

THE APPLICATION OF MATHEWS' FORMULA IN ENZYMATIC STARCH CONVERSIONS¹

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ABSTRACT

Mathews' formula, PT/D , wherein P refers to polarization, T , Lane-Eynon titration, and D , dilution, was originally developed to determine the ratio of fructose to total reducing sugar in glucose-fructose mixtures. The concept has now been applied to the monitoring of the hydrolysis products of the enzymatic conversion of starch with alpha-amylase and amyloglucosidase. In this case the primary product is glucose—small quantities of maltose, isomaltose, and panose being the usual contaminants, depending on the purity of the saccharifying enzyme and its biological source. The use of Mathews' formula allows for the rapid estimation of nonglucose components and, conversely, glucose, with a polarimeter reading and a titration the only data required.

The traditional analytical procedures employed in the starch conversion industry, such as dextrose equivalent (DE), polarization, chromatography, and selective fermentation, are either inadequate to describe the conversion products or too cumbersome to allow for their use as routine monitoring devices, or both. During the development in our laboratory of an amyloglucosidase it became necessary to devise procedures which would enable us to evaluate the degree to which a starch hydrolysis had gone to completion.

In 1932 (1) Mathews described a method employing polarimetry and reducing values for determining glucose and fructose in mixtures. He established that for a constant ratio of fructose to total reducing sugar the polarization (P) will vary with the concentration of total sugar, while the titration (T) will vary inversely as the concentration (1). The insertion of a correction for the dilution (D) required for the titration results in the relationship, PT/D , which is relatively constant for these sugars.

Since the only measurements required were polarization and a Lane-Eynon titration, it appeared that this procedure could offer an easy, rapid, and relatively precise means of examining the hydrolysis products of enzyme conversions. This study was undertaken to establish whether this concept is indeed applicable to starch conversions.

Materials and Methods

Substrates and Standards. It has been established that in addition

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to glucose the minor products produced by commercially available amyloglucosidases can be maltose, isomaltose, and panose, depending on the purity and biological source of the enzyme and the conditions of hydrolysis. Accordingly, these saccharides were prepared and used in this study.

Maltose² was purified according to Pazur (2), with the use of a Darco G-60 column. The product had a specific rotation of +138. Glucose was purified by this same procedure. The product had a specific rotation of +53.

Isomaltose and panose (α -D-glucopyranosyl-(1 \rightarrow 6)- α -D-glucopyranosyl-(1 \rightarrow 4)-D-glucose) were prepared with a modified Pazur and Ando (3) procedure. A crude filtrate of *Aspergillus niger* was employed as the enzyme source, and incubation with maltose was allowed to proceed at pH 4.3, 60°C., for 24 hr. to prepare panose, and 72 hr. to prepare isomaltose. In each case, residual fermentable sugars were removed with bakers' yeast. After clarification each filtrate was passed through a Darco G-60 filter aid column. After thorough washing with water, isomaltose was eluted with 7.5% ethanol and panose with 15% ethanol. The isomaltose had a specific rotation of +120, the panose +151.

The starch employed was commercially available pearl corn starch.

Spray-dried, acid-hydrolyzed, 15-18 DE corn starch sold under the name of Frodex³ was used as substrate in the amyloglucosidase assays.

Enzymes. The commercial preparations employed were AMIGASE, an amyloglucosidase derived from *A. niger*, and ENZYME WC-8,⁴ an alpha-amylase derived from *Bacillus subtilis*.

Analytical. Amyloglucosidase was assayed according to Kerr (4); the unit is defined as that quantity of enzyme responsible for the production of 1 g. of glucose from a 4% solution of a 15-18 DE acid-hydrolyzed starch in 1 hr. at 60°C., pH 4.3.

The Lane-Eynon titration was made in accordance with AOAC recommended procedure (5), employing 25 ml. of the Soxhlet reagent.

The saccharimeter employed was a Schmidt and Haensch double-wedged model, with a dichromate filter. A 2-dm. tube was used and measurements were made at 25°C.

Whatman No. 1 paper was used for chromatography. Development was descending, with n-butanol:pyridine:water, 6:4:3 v./v., for 20-30 hr. at 27°C. The detection spray was silver nitrate-sodium hydroxide (0.5% silver nitrate in acetone, followed by 2% NaOH in methanol).

²Maltose, C.P., obtained from Pfanstiel Laboratories, Inc., Waukegan, Ill.

³Frodex is the registered trademark of American Malze-Products Company, Roby, Indiana.

