OPTIMIZATION OF SUGARCANE FACTORY APPLICATION OF COMMERCIAL DEXTRANASES IN THE U.S.

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ABSTRACT

The application of commercial dextranases to break down dextran in U.S. sugar manufacture is still not optimized, partly because of misinformation about where to add the enzyme and which enzyme to use. Furthermore, there is no uniform method to measure the activity of commercial dextranases by producers/vendors/distributors, which has meant that direct comparison of activities is not possible. In this study, a simple titration method to determine the relative activity of dextranases was identified and modified for easy factory use. All activities were confirmed with an accurate IC-IPAD method using a NaOH/NaOAc gradient. Most commercial dextranase enzymes in the U.S. are from a fungal source: Chaetomium gracile or erraticum, and are available in “non-concentrated” or “concentrated” forms. An approximate 8-10 fold difference in activity exists between the two concentration forms, and activity variations exist within each form. In 2002/03 only “non-concentrated” dextranases were applied in Louisiana to either last evaporator bodies (usually ≤10 ppm/syrup) or juice. “Non-concentrated” and “concentrated” dextranases studied at juice pH 5.4-5.8, showed similar maximum activity at 48.9°C or 120°F, as monitored by IC. Dextranase activities, in last evaporator syrup temperature (~63°C or 145°F) and Brix (~65°) conditions, were dramatically reduced (activity began to decrease after 25-30°Brix). Overall, juice applications were more efficient and economical than adding them to evaporator syrups. Application of “non-concentrated” dextranase to evaporator syrup was uneconomical. However, “concentrated” dextranase can be applied to syrup at levels as low as 10 ppm/solids (equiv. to 45 ppm/juice) to remove up to ~37% dextran which is useful to consider when severe dextran problems occur. Heating juice to 48.9°C in the presence of all dextranases, dramatically removed more dextran (3380 ppm/°Brix) from a juice than at the current ambient temperature of application (32.2°C or 90°F) and was much more economical. For a “non-concentrated” dextranase, after 10 min at 10 ppm/juice and 48.9°C, ~46.3% dextran was removed compared to 13.6% at 32.2°C. For the “concentrated” dextranase, after only 10 min at only 4 ppm/juice, 66.6% dextran was removed at 48.9°C and was considered an overdose.
compared to 29.6% at 32.2°C. Dextranase was shown to work in the presence of dithiocarbamate biocide in juice, and factory studies are being undertaken to check that no adverse dextran formation is occurring at 48.9°C. Under factory storage conditions, over a grinding season (90 days), the activity of “concentrated” dextranase decreased only slightly (~9%), whereas “non-concentrated” dextranase activity had approximately halved (~46%), and even reduced in activity when stored under refrigeration.

INTRODUCTION

The major contributor to sugarcane deterioration in the U.S., particularly Louisiana, where humid conditions prevail, is from *Leuconostoc* lactic acid bacterial infections. Such infections mostly occur after severe freezes, when cut-to-crush times are delayed, and are also impacted by the harvesting method (Eggleston and Grisham, 2003) and because of poor mill hygiene. *Leuconostoc* species (and to a lesser extent some *Lactobacillus* species) produce an extracellular exo-enzyme dextransucrase which catalyzes the production of dextran from sucrose. Dextran is a glucose polysaccharide comprising mainly α-(1-6) linkages, but also contains a small amount of α-(1-4), α-(1-3) and some α-(1-2) linkages (Robyt and Eklund, 1982). Most dextrans in the sugar industry are linear, but some branching may occur (Edye et al, 1995). Moderate and severe dextran (>1000 ppm°Brix in mixed juice) in the factory has long been acknowledged as an interrupter of normal processing operations. Formation of dextran not only causes expensive sucrose losses, but the high viscosity associated with this polysaccharide (especially the high MW portion) often slows evaporator and crystallization rates, raises losses of sucrose to molasses, and distorts factory pol readings. Worse still, the factory is penalized by refineries on dextran in the raw sugar. Although clarification processes remove some dextran (Eggleston et al, 2003), commercial dextranase has been used in sugarcane factories to break down dextran, by hydrolyzing α-(1-6) linkages endogenously into smaller, more manageable molecules, and has been used in sugarbeet processing (DeBruijn, 2002). In some Louisiana factories dextran concentrations ≥800 ppm°Brix in mixed juice cause the staff to add dextranase, whilst other factories just add it when factory processes are obviously suffering.

The application of dextranases in the sugar industry was pioneered in Australia in the 1970s, and the comprehensive review of this work by Inkerman (1980) is recommended. Dextranase activity is governed by the pH, temperature, residence time (R_t), agitation, substrate concentration, type and concentration of enzyme applied, and initial amount of dextran present. Usually, the higher the dextran concentration the more hydrolysis of dextran occurs (Fulcher and Inkerman, 1976). The use of dextranase is a routine procedure in Australian raw sugar factories, when dextran levels are high and detrimentally affect processing. The properties of commercially available dextranases governed the selection of the addition point in the factory (Inkerman, 1980) and dextranase is usually added in holding juice tanks just before clarification, with minimum R_t of 15 min and temps of 55-60°C. Australians have not advocated addition of dextranases in high °Brix syrup evaporators or tanks because dextranases are not as stable at high syrup temperatures as amylases that are routinely used for starch break down in syrup, or at unfavorable syrup pHs (>6.0), and the °Brix has a marked inhibitory effect (Inkerman, 1980). In comparison, in South Africa where diffusers are used, dextranase application in diffuser cane juices was deemed unsuitable mostly because of the high temperatures (Morel du Boil and Wienese, 2002). Consequently, application of dextranases to
evaporator syrups has been advocated (Morel du Boil and Wienese, 2002) but over a 30 min $R_c$ that is not usually available in U.S. factories.

Some U.S. dextranase studies have occurred since the late 1970s. Polack and Birkett (1978) indicated the possibility of using dextranase in plant trials. DeStefano (1988), using laboratory trials, compared the application of several U.S. commercial dextranases for use in mixed juice, final evaporators and syrup storage. DeStefano (1988) advocated the addition of dextranase in syrup storage tanks, as the volumes of material to be treated were smaller and some clean-up had already been achieved via clarification. However, it was acknowledged (DeStefano, 1988) that the pH and °Brix of the syrup are not optimum and relatively higher levels of dextranase would have to be added compared to juice. In the mid 1990s, Edye et al (1997) conducted factory trials of a dextranase from the fungus 

$\textit{Cheatomium gracile}$ that was stated to be more temperature stable. Cuddihy and Day (1999) have discussed some of the financial implications associated with dextranase treatment. However, the problem with these few U.S. dextranase studies is that they never stated the activity of the enzymes they were applying, and this has led to enormous confusion concerning the appropriate concentration of each commercial dextranase to add. Furthermore, there is no uniform or factory usable method for dextranase activity in the sugar industry.

