

A NEW POLARIMETRIC METHOD FOR THE ANALYSIS OF DEXTRAN AND SUCROSE

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ABSTRACT

A new method for dextran quantification has been developed and field-trialled in Jamaica, in association with the Sugar Industry Research Institute. The method uses a near infrared (NIR) polarimeter and a specific dextranase. The dextranase selectively breaks down the dextran into sugars of lesser specific rotations without affecting any other substance present in the juice. The initial dextran concentration is derived from the calibration curve of the change in observed optical rotation (OR) due to enzymatic hydrolysis and output automatically by the polarimeter. Readings are not affected by the molecular weight of the dextrans, the entire procedure takes less than 10 minutes to perform and it is semi-automated. Use of a NIR polarimeter negates the need for lead acetate clarification. The method is suitable for both juice and raw sugar samples.

Keywords: Dextranase, Near Infrared (NIR) polarimeter, Polysaccharides.

INTRODUCTION

Dextran is produced by microorganisms which infect the cane and feed on the sucrose; therefore, the presence of dextran immediately indicates lost sugar. The bacteria are mainly *Leuconostoc* species and are ubiquitous in the soil. They enter the cane at places of exposed tissue caused by machine harvesting, cutting, burning, growth, freezing, disease and pests. Any delay in the kill-to-mill time allows the bacteria to proliferate and the dextran levels to soar, especially in wet muddy cane.

The name dextran refers to a large family of glucose polymers whose structures and subsequent properties can vary widely. Technically the molecular weight (M_r) can range between 1500 and several million; therefore, a dextran of say 1 million M_r has potentially thousands of possible structures due to its branched nature. This massive variation in structure poses a huge challenge for any analyst trying to detect the molecules especially against a substantial background of saccharides with similar structures and properties.

Consequences of Dextran

Dextran is highly dextrorotatory, approximately three times that of sucrose, and, since the farmer is largely paid on the basis of the polarimeter reading, there is an obvious need for assaying for dextran in the core lab. This would allow correction of the falsified reading and identification of the sources of dextran contamination entering the factory. The problems associated with dextran contamination in both the factory and the refinery are well documented in the literature and so are briefly summarised below in Table 1.

Table 1. Summary of the detrimental effects of dextran in terms of the resulting losses.

Production losses	Sucrose losses	Direct financial losses
Increased viscosity leads to reduced throughput due to: -poor filterability -reduced evaporation rate -reduced flocculation rate -slow mud settling Poor crystallization (elongation)	As dextran formed in cane To molasses (melassigenic effect)	False pol reading leads to overpayment to farmer In trade of raw sugar as part of dextran penalty system using unreliable tests

Most dextrans are insoluble in alcohol making sugars and syrups containing it unsuitable for the production of alcoholic beverages. The two most important factors in the purchase of raw sugar are the polarisation and the crystal size distribution. Both of these are dramatically affected by the presence of dextran. The affination rate (removal of molasses from the crystal surfaces) is greatly reduced, leading to further losses of sucrose to the molasses. It is for this reason that high penalties are imposed on dextran contamination when importing raw sugar for refining.

Typically, the problem is treated in retrospect by the addition of crude dextranase enzyme. The enzyme works by hydrolysing the large dextran molecules into smaller oligosaccharide products which do not affect the viscosity as much. This is an expensive treatment largely because of the cost of the enzyme. Without accurate knowledge of the dextran levels in the process, it is impossible to gauge the correct amount of dextranase required.

Dextran detection is and long has been dominated by two equally questionable techniques, namely the haze test (Keniry et al., 1969) and the Roberts test (Roberts, 1983). Both tests exploit dextran's tendency to precipitate out of solution in alcohol. This approach has long been proved unreliable and inaccurate as well as non-specific, costly and time-consuming (Kubik et al.; 1994, DeStefano and Irej, 1986; Curtin and McCowage, 1986; and Brown and Inkerman, 1992).

Many alternative tests have been proposed and investigated, often as modifications on the theme of alcohol precipitation with various chemical and/or enzymatic inclusions. Although these tests are often arguably more accurate and reproducible, they are generally expensive and labor-intensive to perform. Hence, they are unattractive to the majority of sugar technologists. There is a longstanding need for a fast, accurate, simple and inexpensive method for the detection and quantification of dextran.