⁴Amigase and Enzyme WC-8 are registered trademarks of Wallerstein Company, Div. of Baxter Laboratories, Inc.

Establishment of the PT/D for a given sample was done in accordance with the following example:

The polarization (P) of a 10% solution of glucose is 30.6° Ventzke (a polarimeter can be employed in place of a saccharimeter and the direct readings used to establish the standard curves, etc.). The Lane-Eynon titration (T) of a 1:25 dilution (D) of this sample is 30.2 ml. The PT/D of this solution is then

$$30.6 \times 30.2 \div 25 = 37.0$$

Experimental. A series of mixtures of glucose with maltose, isomaltose, and panose were prepared, and P and T were determined on solutions with a total carbohydrate concentration of 10%. Standard curves were plotted with percent nonglucose the ordinate and PT/D the abscissa. Figure 1 shows such curves, and it can be seen that the

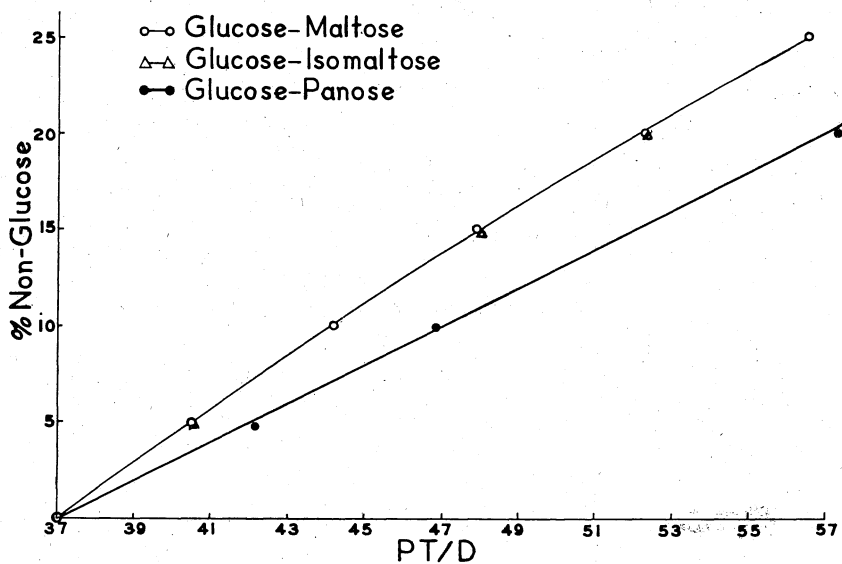


Fig. 1. PT/D of glucose-maltose, glucose-isomaltose, and glucose-panose mixtures.

PT/D remains fairly constant for mixtures of glucose and maltose and of glucose and isomaltose, and that the curves are superimposable. However, the PT/D for glucose-panose mixtures is higher, because of the high specific rotation of this trisaccharide (+151) and lower relative reducing power (0.4). Since the panose produced by a contaminating transglucosidase is normally hydrolyzed within the typical 72-hr. conversion period, the PT/D standard curves for the glucose-maltose

and glucose-isomaltose mixtures are, from the practical standpoint, the only ones applicable, and are the curves employed in this study.

To check the reliability of the standard curve, mixtures of pure glucose, maltose, isomaltose, and panose were prepared so as to approximate "typical" enzyme hydrolysates. Table I shows the results

TABLE I
RECOVERY OF GLUCOSE FROM ARTIFICIAL MIXTURES
(Values for mixtures 1, left, and 2, right, in each column)

COMPONENT	CONCENTRATION	P	T ^a	PT/D	NON-GLUCOSE ^b	GLUCOSE ^c
	%				%	%
Glucose	93.5, 95.3					
Maltose	2.5, 2.0	39.5, 36.7	26.5, 27.2	41.8, 40.0	6.8, 4.4	93.2, 95.6
Isomaltose	2.0, 1.8					
Panose	2.0, 0.9					

^aThe titration was performed on samples diluted 25-fold. Thus, D = 25 for all calculations.

^bNonglucose was determined from the standard glucose-maltose or glucose-isomaltose curve. This value designated (X).

^cGlucose equals (100 - X).

obtained with two such artificial mixtures. Recovery of the glucose was essentially complete, and the data indicate that concentrations of panose as high as 2% do not introduce significant errors.

