochloric acid solution. S, response when standard is mixed with buffered salt solution.

from such a standardization. In this method of operation, the change in absorbance shown on the recorder chart (corresponding to the amount of ferricyanide reduced) is used as the criterion of the reducing value of the hydrolyzed and nonhydrolyzed samples. That it is not necessary to standardize with dextrose is seen from consideration of the following equation, which is essentially equation 3 rearranged.

$$\frac{DE}{a - b(DE)} = \frac{x'}{y'} \qquad (4)$$

where x′ and y′ are absorbance changes from the recorder chart analogous to the x and y values of equation 3. The constants a and b were found equal to 152 and 0.49, respectively, and differed from those derived for equation 3 (111.1 and 0.1111, respectively), chiefly because absorbance is not a linear function of ferricyanide concentration over the entire range; i.e., Beer's Law is not strictly followed.

It is also evident from equation 4 that it is not necessary to know exactly the concentration of carbohydrate in the sample being analyzed, for DE depends only on the x′/y′ ratio.

In practice, x′/y′ ratios obtained with the syrup standards are plotted against their DE's as determined by the Lane and Eynon procedure to form a standard curve (Fig. 9). DE's of unknown syrups are then computed by reference to the standard curve.

Table II shows results for acid-hydrolyzed syrups and sugar liquors. Excellent agreement between the two methods of DE determination

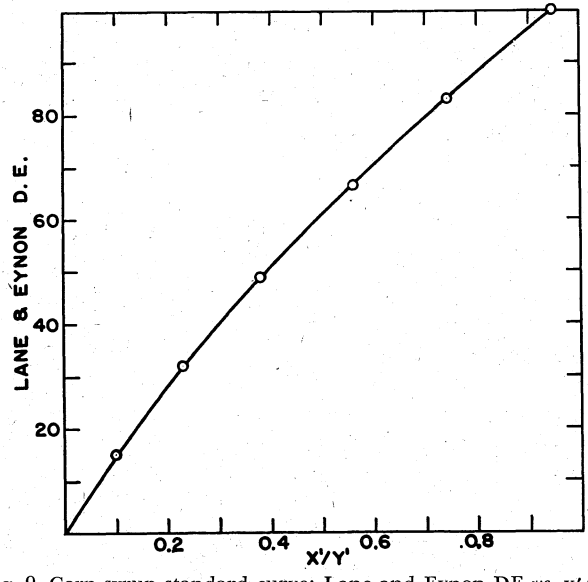

Fig. 9. Corn syrup standard curve: Lane and Eynon DE *vs.* x'/y'.

TABLE II
COMPARISON OF DE'S OF ACID-HYDROLYZED SYSTEMS

PRODUCT	LANE-EYNON DE	AUTOMATED DE
Corn syrup solids	16.2	15.7
	18.2	18.4
Corn syrups	43.4	42.5
	49.9	49.8
	56.0	55.7
	60.0	59.8
Sugar liquor[a]	93.2	93.6
Greens[a,b]	81.6	88.5

[a] Ash-free basis.
[b] "Greens" is the mother liquor from the dextrose crystallization process after the dextrose has been removed. Composition (dry basis) of this particular sample, established by quantitative paper chromatography (3,12) was as follows: HMF, 2.2%; levoglucosan, 4.2%; fructose, 2.8%; dextrose, 56.3%; DP_2, 19.3%; DP_3, 3.6%; carbohydrate balance, 1.7%; and protein and ash, 9.9% (total, 100%).

was found for all but the greens sample. Its content of HMF and fructose explains in part the higher value obtained by the automated procedure (see Table II). Yet another possible reason for lack of agreement may be the composition of the disaccharide fraction; this is rich in gentiobiose and isomaltose (16), which are hydrolyzed in acid at a slower rate than is maltose (15). Incomplete hydrolysis of such disaccharides in the automated procedure would lead to erroneously high results.