Since 1996 many Louisiana factories have been utilizing dextranase, but the point of application is extremely varied and reports from some factories have indicated that dextran degradation is very limited and the economical use of the enzymes has been questioned. Furthermore, our initial factory studies of current dextranase applications (20 ppm/solids) in last evaporator syrups (65.5°C; $R_c=20$ min) indicated there were no differences in viscosity changes compared to the 0 ppm control. This study was, therefore, undertaken at the request of the Louisiana raw sugar manufacturing industry to optimize the addition and conditions of dextranase in U.S. factories.

## EXPERIMENTAL

### Commercial Dextranases

A variety of commercial dextranases currently in use in Louisiana and Florida sugarcane factories, or available in the U.S., were studied and are listed in Table 1. Because the United States Department of Agriculture does not endorse one brand over others of a similar nature, the enzymes have been denoted with numbers. Commercial dextranase 1 was a gift from Dr. Ron DeStefano who had stored it at 4°C. Dextranase 2 was donated by the suppliers. Dextranase 3 was obtained from two Louisiana sugar factories on the day of consignment, as well as the supplier. Dextranase 4 was obtained from one Louisiana factory on the day of consignment.

### Dextranase Activity

This was initially determined by three different methods. A spectrophotometric method (Anon, 2002a) measured reducing sugar from dextranase action on dextran (T2000™, Amersham MW $\geq$ 2,000,000 Da), which reacts with 3,5 dinitrosalicyclic acid to give a yellow-brown color, which is measured at 540 nm. One dextranase unit (DU) is the amount of enzyme which degrades dextran to produce reducing sugar equivalent to 1 mg maltose per hour at 40°C and pH 5.4. A titration method (Anon, 2002b) was modified (see Appendix 1) to measure reducing sugar from
dextranase action on dextran T2000™, and the reducing sugar is determined by the Hanes method. One dextranase unit (DU/mL) is the amount of enzyme which degrades dextran T2000™ to produce reducing sugar corresponding to the reducing power of one micromole of sodium thiosulfate in one min at 37°C and pH 5.8. Relative dextranase activities of the different enzymes studied were confirmed by comparison of ion chromatograms, after dextran (T2000™, 500ppm) was digested by the dextranase (0.015g/100ml acetate pH 5.4 buffer [13.5g sodium acetate trihydrate was dissolved in 900ml of deionized water, and the pH adjusted to pH 5.4 with 1N acetic acid]) for 30min at 40°C in a shaking (90 rpm) water bath.

**Haze Dextran in Sugarcane Syrups**

Haze dextran in sugarcane syrups was determined following an alcohol method (ICUMSA GS1-15 [1994]) with modifications. Dextran T2000™ was the standard and dextran was precipitated with 100% absolute ethanol.

**Haze Dextran in Sugarcane Syrups**

Haze dextran in sugarcane juices was based on ICUMSA GS1-15 [1994] and rapid haze (Clarke et al, 1987) methods with modifications. Juice (35ml) was pipetted into a conical flask and Termamyl™ (Novo, U.S.) amylase (0.1 ml) added at 55°C for 15 min to degrade starch. The mixture (10 ml) was then pipetted into a 25 ml plastic syringe with a filter holder attached, containing a coarse glass filter (25 mm). TCA (10%) solution (2 ml) was then pipetted into the syringe body and 0.5 g high-grade celite filter aid (Aldrich, U.S.). The plunger was placed in the syringe, and inverted at least five times. The first 2 ml of filtrate was discarded, and then 5 ml filtrate was added to 5 ml absolute ethanol, mixed and left for 2 min. The absorbance at 720 nm was immediately read in a 1 cm cell on a Shimadzu UV/VIS-1201 spectrophotometer. The amount of dextran was calculated using the same standard curve as for the ICUMSA haze dextran method above.

°Brix  The mean °Brix of triplicate samples was measured using an Index Instruments TCR 15-30 temperature controlled refractometer accurate to ± 0.01°Brix.

**Dextran Breakdown Products by Ion Chromatography with Integrated Pulsed Amperometric Detection (IC-IPAD)**

See (Eggleston, 2002) for method. Duplicate samples were diluted (1g/25ml) then filtered through 0.45 μm filters. All compounds analyzed were quantitated in reference to standards.

**Viscosity**

The viscosity of syrup was measured on a Brookfield (Middleboro, U.S.) DV-II⁴ rotational viscometer at 25°C using spindle no. 18 (see Eggleston et al, 2004).

**Temperature Effect Studies**

**Pure Dextran**: Dextran T2000™ (500ppm) was prepared in pH 5.4 acetate buffer. Dextranase enzymes 2, 3 & 4 were first diluted (0.015g/100ml pH 5.4 acetate buffer). The "concentrated" dextranase 2 had also been previously diluted 4.6X to make it economically equivalent to the nearest priced "non-concentrated" dextranase. Diluted enzyme (1ml) was added to the dextran (2 ml) in a test-tube, covered with aluminum foil, and thoroughly mixed. For the control, deionized water (1 ml) was added instead of dilute dextranase. The test-tubes were placed in a shaking
waterbath (90 rpm) at different temperatures ranging from 26.6 - 65.5°C for 25 min. After incubation the test-tubes were immediately placed in a boiling water-bath for 2.5 min to denature the enzyme, and cooled on ice. Preliminary experiments were undertaken to ensure that 2.5 min boiling was sufficient time to stop further dextranase action. Undiluted duplicate aliquots were analyzed on IC-IPAD.

**Sugarcane Juice:** Mixed juice (12.7°Brix; pH 6.1; 3177 ppm haze dextran/°Brix) was obtained from a Louisiana factory and stored in a -80°C freezer until used. Dextranases 2, 3 & 4 were first diluted (0.03g/100ml) in pH 5.4 acetate buffer. The "concentrated" enzyme 2 had also been previously diluted 4.6X to make it economically equivalent to the nearest priced "non-concentrated" dextranase. Diluted enzyme (1 ml) was added to the juice (2 ml) in a test-tube, covered with aluminum foil, and thoroughly mixed. For the control, de-ionized water (1 ml) was added instead of dilute enzyme. The test-tubes were placed in a shaking water bath (90 rpm) at different temperatures ranging from 26.6-65.5°C for 25 min. After incubation the test-tubes were immediately placed in a boiling water-bath for 2.5 min, and cooled on ice. Duplicate aliquots were diluted (0.75ml/50ml de-ionized water) and filtered before IC-IPAD analyses.