The Optical Activity Dextran Kit

Until recently, most polarimeters used the sodium wavelength of 589nm, which is yellow light. To achieve accurate results sugar samples had to be clarified and largely decolourised using lead subacetate. Now multi-wavelength instruments are readily available. Measurements of the sucrose content of cane juices by NIR polarimetry at 880nm are not affected by the yellow/brown color remaining after conventional filtration using a filteraid. Readings obtained using NIR polarimetry in comparison to those at the sodium wavelength have been previously shown to be more reproducible and more sensitive to interference by high dextran concentrations (Wilson, 1996).

Not only does the poisonous and environmentally unsound lead subacetate treatment damage enzymes; it also removes an unknown portion of the dextrans, making it an unsuitable clarifier in both this and other dextran methods. This latter point, of dextran removal, is also the case with a number of the more recent commercial clarifiers. In this method a conventional filter-aid is employed which successfully clarifies the juice or sugar solution without removing dextran. This filter-aid is paramount to the successful clarification of the juice sample.

This procedure is centered on the use of a NIR polarimeter manufactured by Optical Activity Ltd. in conjunction with a specific dextranase totally free of invertase activity. The dextran is hydrolysed into smaller dextrans and constituting smaller units such as isomaltotriose, isomaltose and glucose, each of which is less optically active than dextran. The hydrolytic reactions are rapid when the enzyme is used in excess. The change in rotation between that of the original sample and that observed at a predetermined time after the addition of dextranase can be calibrated to the original concentration of dextran present in the sample.

MATERIALS AND METHODS

The NIR polarimeter used was a SacchAAr 880, manufactured by Optical Activity Ltd. The polarimeter sample tube (also manufactured by Optical Activity Ltd.) was an A2 with a bore of 4mm and 200mm path length. The tube is jacketed and the temperature maintained at 20°C using an Index Instruments Ltd. thermocirculator.

The enzyme concentration in the sample and the total sample volume were previously optimised for this procedure and are 1 ml enzyme solution (see below) added to 19 ml sample. A selected pure dextranase preparation with activity of 30,400 units/ml is diluted 1:5 in distilled water. It is always used at this dilution, except for those experiments that involve the use of impregnated filter papers. In order to assist the user and prevent any error in measuring quantities of liquid, the enzyme will be available commercially in this form. These papers will consistently carry the required amount of dextranase to carry out the reaction within the desired time limit and have already been tested in field trials during the work with the Sugar Industry Research Institute of Jamaica.

RESULTS

Effect of Molecular Weight

It was necessary to determine if the extent of the change in rotation due to hydrolysis is influenced by molecular weight. The following different molecular weight range dextrans were dried for a week in a desiccator containing P₂O₅ and then made up to 4000ppm in distilled water:

- 9,5kDa (Sigma Cat. No. D-9260)
- 71.4kDa (Sigma Cat. No. D-3759)
- 2,000kDa (Sigma Cat. No. D-5376)

After quantifying the control readings, 1ml of dextranase solution was added to 19ml of dextran solution, rapidly shaken and injected into the sample tube. The results (Table 2) were recorded when the readings had reached a stable minimum. It can be observed that there is no systematic or significant effect of M_r on the change in OR due to enzymatic hydrolysis. The variability in the results is thought to be due to structural and preparative differences between the commercially available dextrans reflected by differences in appearance (powders / flakes).

Table 2. The change in OR due to enzyme action for three different molecular weight dextrans.

M_r of Dextran (Daltons)	Change in OR °Z due to enzyme action
9.500	1.26
71.400	1.19
2.000.000	1.34

Confirmation of Enzymatic Specificity

Many commercial enzyme preparations contain several enzyme activities in addition to the major activity that is purchased. It was necessary to ensure that the dextranase preparation was unable to hydrolyse sucrose and non-dextran polysaccharides.

A selection of possible alternative saccharides were chosen and 5% solutions made up in distilled water. 1ml of dextranase solution was added to 19ml of the analyte solution and the OR observed for 20 minutes. Little or no change in the reading over time (other than that accounted for by the controls and the accuracy of the instrument) indicates no reaction (Table 3).

Table 3. The effect of dextranase on other possible analytes.

Analyte	Result
Sucrose	No reaction
Dextrin	No reaction
Xylan	No reaction
Pectin	No reaction

Although the above list is non-exhaustive, there are no apparent reactions with these substances, which form the majority of dissolved carbohydrates constituent in sugar samples.