Table III shows DE's of dual converted hydrolysates. In the preparation of these products, two hydrolytic steps are involved: 1) partial

TABLE III
COMPARISON OF DE'S OF DUAL CONVERTED SYSTEMS

PRODUCT	LANE-EYNON DE	AUTOMATED DE
Corn syrups	44.6[a]	43.7
	45.5[a]	43.5
	42.5[a]	39.9
	43.7[a]	40.4
	61.7	61.2
	66.6	66.7
	70.0	69.5
	71.1	71.1
	72.1	71.5
First sugar	100	99.5
Second sugar	100	99.4
Sugar liquor	97.1	96.5
	91.6	93.1
Greens	84.9	87.5

[a] High-maltose syrups (44%).

hydrolysis with either acid or enzymes, and 2) further hydrolysis to the extent desired with enzymes. Over-all agreement between automated and Lane and Eynon DE's is good, there being in no case divergences greater than 4 DE. The lower results by the automated procedure for the first four syrups in the table reflect their high maltose content. The reason for the difference is the response of maltose to the reagents (Fig. 7 and Table I). The response of maltose to the ferricyanide reagent as employed in the automated method furnishes a value more nearly in line with that which it should be (52.6 DE, from theoretical considerations). A higher value (57.2 DE) was obtained with the Lane and Eynon reagent.

All the automated DE's in Tables II and III are averages of four determinations made on one sample preparation on at least four different days. The standard deviation of a single determination computed with 66 degrees of freedom was determined as $\pm$ 0.7 DE.

Acknowledgment

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Literature Cited

1. EVANS, J. W., and FETZER, W. R. Determination of moisture in sugar products. Ind. Eng. Chem., Anal. Ed. **13:** 855–858 (1941).
2. FETZER, W. R., EVANS, J. W., and LONGNECKER, J. B. Determination of dextrin, maltose, and dextrose in corn sirup. Ind. Eng. Chem., Anal. Ed. **5:** 81–84 (1933).
3. OUGH, L. D. Chromatographic determination of saccharides in starch hydrolyzates. *In* Methods in carbohydrate chemistry, ed. by R. L. Whistler, vol. IV, pp. 91–98. Academic Press: New York (1964).

4. CORN INDUSTRIES RESEARCH FOUNDATION, INC. Standard analytical methods of the member companies, Method E–26. Washington, D.C. (1952).
5. HODGE, J. E., and DAVIS, H. A. Selected methods for determining reducing sugars, p. 30. U.S. Dept. Agr., Northern Regional Research Lab.: Peoria, Ill. (1952).
6. CLELAND, J. E., and FETZER, W. R. Determination of moisture in sugar products. Ind. Eng. Chem., Anal. Ed. **13:** 858–860 (1941).
7. WOOD, W. B., JR. A preliminary physiochemical study of the reducing action of glucose. J. Biol. Chem. **110:** 219–232 (1935).
8. NUSSENBAUM, S., and HASSID, W. Z. Estimation of molecular weight of starch polysaccharides. Anal. Chem. **24:** 501–503 (1952).
9. HILL, J. B., and KESSLER, G. An automated determination of glucose utilizing a glucose oxidase-peroxidase system. J. Lab. Clin. Med. **57:** 970–980 (1961).
10. NATH, N., and SINGH, M. P. Studies in the kinetics of oxidation of the reducing sugars D-glucose and lactose by alkaline ferricyanide. Z. Physik. Chem. **221:** 204–210 (1962).
11. KERR, R. W. Chemistry and industry of starch (2nd ed.), p. 375. Academic Press: New York (1950).
12. OUGH, L. D., and ROHWER, R. G. Presence of levoglucosan in cornstarch hydrolyzates. J. Agr. Food Chem. **4:** 267–271 (1956).
13. OUGH, L. D. Paper chromatographic analysis of the disaccharide fraction of 60 dextrose equivalent acid hydrolyzed corn syrup. Anal. Chem. **34:** 660–664 (1962).
14. PIGMAN, W. W., and GOEPP, R. M., JR. Chemistry of the carbohydrates, p. 437. Academic Press: New York (1948).
15. WOLFROM, M. L., THOMPSON, A., and TIMBERLAKE, C. E. Comparative hydrolysis rates of the reducing disaccharides of D-glucopyranose. Cereal Chem. **40:** 82–86 (1963).
16. MONTGOMERY, E. M., and WEAKLEY, F. B. Carbohydrate composition of hydrol. J. Assoc. Offic. Agr. Chemists **36:** 1096–1108 (1953).