**°Brix Effect Study**
Solutions of dextran T2000™ (1000 ppm) were prepared in pH 5.4 acetate buffer with differing °Brix levels. Dextran was added to the buffer first and completely dissolved by boiling for 2 min, then sucrose was added to adjust the °Brix, and the final °Brix noted. A range of °Brixes was achieved: 0.0-70.6. Dextranases 2, 3 & 4 were first diluted (0.03g/100ml) in pH 5.4 acetate buffer; the "concentrated" dextranase 2 had been previously diluted 4.6X to make it economically equivalent to the nearest priced "non-concentrated" dextranase. Diluted enzyme (1 ml) was added to the dextran/sucrose solution (2 ml) in a test-tube, covered with aluminum foil, and thoroughly mixed. For the control, de-ionized water (1 ml) was added instead of dilute enzyme. The test-tubes were placed in a shaking water bath (90 rpm) at 48.0°C for 25 min. After incubation the test-tubes were immediately placed in a boiling water-bath for 2.5 min, and cooled on ice. Diluted aliquots (dilution depended on the °Brix level) were analyzed on IC-IPAD.

**Laboratory Dextranase Studies on Factory Syrups and Juices**

**Dextranase/Syrup Reactions:** A final evaporator syrup (FES) was obtained from a Louisiana factory (°Brix 58.8; pH 6.4; 7230 ppm haze dextran/°Brix) at the end of the 2002 grinding season, and stored in a -80°C freezer until used. Levels of dextranase addition were calculated as ppm/solids (ppm/solids x 4.5 = ppm/juice). "Non-concentrated" dextranase 3 (0, 20, 40 and 80 ppm/solids) was added to 200 ml of syrup and vortex mixed for 40 sec to ensure thorough mixing. The syrup/dextranase mix was then immediately placed in a shaking water bath at 63°C and 90 rpm. Aliquots (40 ml) were removed after 0, 10, 15 and 20 min, and boiled immediately for 2.5 min. After cooling, the samples were analyzed, in duplicate, for haze dextran and viscosity. Preliminary results showed that boiling did not affect the haze dextran or viscosity. For the "concentrated" dextranase 2, the reaction conditions were the same, except 10 ppm/solids was also analyzed.

**Dextranase/Juice Reactions:** A sugarcane juice (15.0°Brix; pH 5.6) was obtained for this study by allowing a sugarcane pile to deteriorate at ambient conditions for 3 days outside a Louisiana factory. Juice was extracted in the factory laboratory core press, and biocide added (20 ppm/juice; Bussan881™, Buckman Labs.) to prevent further dextran formation reactions. A required level of
haze dextran (3380 ppm/°Brix) was achieved by diluting this deteriorated juice with fresh crusher juice. "Non-concentrated" dextranase 3 (0, 10, 20, 40 and 80 ppm/juice) was added to 250ml of juice and mixed thoroughly for 40sec. The juice/dextranase mix was then immediately placed in a shaking water-bath at 32.2°C and 90 rpm. Aliquots (40 ml) were removed after 0, 5, 10, 15 and 20 min, and boiled immediately for 2.5 min. After cooling, the samples were analyzed, in duplicate, for haze dextran. Preliminary results showed that boiling did not affect the haze dextran. This experiment was repeated at 48.9°C. For the "concentrated" dextranase 2, the reaction conditions were the same.

Effect of Biocide on Reactivity of Dextranase
The method described above for dextranase/juice reactions was used to measure the effect of adding 0, 10 and 20 ppm biocide (dithiocarbamate; Bussan881™) to juice (3380 ppm/°Brix) in the presence of 10 ppm/juice "concentrated" dextranase 2.

Storage Characteristics of Dextranases
Fresh “non-concentrated” and “concentrated” dextranases (150ml) were stored in dark brown bottles in a cool and dark laboratory corner (ambient temperatures ranged from 23-27°C). The activity of the dextranases was measured periodically over a 90 day period using the simple titration method (Appendix 1). As a control, samples of the same dextranases were stored in a refrigerator (4°C) and periodically analyzed as well.

RESULTS AND DISCUSSION

Dextranases Available in the U.S.
Most commercial dextranases currently available in the U.S. are produced from a fungal source, Chaetomium gracile or erraticum, and have GRAS (Generally Recognized As Safe) status. The U.S. Food and Drug Administration does not approve of dextranases from Penicillium or bacterial sources which are sold overseas. Dextranases from Chaetomium have been shown to hydrolyze faster than dextranases from Penicillium and Bacillus sources (Taylor et al, 1990). One of the greatest sources of confusion for factory staff about dextranase is that it is not possible to directly compare the activities (or strengths) of commercial dextranases because each dextranase producer/vendor/distributor uses a different method to measure activity. For example, units of dextranase activity can be u/g, Du/g, U/ml and Du/ml. This is further exacerbated by the fact that the dextranase market is very dynamic and dextranase activities and prices can change regularly.

Consequently, there is an urgent need for a uniform method to measure dextranase activities at the factory. For this reason, a simple titration method to determine the relative activity of dextranase was identified (Anon, 2002b) and modified for easy use at the factory (see Appendix 1). The method does not need any sophisticated equipment, such as a spectrophotometer, and there is no need for standards and a standard curve. Having an easy-to-use method to measure activity will allow factory staff to compare commercial dextranases and make more informed decisions on which dextranase to use. The stability of the dextranase stored across the grinding season can also be monitored.

To undertake a study of factory optimization of dextranase application, it was first necessary to
understand the differences in the activities and properties of the different commercial dextranases available in the U.S. Relative activities and some physico-chemical properties of commercial dextranases that are currently available in the U.S. are listed in Table 1. Initially, a spectrophotometric (Anon, 2002a) and the titration method were used to compare the activities of commercial dextranases (Table 1), and the correlation between the two was excellent ($R^2=0.999$) which confirms their accuracy. It was also found that the relative activities of commercial dextranases can be accurately confirmed using IC-IPAD profiles of dextran/dextranase mixtures, and these are illustrated in Figure 1. Dextranase breaks high MW dextran down into smaller, more manageable MW dextran and eventually oligosaccharides, with isomaltotriose, isomaltose and glucose being the final major products (Figure 1).