It was also desirable to check the PT/D procedure against DE measurements of artificial mixtures containing rather large concentrations of a nonglucose component. Accordingly, the DE of the glucose-maltose mixtures used for the standard curve was determined; results are shown in Table II. It can be seen that, as is generally recognized,

TABLE II
COMPARISON OF PT/D ANALYSIS OF GLUCOSE-MALTOSE MIXTURES WITH DE

GLUCOSE	P	T ^a	PT/D	NON-GLUCOSE ^b	GLUCOSE ^c	DE
%				%	%	
100	30.6	30.2	37.0	0	100	100
95	33.1	30.6	40.5	5	95	98.7
90	35.4	31.2	44.2	10	90	96.8
85	37.7	31.8	47.9	15	85	95.0
80	40.0	32.6	52.2	20	80	92.6
75	42.5	33.3	56.6	25	75	90.8

^{a, b, c} See corresponding footnotes, Table I.

DE is not a good indication of the extent to which starch has been converted to glucose. The presence of as much as 15% maltose will still provide for a DE as high as 95.

To establish the utility of the procedure in the monitoring of enzymatic starch conversions, a dual-enzyme hydrolysis of pearl corn

starch was made. Liquefaction was made at pH 7.0, at a substrate concentration of 30% with the *B. subtilis* alpha-amylase.

When the liquefied mass tested negative with iodine, the pH was adjusted to 4.3 and the temperature lowered to 60°C. AMIGASE, with an activity of 200 AG units per g., was added at the level of 20 AG units per 100 g. of starch.

The conversion mixture was sampled every 12 hr. from 24 to 72 hr. To stop the reaction, the pH was adjusted to 2.0 and the sample was placed in a boiling-water bath for 15 min. P, T, solids (refractometer), and true glucose as measured with the Glucostat⁵ procedure were determined on filtrates diluted threefold. While solids measurements are required for the true glucose and DE determinations, this is not a requirement of the PT/D procedure.

Results

Table III shows a summary of the results obtained with a typical

TABLE III
DUAL-ENZYME STARCH CONVERSION USING PURIFIED AMYLOGLYCOSIDASE. COMPARISON OF PT/D ANALYSIS WITH GLUCOSE VALUES OBTAINED WITH GLUCOSE OXIDASE

TIME OF SACCHARIFICATION	P	T ^a	PT/D	Non-GLUCOSE ^b	GLUCOSE ^c	GLUCOSE ^d	DE
hr.				%	%	%	
24	34.4	33.5	46.1	12.6	87.4	87.5	90.2
36	33.5	32.4	43.4	9.0	91.0	91.8	93.2
48	32.3	32.1	41.5	6.4	93.6	93.4	94.1
60	32.2	31.7	40.8	5.4	94.6	94.1	95.3
72	32.0	31.4	40.2	4.8	95.2	95.0	96.3

^{a,b,c} See corresponding footnotes, Table I.

^d Glucose value obtained with Glucostat procedure.

starch conversion. It can be seen from these data that the PT/D is an accurate reflection of the changes taking place during this hydrolysis. The glucose content calculated from the maltose or isomaltose curve established with the PT/D measurement agrees remarkably well with the glucose value obtained with glucose oxidase, the differences being statistically insignificant. Once again, the data show the relatively poor reliability of DE as a criterion of the quality of a conversion.

During this investigation some observations were made with a crude, transglucosidase-contaminated sample of amyloglycosidase. This preparation contained approximately 7% of the transferring enzyme, according to our assay.⁶

A dual-enzyme conversion of pearl corn starch was made as pre-

⁵ Registered trademark of Worthington Biochemical Corp., Freehold, N.J.

⁶ Details of this transglucosidase assay to be published elsewhere.

viously described; the crude enzyme system was used for saccharification. Samples of the hydrolysate were withdrawn every 12 hr. from 24 to 96 hr.

Figure 2 shows the PT/D measurements during the course of the conversion. It was observed that maximum conversion to glucose

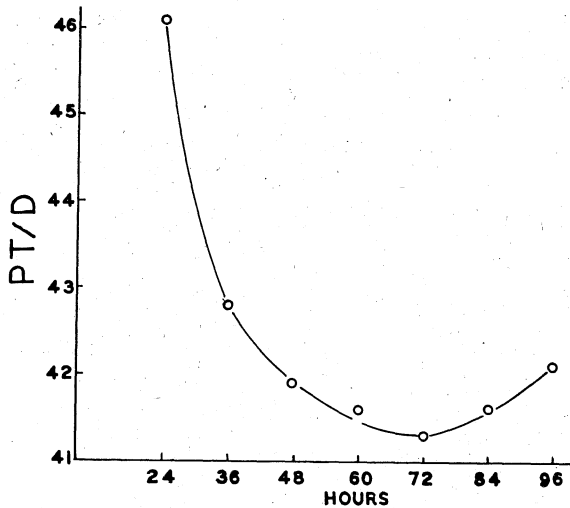


Fig. 2. PT/D analysis of dual-enzyme corn starch hydrolysate saccharified with amyloglucosidase contaminated with transglucosidase.