Calibration Curve Constructed in 15% Sucrose

Using the calibration curve and the preloaded filter papers, it becomes possible to transform the assay from a fairly technical laboratory assay into a kit for use by unskilled workers. The calibration data will be incorporated into the software of the polarimeter negating the need for lengthy calculations and reducing the chances of operator error.

Using an 188kDa dextran (Sigma Cat No, D4876), solutions of 8000ppm, 4000ppm, 2000ppm, 800ppm, 400ppm and 200ppm were made up in 15% sucrose (since sucrose is known to mildly retard the rate of the reaction with dextran via non-competitive inhibition).

The dextranase solution was added to the dextran just prior to injection into the polarimeter and the OR followed for 15 minutes. The readings were recorded at 5-second intervals by a data collection program.

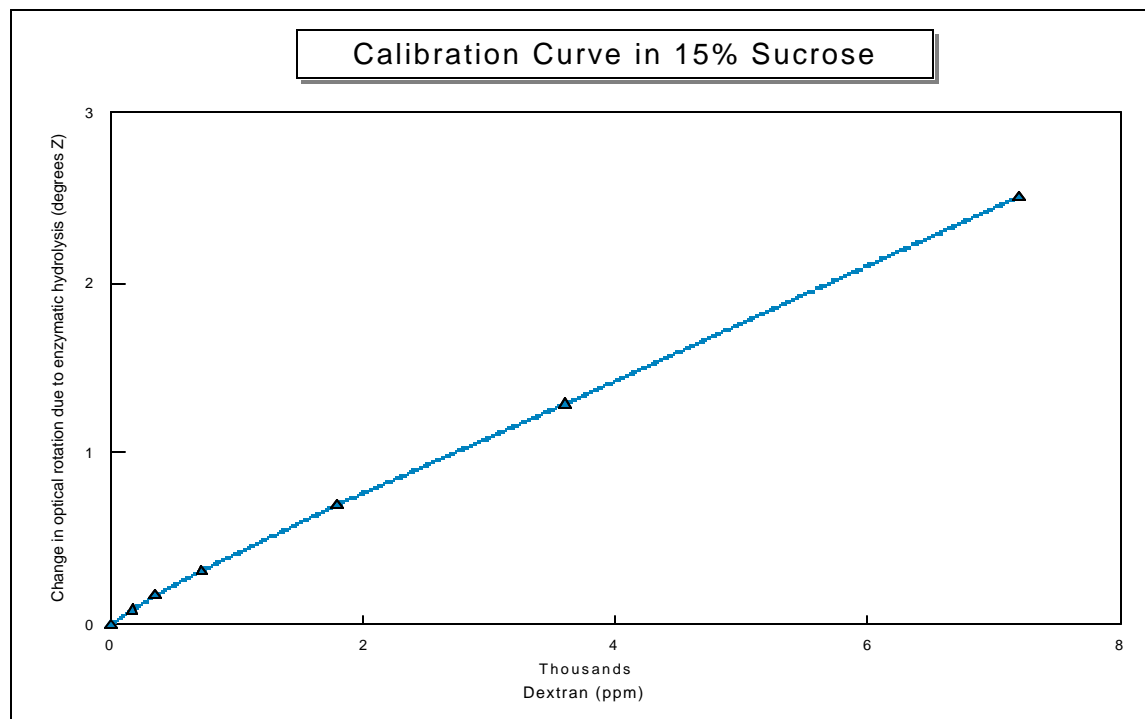


Figure 1. The relationship between dextran concentration and change in OR due to hydrolysis by dextranase enzyme.

The relationship shown in Figure 1 is clearly linear in character but has a slight curve (which in this data is a 39.5% change in x/y). This relationship is reproducible on a day-to-day basis and has been curve-fitted and the algorithm incorporated into the instrument's software to allow accurate automatic readings of dextran concentration to be instantly generated.

Detecting Spiked Dextran in Cane Juice

"Dextran-free" cane juice was obtained and subjected to standard addition with a known mass of dextran to demonstrate that dextran could be detected and quantified in the cane juice as effectively and accurately as in distilled water.

A 2000ppm solution of dextran (71.4kDa) was made up in distilled water and the OR determined. 200ml of cane juice were vacuum filtered with filteraid (2g/100ml) and the OR determined. 0.1g dextran was weighed into a 50ml flask, which was filled to the mark with cane juice and the OR determined. All three samples were then subjected to the new dextran method (Table 4).

Table 4. Change in OR ($^{\circ}$ Z) due to enzyme treatment in spiked samples of water and cane juice.