Table 1. Relative Activities of Dextranases and Some Physico-Chemical Properties

<table>
<thead>
<tr>
<th>Commercial Dextranase</th>
<th>Source</th>
<th>Rel. Specific Activity DU/g&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Rel. Specific Activity DU/ml&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Color</th>
<th>Brix</th>
<th>Protein % w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Concentrated&quot; 1</td>
<td>Chaetomium gracile</td>
<td>60,603.3</td>
<td>48,072.1</td>
<td>Clear, light</td>
<td>45.13</td>
<td>0.84</td>
</tr>
<tr>
<td>&quot;Concentrated&quot; 2</td>
<td>Chaetomium erraticum</td>
<td>n.d.&lt;sup&gt;c&lt;/sup&gt;</td>
<td>57,686.5</td>
<td>Clear, light</td>
<td>41.96</td>
<td>n.d.</td>
</tr>
<tr>
<td>&quot;Non-concentrated&quot; 3&lt;sup&gt;d&lt;/sup&gt; (Factory 1)</td>
<td>Chaetomium gracile</td>
<td>8,402.8</td>
<td>5,999.4</td>
<td>Clear, slightly yellow</td>
<td>26.57</td>
<td>0.26</td>
</tr>
<tr>
<td>&quot;Non-concentrated&quot; 3&lt;sup&gt;d&lt;/sup&gt; (Factory 2)</td>
<td>Chaetomium gracile</td>
<td>8,290.8</td>
<td>5,499.5</td>
<td>Clear, slightly yellow</td>
<td>26.64</td>
<td>0.15</td>
</tr>
<tr>
<td>&quot;Non-concentrated&quot; 4</td>
<td>Chaetomium gracile</td>
<td>6,356.3</td>
<td>4,783.2</td>
<td>Clear, v. slightly yellow</td>
<td>37.45</td>
<td>0.12</td>
</tr>
</tbody>
</table>

<sup>a</sup> See Anon (2002a)

<sup>b</sup> See Anon (2002b) and Appendix I

<sup>c</sup> n.d. = not determined

<sup>d</sup> "Non-concentrated" dextranases were obtained from barrels which had been delivered to Factory 1 and 2 that day, and stored at 4°C immediately. A sample of this enzyme was also sent from the supplier and a similar 5,499.5 DU/ml obtained.

The greater the dextranase activity, the greater the number and size of the breakdown product peaks that occur on the ion chromatograms. Considerable variation existed among the enzymes currently being used in Louisiana and others available in the U.S. (Table 1). Dextranases could be categorized into "concentrated" and "non-concentrated" forms, and an approximate 8-10 fold difference in activity existed between the two forms, but this did not always reflect the corresponding differences in price and highlights the different practices of distributors. Activity variations also existed among dextranases within each concentration form. As expected, the
“concentrated” dextranases produced more and larger IC peaks than the “non-concentrated” dextranases currently being used in Louisiana (Figure 1). Because of the ease and simplicity of the titration method compared to the spectrophotometric method, subsequent enzyme activities in this research were only measured using the titration method.

Current Addition of Commercial Dextranases in the U.S. - Survey Results
To gain information on the current status of dextranase applications in Louisiana a questionnaire/survey was sent out to all Louisiana factories in 2002. Eleven out of sixteen factories responded and results are summarized in Table 2. All applications varied considerably with factory. Some factories chose not to use dextranase, but rather opted to manage dextran by managing cane cut-to-crush times. Only “non-concentrated” dextranases were being used in Louisiana factories in 2002 and 2003. Most dextranase applications were in the last evaporator bodies at very low ppm levels (2-10 ppm/syrup), with one factory adding it to massecuites in a vacuum pan (Table 2), and the others to juice. Only one factory had an incubation tank (R1=12 min) for dextranase addition, although it was installed to aid the natural amylase activity of sugarcane (Eggleston et al, 2003).

Factors Which Affect Dextranase Activities in the Factory
The optimum pH range for dextranase activity is pH 5.0-6.0, with the lower end of the range more preferable, and this coincides with the typical pH operating range of juices before clarification in U.S. sugarcane factories. Therefore, there was no need to manipulate juice pH. Addition of lime in the clarification process increases the pH well above pH 6.0, indicating that dextranase should never be added to limed juice. Syrup pH is often between pH 6.0-6.5, which is less optimal than natural juice pH. For these reasons, pH effects were not studied; instead we focussed on the effects of temperature and °Brix.

Dextranase Activity at Different Temperatures
Temperature is known to have a critical effect on the activity of dextranases (Inkerman, 1980). We undertook studies on the effect of temperature on the activity of dextranases using IC-IPAD with relative dextranase activity being measured as the isomaltotriose peak height. The pattern of temperature/dextranase effects was close for pure dextran (Fig. 2a) and dextran formed in juice (Fig. 2b), indicating T2000 was an adequate standard. The “concentrated” enzyme was added after a 4.6 dilution to make it economically equivalent to the nearest “non-concentrated” dextranase. However, even at this dilution the activity was still consistently higher across the temperature range studied than for the “non-concentrated” dextranases (Fig. 2), which highlights the differential between relative economic price and activity. The remarkable similarity of the temperature effects patterns for the “concentrated” and “non-concentrated” dextranases studied (Fig. 2), most likely highlight the same fungal source of the dextranases (Table 1).

For all the dextranases studied the maximum activity was distinctly ~48.9°C, with 43.3-54.4°C being the optimum activity range. It has been reported by Morel du Boil and Wienese (2002) that high °Brix levels may increase the stability of dextranase to slightly higher temperatures. The lowest activity occurred at ~65.5°C, because of partial denaturation of the dextranase enzyme. Dextranase activities were also low at 26.7-32.2°C, but were still better than at ~65.5°C. This has dramatic consequences for the factory application of dextranases. Most last evaporators, where many factories have been adding dextranase (Table 2) have syrup temperatures ~65.5°C, but the activity has dropped off dramatically at this temperature (Figure 2). Even for those factories that
activity has dropped off dramatically at this temperature (Figure 2). Even for those factories that are adding dextranase to juice (Table 2) where the ambient juice temperatures are ~26.7-32.2°C, the dextranase activity is still relatively low (Figure 2).