TABLE IV
DUAL-ENZYME STARCH CONVERSION USING AMYLOGLUCOSIDASE
CONTAMINATED WITH TRANSGLUCOSIDASE

TIME OF SACCHARIFICATION	P	T ^a	PT/D	Non-glucose ^b	Glucose ^c
hr.				%	%
24	34.5	33.4	46.1	12.6	87.4
36	33.0	32.4	42.8	8.2	91.8
48	32.6	32.1	41.9	6.9	93.1
60	32.5	32.0	41.6	6.5	93.5
72	32.4	31.9	41.3	6.1	93.9
84	32.7	31.8	41.6	6.5	93.5
96	33.0	31.9	42.1	7.2	92.8

^{a, b, c} See corresponding footnotes, Table I.

occurred at 72 hr., after which reversion appeared to take place. Table IV shows the apparent disappearance of glucose and the appearance of nonglucose products. This was confirmed by paper chromatography, as can be seen in Fig. 3. At 24 hr., glucose was the primary product, the other 12.6% of the total carbohydrate comprising maltose,

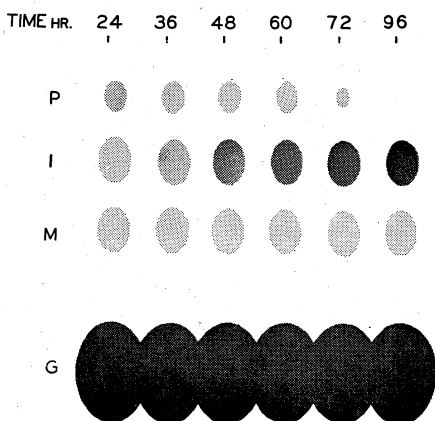


Fig. 3. Saccharification of liquefied corn starch with amyloglucosidase contaminated with transglucosidase. Chromatogram of hydrolysis products (shaded tracing). G = glucose; M = maltose; I = isomaltose; P = panose.

isomaltose, and panose. At each succeeding sampling time the panose component diminished, whereas the isomaltose increased. Maltose appears to have remained rather constant, whereas beyond 72 hr. enough transfer has taken place at the expense of glucose to cause a reversal of the PT/D curve.

These data also indicate the extent to which a 7% contamination by transglucosidase can contribute to reducing the yield of glucose obtainable from enzymatically converted starch. From Table III it was seen that a refined amyloglucosidase system produced glucose with a 95.2% conversion yield, whereas the transglucosidase-contaminated system produced glucose with a maximum yield of 93.9%. Obviously, the higher the contamination, the lower the maximum yield obtainable.

Discussion

The most efficient process for preparing glucose from starch involves the use of an enzymatic liquefaction and saccharification. While the highest yield is realized with the dual-enzyme process, invariably there are minor contaminating by-products which interfere with the simple, direct measurement of the glucose formed.

The data presented in this paper indicate that Mathews' formula, PT/D, can be used to monitor these starch conversions, and that the glucose formed can be determined directly with only a polarimeter reading and a Lane-Eynon titration.

Though it is general knowledge that DE is not a reliable indicator of the progress of a starch conversion, this fact is called to the reader's attention.

It also appears from these data that the user of amyloglucosidase will have to make certain that his conversion is being controlled in accordance with the demands of his particular enzyme system, since it is possible to exceed the time required to provide the optimum yield of glucose with certain preparations.

Literature Cited

1. JACKSON, R. F., and MATHEWS, J. A. Some physical properties of levulose and its estimation by copper reduction methods. *Bur. Standards J. Research* **8**: 433-442 (1932).
2. PAZUR, J. H. β -Maltose monohydrate. *In Methods in carbohydrate chemistry*, vol. I. Academic Press: New York (1962).
3. PAZUR, J. H., and ANDO, T. Isomaltose. *In Methods in carbohydrate chemistry*, vol. I. Academic Press: New York (1962).
4. KERR, R. W. Method of making dextrose using purified amyloglucosidase. U.S. Patent 2,967,804 (1961).
5. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and tentative methods of analysis (9th ed.), Sec. 29.053, p. 430. The Association: Washington, D.C. (1960).