Sample	OR $^{\circ}$ Z before enzyme treatment	OR $^{\circ}$ Z after enzyme treatment	Change in OR $^{\circ}$ Z
Water + dextran	2.13	1.52	0.61
Juice	50.47	50.47	0.00

Juice + dextran	52.60	52.00	0.60
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The assay behaves the same in cane juice as in water as shown by the essentially identical values of change in rotation due to dextranase addition.

Confirmation of the Analytical Precision and Reliability

Using a 40% raw cane sugar solution high in natural dextran the assay was performed 10 times on the same sample to demonstrate the precision of the test and therefore the reliability of a single measurement approach.

The results showed absolutely no variance within the accuracy range of the instrument, which is $\pm 0.02^\circ\text{Z}$. This indicates the measurements are entirely repeatable under standard laboratory conditions.

Observation of Dextran Growth Over Time

The following work was carried out during field trial work in association with SIRI at their Central Laboratory, Mandeville, Jamaica. Using green cane deliberately contaminated with dextran-producing bacteria, the test was performed repeatedly over a 4-day period to demonstrate the growth of dextran over time.

Enough cane was crushed from the pile to collect 500ml of raw juice. Filter-aid was added in the concentration of 2g/100ml and after stirring, the mixture was vacuum filtered through a Millipore AP20 prefilter (as before). The OR of the clear cane juice was determined on the polarimeter. 60ml of juice were incubated on a shaker for 7 minutes with 1 dextranase-impregnated filter / 30ml and the OR determined at 10 minutes (after addition of impregnated filters).

The increase of dextran levels is clearly seen in the rising values of the difference between the control and test readings (Table 5). The dextran is calculated by using the quadratic equation fitted to the calibration curve. The lack of exposure of the cane to mud and rain during the test period would explain why the increase of dextran is less than that expected in an average cane yard.

Table 5. Increase in dextran over time. The dextran is calculated by using the quadratic equation fitted to the calibration curve.

Day	Control (OR ^o Z)	Test OR (°Z)	Difference OR (°Z)	Dextran (ppm)	Corrected OR (°Z)
1	60.35	59.61	0.74	1431.72	58.80
2	59.43	58.57	0.86	2279.23	56.97
3	61.80	60.85	0.95	2551.58	59.04
4	60.12	59.00	1.12	3064.16	56.81

SUMMARY

From the above set of experiments, it is evident that the theoretical basis of the assay remains sound when put into practice. The enzyme selected for this work appears to be specific for a single substrate, namely dextrans. The calibration curve has been previously shown to be unaffected by factors such as molecular weight of the substrate and the pH of the medium in which measurements are made with detection limits that cover the entire range of market requirements. This assay procedure is robust, rapid, simple to perform and through subsequent development of the instrument is now semi-automated. The presence of dextran in sugar represents financial losses at almost every stage of the process from cane to cube. It is hoped that this new analytical method will now make it possible for both the factory and the refinery to identify dextran sources and take an informed approach to employing the correct remedial actions in both the short and long term.

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REFERENCES

1. Brown, C. F. and Inkerman, P. A. 1992. Specific method for quantitative measurement of the total dextran content of raw sugar. *J. Agric. Food Chem.* 40:227-233.
2. Curtin, J. H. and McCowage, R. J. 1986. Dextran measurement in cane products. *Proc. ISSCT.* 19:755-764.
3. DeStefano, R. P. and Irely, M. S. 1986. Measuring dextran in raw sugars - historical perspective and state of the art. *J. Am. Soc. Sugar Cane Technol.* 6:112-120.
4. Imrie F. K. E. and Tilbury R. H. 1972. Polysaccharides in sugar cane and its products. *Sugar Technol. Rev.* 1:291-361.
5. Keniry, J.S., Lee, J. B. and Mahoney, V.C. 1969. Improvements in the dextran assay of sugar cane materials. *Int. Sugar J.* 71:230-233.
6. Kubik, C., Galas, E. and Sikoro, B. 1994. Determination of dextran in raw beet juices by the

- haze / enzymatic method. *Int. Sugar J.* 96(1149):358-360.
7. Muller, E.G. 1981. Dextran. *Tate and Lyle's SIA.* 43(5):147-148.
 8. Roberts, E. J. 1983. A quantitative method for dextran analysis. *Int. Sugar J.* 85:10-13.
 9. Wilson, T. E. 1996. A comparison of raw sugar polarisation methods. *Int. Sugar J.* 98(1168):169-174.