Table 2. Results of dextranase factory application survey conducted in 2002

<table>
<thead>
<tr>
<th>Factory</th>
<th>Dextranase used</th>
<th>Level added (ppm/juice or syrup)</th>
<th>Point of Factory Addition in 2002</th>
<th>Temp. °C</th>
<th>Resid. Time (min)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>&quot;Non-Concentrated&quot; 4</td>
<td>5-10</td>
<td>Last evaporator body</td>
<td>62.8</td>
<td>20</td>
<td>6.5</td>
</tr>
<tr>
<td>2.</td>
<td>&quot;Non-Concentrated&quot; 3</td>
<td>8</td>
<td>Low grade vacuum pan</td>
<td>65.5</td>
<td>not known</td>
<td>5.9</td>
</tr>
<tr>
<td>3.</td>
<td>&quot;Non-Concentrated&quot; 5</td>
<td>5-20</td>
<td>Juice incubator tank</td>
<td>65.5</td>
<td>12-14</td>
<td>5.0-5.4</td>
</tr>
<tr>
<td>4.</td>
<td>&quot;Non-Concentrated&quot; 3</td>
<td>10</td>
<td>3rd evaporator body</td>
<td>60</td>
<td>30</td>
<td>6.0</td>
</tr>
<tr>
<td>5.</td>
<td>&quot;Non-Concentrated&quot; 3</td>
<td>2-4 ppm/ton juice</td>
<td>Juice tank under cush-cush</td>
<td>29.4</td>
<td>10</td>
<td>5.4-5.6</td>
</tr>
<tr>
<td>6.</td>
<td>&quot;Non-Concentrated&quot; 3</td>
<td>2-4 ppm/ton</td>
<td>Between 3-4th evaporators</td>
<td>76.7-79.4</td>
<td>not known</td>
<td>6.4</td>
</tr>
<tr>
<td>7.</td>
<td>&quot;Non-Concentrated&quot; 5</td>
<td>5</td>
<td>3rd effect evaporator</td>
<td>60-65.5</td>
<td>not known</td>
<td>6.5</td>
</tr>
<tr>
<td>8.</td>
<td>&quot;Non-Concentrated&quot; enzyme 5</td>
<td>2</td>
<td>Syrup body</td>
<td>60</td>
<td>10-30</td>
<td>6.1-6.2</td>
</tr>
<tr>
<td>9.</td>
<td>NONE</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

*Two other factories also responded that they did not add dextranase*
Effect of °Brix on Dextranase Activity

Similar to the temperature effects (Figure 2), even though the "concentrated" enzyme was prediluted to the economic equivalence of the nearest priced "non-concentrated" dextranase, the activity was still much higher at different °Brixes, which further highlights the differential between economic price and activity. The activity of the dextranases was stable up to ~25-30°Brix, but afterwards decreased dramatically. This is because as the °Brix increases there is a depletion of water, i.e., the water activity decreases, which is the second substrate in the hydrolysis of dextran by dextranase. Another contributing factor could be the concentration of impurities which inhibit dextranases. As can be seen in Figure 3, the dextranase activity at °Brixes of evaporator syrups was very low. This, compounded with the high temperatures that are known to inhibit dextranase (Figure 2), strongly indicates that the last evaporator is not the best point to add dextranase in the U.S. Most certainly, dextranase cannot be added in massecuites where the °Brix is often ~80. In contrast, juices before clarification occur from ~12-15°Brix and would, therefore, not suffer such °Brix inhibitory affects on dextranases.

Dextranase Studies on Factory Syrups and Juices

Application of Dextranases to Last Evaporator Effect Syrup

Because of the lack of adequate residence times for the addition of juices in U.S. factories, dextranase has been added to the last evaporators, mostly because of the availability of up to 20 min calculated R (Eggleston and Monge, 2002). In raw sugar factories, solids are concentrated from approximately 15.0°Brix in juice to ~65.0°Brix in final evaporator syrup. As a consequence, the dextranase has to act upon ~4.5 times as much dextran in syrup than in juice.

For this reason, we added dextranase to syrups as ppm on solids (i.e., equivalent to 4.5 times as much as if it had been added on juice). Dextran removal was measured using a modified haze dextran method because of its ease of use and because it gives a reliable estimate of high MW dextrans which are responsible for the major processing difficulties associated with this polysaccharide (Inkerman, 1980). However, the haze method is a reflection of all haze forming material, and although proteins are precipitated with trichloroacetic acid and starch is removed with amylase, other polysaccharides such as indigenous cane polysaccharide could have contributed to the haze.

Therefore, although results are relative, they are most likely underestimations of dextran removal. Viscosity was monitored as well as % dextran removal, to ensure that processing efficiency was definitely improved upon by degrading dextran in syrup with dextranase. The effect of various concentrations of a "non-concentrated" dextranase to a final evaporator syrup containing dextran (7230 ppm/°Brix) at 63°C are shown in Figure 4. It can be seen in Figure 4a that 20 ppm/solids dextranase had very little effect on dextran degradation, with only 7.4% being removed after 20 min. Slight improvements occurred at 40 ppm (Figure 4a) with 15.8% dextran removal after 20 min. Dextran degradation with 80 ppm/solids dextranase was better, with 13.5 and 25.2% dextran removal after 15 and 20 min, respectively. However, "non-concentrated" dextranase at 80 ppm/solids is equivalent to 360 ppm/juice, and for such a limited breakdown in dextran at this high level, it is not economically viable to add "non-concentrated" dextranase to syrup. The effect of the "non-concentrated" dextranase on viscosity reduction in the syrup is illustrated in Figure 4b. At 0 ppm dextranase, the viscosity increased slightly across 20 min R., most likely because of
evaporation. No significant affect on viscosity was found at 20 ppm/solids. It was only at 40 and 80 ppm/solids addition of the “non-concentrated” dextranase, that viscosity reductions were found (Figure 4b). Similar small viscosity reductions of syrup in the presence of dextranase were found by Hidi and Staker (1975), as the viscosity of syrups is mostly caused by sucrose. These results agree with initial factory studies that we conducted where no differences were observed in viscosity reductions by adding 20 ppm/solids of another “non-concentrated” dextranase to a last evaporator body (65°C; \( R = 20 \text{ min} \)) over the control of 0 ppm/solids.

Compared to the “non-concentrated” dextranase (Figure 4), a much lower level (10 ppm/solids) of the “concentrated” dextranase was able to remove 37% of dextran after 20 min \( R \) (Figure 5a). As expected, the higher the level of “concentrated” dextranase applied, the more dextran removal occurred (Figure 5a), but at levels >10 ppm/solids the economics of the applications are not favorable. Furthermore, even at 10 ppm/solids, after 15 min \( R \), a significant reduction in viscosity was observed (Figure 5b). 10 ppm/solids is equivalent to 45 ppm/juice, which is much higher than the levels at which factories have been adding “non-concentrated” dextranases in Louisiana (Table 2).

Application of Dextranases to Juice

Current factory applications of dextranase to juice occur at ambient temperatures ~32.2°C (90°F), but the maximum activity of dextranases in juice was ~48.9°C (120°F; Figure 2). Consequently, we investigated the application of both the “non-concentrated” and “concentrated” dextranases that were previously studied on syrup, to juice (3380 ppm dextran/°Brix) at both 32.2 and 48.9°C. Rs up to 25 min were also studied because one Louisiana factory had expressed interest in building a large incubation tank with a such a \( R \). However, most applications of “non-concentrated” dextranases to juice occur in Louisiana with available \( R \) of only ~10 min or less (Table 3).

Current factory applications of “non-concentrated” dextranases to juices at 32.2°C have mostly occurred at 2-4 ppm/juice levels (Table 2). However, as can be seen in Figure 6a, at 4 ppm/juice and after 10 min \( R \), only 11.8% dextran had been removed. Increasing the dextranase level to 10 ppm/juice slightly improved dextran removal (Figure 6a), and even at 20 ppm/juice only 26.7% dextran was removed after 10 min. Markedly more dextran was removed from the juice by the “non-concentrated” dextranase when the reaction occurred at 48.9°C (Figure 6b). After 5 min at 4 ppm/juice and 48.9°C, ~19.6% dextran was removed compared to only 4.7% at 32.2°C (Figure 6a). After 5 min at 10 ppm/juice and 48.9°C, ~23.1% dextran was removed compared to 11.4% at 32.2°C, and so on (Figure 6). Results, therefore, indicate that heating the juice to the maximum dextranase activity temperature of 48.9°C, markedly improves the efficiency of application and, to some extent, overcomes the limited availability of \( R \) in many factories.

In comparison to the “non-concentrated” dextranase at 32.2°C (Figure 6a), the “concentrated” dextranase performed markedly better at this ambient juice temperature (Figure 7a). At just 4 ppm/juice and after 5 min \( R \), at 32.2°C, a remarkable 29.6% dextran was removed by the “concentrated” dextranase (Figure 7a) compared to only 4.7% for the “non-concentrated” dextranase under the same conditions (Figure 6a). Increasing the “concentrated” dextranase level to 8 ppm/juice markedly increased dextran removal to ~50% at 32.2°C and 5 min \( R \) (Fig. 7a). Little
further effect was obtained at >8 ppm/juice of the “concentrated” dextranase (Figure 7a), with most of the dextran had been broken down after 5 min by 10 ppm/juice.

Heating the juice and “concentrated” dextranase mixture to 48.9°C also caused a dramatic improvement (Figure 7b), and for this reason we could study the effects over a shortened Rₙ of 15 min. Even after 5 min at 4 ppm/juice and 48.9°C, most of the dextran measured by the haze method was removed (Figure 7b). “Concentrated” dextranase >4 ppm/juice at 48.9°C had very little further effect (Figure 7b) as most of the dextran substrate had been depleted and, therefore, 4 ppm/juice was an overdose because of asymptotic behavior under the stated conditions. Consequently, levels as low as 2 ppm/juice of the “concentrated” dextranase after only 5 min will be sufficient in juice at 48.9°C. Before addition at such a low levels, the “concentrated” dextranase should be first diluted with stabilizing sucrose solution to increase enzyme-substrate contact time. These results, therefore, strongly suggest that heating the juice to the max. dextranase activity temperature of 48.9°C, markedly improves the efficiency and economics of application and, to some extent, overcomes the limited availability of R, in many factories.

Addition of Dextranases to Juice in the Presence of Biocide
One question that arises about the application of dextranases at the higher temperature of 48.9°C is will such a temperature stimulate adverse microbial growth and possible dextran formation? It may be necessary to simultaneously add biocide and dextranase to the juice. For this reason we investigated the effect of dextranase in the presence of a biocide (dithiocarbamate) commonly used in U.S. factories at approximately 10 ppm/juice levels. Results shown in Figure 8 show that the “concentrated” dextranase still worked in the presence of the biocide, and 10 ppm levels of biocide may be slightly more favorable than 20 ppm additions. When severe dextran problems occur, an option in the factory could be to add 10 ppm/juice to the mill tandem and 10 ppm to mixed juice.

Storage Characteristics of “Non-Concentrated” and “Concentrated” Dextranases Under Simulated Factory Conditions
Dextranase vendors/distributors routinely recommend that barrels of dextranase should be stored in the coolest and shadiest area of the factory in order to prevent loss of activity at higher temperatures. To simulate such factory storage conditions we stored a “non-concentrated” and “concentrated”dextranase in a cool and dark corner of a laboratory (ambient temperatures range from 23-27°C), and analyzed the effect of storage time on the dextranase activity across the approximate length of a grinding season (~90 days). By the end of ~90 days, the activity of the “concentrated” dextranase had decreased only slightly (~9%; Figure 9). In dramatic contrast, the activity of the “non-concentrated” dextranase decreased dramatically across the storage time under these simulated factory conditions (Figure 9), and the activity was approximately half (~46%) of what it was at the beginning. This means that the level of application of the “non-concentrated” dextranase in the juice would have to be doubled in the late season if the same break down of dextran was to be achieved. This is of concern to Louisiana factories in particular, as freezes often occur that can cause severe dextran problems. The dramatic decrease in activity for the “non-concentrated” dextranase is most likely due to excessive dilution water de-activating/denaturing the enzyme protein structure, or by increasing conformational mobility of the protein.
As a control, the dextranases were also stored in a refrigerator (4°C; Figure 10). Whereas there was no change in activity for the “concentrated” dextranase stored under refrigeration, for the “non-concentrated” dextranase the activity still decreased significantly (Figure 10) but to a lesser extent than the simulated factory conditions conditions (Figure 9). There are no ideal or easy storage conditions for “non-concentrated” dextranase at the factory, and factories may want to consider not purchasing all their dextranase early in the season. Furthermore, this problem of the storage of “non-concentrated” dextranase highlights the need for the factory staff to be able to monitor the activity of their dextranase across the season.

Economic Costs of Different Dextranase Applications

The relative equivalent cost for the “concentrated” versus the “non-concentrated” was greatest in the syrup (Table 3), with $2.55 spent on the “non-concentrated” dextranase for every US dollar spent on the “concentrated” one. Irrespective of the dextranase concentration form used, much higher levels of dextranase had to be added to syrup compared to the juice at either 32.2°C or 48.9°C (Table 3). Cost calculations of dextranase juice addition after 5 min R, were undertaken because of the limited amount of R currently available in U.S. factories. At 32.2°C, a 4 ppm/juice level of the “concentrated” dextranase was approximately two-fold less expensive compared to the “non-concentrated” dextranase (Table 3). Although the difference in cost-effectiveness between the two concentration forms of dextranase apparently became much smaller at 48.9°C and 4 ppm/juice levels (Table 3), this is misleading. This is because at 4 ppm/juice the “concentrated” dextranase was overdosed (Figure 7b), with very little extra dextran substrate being removed at higher ppm levels or R.s. The “concentrated” dextranase can, therefore, be added at even lower levels, i.e. <4 ppm/juice, compared to the “non-concentrated” dextranase (Figure 6), which is more economical. Furthermore, these cost calculations of application do not take into account the extra freight cost of the “non-concentrated” dextranase which contains more water.

SUMMARY AND FURTHER DISCUSSION

In the U.S., most commercial dextranase is from a fungal (Chaetomium) source and is available in “non-concentrated” or “concentrated” forms. An approximate 8-10 fold difference in activity exists between the two forms, and activity variations exist among dextranases within each form. Currently, there is no uniform method to measure dextranase activity used by commercial dextranase producers/vendors/distributors, which has meant that direct comparison of activities is not possible. A simple titration method to determine the relative activity of dextranases has been identified and modified for easy factory use. This method will allow factory staff to compare commercial dextranases and make more informed decisions on which dextranase to use, as well as monitor the stability of the dextranases stored across the grinding season, as some have been shown to change. Under factory storage conditions over the typical length of a Louisiana grinding season, the activity of a “concentrated” dextranase decreased only slightly (~9%). In strong contrast, the activity of a “non-concentrated” dextranase had approximately halved (~46%) and even reduced in activity when stored under refrigeration.
Table 3. Cost-effective calculations for different dextranase applications.

"Concentrated" dextranase based on $18.36 per lb
"Non-concentrated" dextranase based on $6 per lb

Syrup (7230ppm/Brix) Application
63°C and 20 min R

<table>
<thead>
<tr>
<th>Dextranase</th>
<th>ppm/solids</th>
<th>Dextran Breakdown</th>
<th>Breakdown Ratio</th>
<th>Relative Equivalent Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Concentrated&quot;</td>
<td>20</td>
<td>57.3%</td>
<td>1</td>
<td>$1</td>
</tr>
<tr>
<td>&quot;Non-concentrated&quot;</td>
<td>20</td>
<td>7.4%</td>
<td>7.79</td>
<td>$2.55</td>
</tr>
</tbody>
</table>

20 ppm/solids is equivalent to 90ppm/juice

Juice (3380ppm/Brix) Application
32.2°C and 5 min Rt

<table>
<thead>
<tr>
<th>Dextranase</th>
<th>ppm/juice</th>
<th>Dextran Breakdown</th>
<th>Breakdown Ratio</th>
<th>Relative Equivalent Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Concentrated&quot;</td>
<td>4</td>
<td>29.6%</td>
<td>1</td>
<td>$1</td>
</tr>
<tr>
<td>&quot;Non-concentrated&quot;</td>
<td>4</td>
<td>4.7%</td>
<td>6.30</td>
<td>$2.06</td>
</tr>
</tbody>
</table>

Juice (3380ppm/Brix) Application
48.9°C and 5 min R

<table>
<thead>
<tr>
<th>Dextranase</th>
<th>ppm/juice</th>
<th>Dextran Breakdown</th>
<th>Breakdown Ratio</th>
<th>Relative Equivalent Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Concentrated&quot;</td>
<td>4</td>
<td>61.2%</td>
<td>1</td>
<td>$1*</td>
</tr>
<tr>
<td>&quot;Non-concentrated&quot;</td>
<td>4</td>
<td>19.6%</td>
<td>3.12</td>
<td>$1.02</td>
</tr>
</tbody>
</table>

The "concentrated" dextranase was overdosed at 4 ppm/juice because of an asymptotic response - see text for full details.

Overall, applications of dextranases to juice were much more efficient and economical than adding them to evaporator syrups, and application of "concentrated" dextranase was more economical than application of "non-concentrated". Some sugar technologists (DeStefano, 1988) have advocated dextranase addition to syrup rather than juice because it is "wasteful to treat contaminated material before its clarification". However, recent large factory studies (Eggleston et al, 2003) have shown...
that dextran removal is very dependent on the clarification system in use, and if no pre-heating of the juice occurs, as in cold liming, dextran can even form. Furthermore, Eggleston et al (2003) found much less dextran removal across factory clarification processes than DeStefano (1988) reported in three samples. Application of "non-concentrated" dextranases to final evaporator syrups is not economical and confirms initial factory studies. However, "concentrated" dextranase can be applied at levels as low as 10 ppm/solids (equivalent to 45 ppm/juice), and although this is higher than levels required for addition to juice, factory staff could consider adding it to both syrup and juice when severe dextran problems occur, such as after a severe freeze or storm. Heating the juice to 48.9°C, dramatically removed more dextran from a juice than at the current ambient temperature of application (32.2°C) and was more economical.

In practice at the factory, to bring the juice temperature up to 48.9°C (or at least in the optimum temperature range of 43.3-54.4°C), heated juice could be recirculated into small tanks with as little as 5 min Rt for the "concentrated" dextranase to work at <4 ppm/juice levels. Such tanks could include tanks which were previously used for cold liming. Accurate temperature control of the juice must be maintained to prevent inactivation of the dextranase. Leuconostoc and lactobacillus growth does not occur readily at or above a temperature of 50°C (Inkerman, 1980), therefore, the possible problem of simultaneous dextran formation and breakdown is expected to be limited. This is further evidenced by the formation of dextran being catalyzed by an exogenous enzyme dextranucrase, compared to the endogenous mechanism of dextranase to breakdown dextran. Moreover, dextranase was shown to work in the presence of biocide (dithiocarbamate) in juice. Nevertheless, factory studies are being planned to check that no adverse dextran formation is occurring at 48.9°C, as well as optimize conditions for factory conditions as extrapolation from the laboratory to the factory is not always simple.

ACKNOWLEDGEMENTS

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APPENDIX 1

Easy Measurement of Dextranase Activity at the Sugarcane or Sugarbeet Factory/Refinery

Modified (Anon, 2002b) Thiosulfate Titration Assay

**Principle:**
Dextranase hydrolyzes the α1,6 glucosidic linkages in dextran. Dextranase activity is assayed by letting it hydrolyze dextran and measuring the amount of reducing sugar formed. The reducing sugar is determined by the Hanes method. Equations for the reactions are as follows:

<table>
<thead>
<tr>
<th>Reaction End Color</th>
<th>Equations</th>
</tr>
</thead>
<tbody>
<tr>
<td>yellow</td>
<td>1. $K_3Fe(CN)_6 + \text{Reducing Sugar} \rightarrow Na_2CO_3 \rightarrow K_4Fe(CN)_6$</td>
</tr>
<tr>
<td>orange</td>
<td>2. $2K_3Fe(CN)_6 + 2KI \rightarrow \text{Acetic acid} \rightarrow 2K_4Fe(CN)_6 + I_2$</td>
</tr>
<tr>
<td>orange</td>
<td>3. $2K_4Fe(CN)_6 + 3ZnSO_4 \rightarrow K_2Zn[Fe(CN)_6]_2 \rightarrow 3K_2SO_4$</td>
</tr>
<tr>
<td>dark blue</td>
<td>4. $I_2 + \text{starch indicator} \rightarrow \text{starch-I}_2 \text{ complex}$</td>
</tr>
<tr>
<td>white</td>
<td>5. $\text{Starch-I}_2 \text{ complex} + 2NaS_2O_3 \rightarrow Na_2S_4O_6 + 2NaI$</td>
</tr>
</tbody>
</table>

**Reaction conditions:** Temperature - 37°C, pH - 5.8, Reaction time - 30 minutes

**Definition of Units**
1 unit of dextranase activity (DU) is defined as the amount of enzyme which degrades dextran to produce reducing sugar corresponding to the reducing power of one μM of sodium thiosulfate in one min at the conditions stated above.

**Simple Equipment Required:**
Water bath or constant temperature oven at 37°C
Metal pan for a boiling water bath
Burette (25 ml)

**Reagents:**

**Dextran Solution**
Weigh out the dry weight equivalent of 0.5 g Dextran T2000™ (Amersham) into a 100 ml volumetric flask. Make to mark with distilled or deionized water and dissolve. Boil if necessary to dissolve.

**Reagent A**
Separately, weigh out 8.25 g of potassium ferricyanide and 10.6 g of anhydrous sodium carbonate into weigh boats. Rinse both compounds into a 1 L volumetric flask with distilled or deionized water. Make up to the mark with distilled or deionized water. Pour the solution into a dark brown bottle and allow to stand in the dark for 2 or 3 days.

**Reagent B**
Separately, weigh out 25 g potassium iodide, 50 g zinc sulfate heptahydrate, and 250 g sodium chloride. Rinse all compounds into a 1L volumetric flask with distilled or deionized water. Make up to the mark with distilled or deionized water and allow the compounds to dissolve.
Reagent C
Put 50 ml of glacial acetic acid into a 1 L volumetric flask. Make up to the mark with distilled or deionized water.

1% Starch Indicator
Weigh out 1 g of soluble potato starch (Sigma) into a 100 ml volumetric flask. Rinse in with distilled water. Make up to about the 80 ml mark. Boil to dissolve then cool. Make up to the mark with distilled or deionized water.

0.1M Acetate Buffer, pH 5.8
Weigh out 13.5 g sodium acetate trihydrate into a boat. Wash this into a 1 L beaker with 900 ml millipore water. Adjust the pH to 5.8 with 1N acetic acid (approx. 11 ml). Transfer to a 1 L volumetric flask. Label and store at room temp. Replace after 4 weeks.

0.01N Sodium Thiosulfate
Accurately weigh out 1.241 g sodium thiosulfate pentahydrate into a boat. Rinse with distilled or deionized water into a 1 L volumetric flask. Make up to mark with distilled or deionized water.

Dilution of Dextranase Enzyme
The more active the enzyme is, the more dilution is required. Conversely, the less active an enzyme is, the less dilution is required. It is recommended that factories/refineries start at a dilution of 0.5g/L in de-ionized water:

Weigh out 0.5 g enzyme into a weigh boat. Rinse this into a 1 L volumetric flask with distilled water. Make up to the mark with distilled water.

For “concentrated” dextranases, a dilution of 0.065g/L de-ionized water is recommended.

Procedure

A. Pipette 10 ml of dextran solution into three test-tubes then pipette in 4 ml of 0.1M Acetate buffer, pH 5.8. Cover the test-tube in aluminum foil to prevent evaporation. Shake well. Label one test-tube as replicate (1), one test-tube as replicate (2), and the final test-tube as the control.

B. Add 1 ml of distilled water to the control test-tube. Add 1 ml of the dilute enzyme solution to test-tube (1) and (2). Shake well. Put in a water bath at constant temperature of 37°C and allow to incubate for 30 min.

C. Label three stoppered conical flasks the same as the three test-tubes.

D. Into each of the three stoppered conical flasks, pipette 5 ml of Reagent A and 3 ml of distilled or deionized water.

E. After 30 min of incubation, remove the test-tubes from the incubator/water bath. Pipette 2 ml of the incubated test-tube solution into the correct stoppered conical flasks and solutions. Close the conical flasks with their stoppers, shake, then place them in a boiling water bath for 15 min. Allow to cool.
F. To the stoppered conical flasks pipette in 5 ml of Reagent B first, then and 3 ml of Reagent C. Add 5 drops of the soluble starch solution. The solutions will turn dark blue after mixing. This is solution X.

G. 0.01N sodium thiosulfate has already been put into a burette. Titrate sodium thiosulfate into solution X until the blue color of the starch-iodine complex completely disappears. Make sure to swirl. Accurately write down the ml of sodium thiosulfate that were needed for the titration. Take the average ml of replicates (1) and (2).

Calculation of Dextranase Activity:

Units/ml = (C - T) x 0.01 x 1000 x 1/30 x 15/2 x N

C = Titration ml of the control
T = Titration ml of the enzyme
N = Dilution multiple of enzyme
Fig. 1. Use of ion chromatography profiles to accurately determine relative dextranase activities

* At the time of the experiment the "conc" dextranase cost 4.6X more than the "non-conc" dextranase. Since the experiment the difference is only 3.1X.

Fig. 2. Effect of temperature on dextranase activity to breakdown (a) dextran T2000™ and (b) cane juice dextran
Fig. 3. Effect of °Brix on dextranase activity

Fig. 4. Effect of various levels of “non-concentrated” dextranase in a final evaporator syrup (7230ppm/°Brix Dextran) on (a) the removal of dextran and (b) reduction in viscosity. (Note: ppm/solids is equivalent to 4.5 x ppm/juice).
Fig. 5. Effect of various levels of “concentrated” dextranase in a final evaporator syrup (7230 ppm/Brix Dextran) on (a) the removal of dextran and (b) reduction in viscosity. (Note: ppm/solids is equivalent to 4.5 x ppm/juice).

Fig. 6. Effect of various levels of “non-Concentrated” dextranase on removal of dextran from cane Juice (3380 ppm/Brix). Application = ppm on juice.
Fig. 7. Effect of various levels of "concentrated" dextranase on removal of dextran from cane juice (3380 ppm\(^\circ\)Brix). Application = ppm on juice.

Fig. 8. Effect of Biocide (Carbamate) on dextranase action. (10 ppm dextranase on cane juice at 48.9\(^\circ\)C (120\(^\circ\)F) and pH 5.4).
Fig. 9. Changes in activity of dextranases under simulated factory storage conditions. Stored at room temperature (~23-27°C) in shaded place over a 90 day grinding season.

Fig. 10. Changes in activity of dextranases stored under refrigeration (4°C) over a grinding season period. Diamonds represent the "concentrated" dextranase, and triangles represent the "non-concentrated" dextranase.