Factory trials to optimize the application of dextranase in raw sugar manufacture: Part II

By **Gillian Eggleston[1*], Adrian Monge[2], Belisario Montes[3] and David Stewart[3]**

[1*] Contact author: SRRC-USDA-ARS, New Orleans, LA 70124, U.S.A.
Tel: + 1 504 286 4446 Fax: + 1 504 286 4367 Email: gillian@srrc.ars.usda.gov

[2] Cora Texas Manufacturing Co., White Castle, LA 70788, U.S.A.

[3] Alma Factory Plantation LLC, Lakeland, LA 70752, U.S.A.

Abstract

Application of commercial dextranase to hydrolyze dextran in U.S. sugar manufacture is still not optimized, partly because of confusion about which enzyme to use, and how and where to add the enzyme. This Part II of a 2004 dextranase study reports optimization trials at a factory that applied dextranase to a 17 min retention time incubation tank. Working solutions of "concentrated" dextranase in water were required to improve contact between the enzyme and substrate (dextranase/dextran) and are more cost-effective than adding "non-concentrated" dextranase undiluted. The factory had relatively low levels of antibody dextran (<300 ppm/°Brix) compared to those at the factory reported in Part I of this study (Eggleston *et al,* 2006). The application of dextranases was more problematic because of lower contact between dextranase and dextran. Addition of a 5-fold working solution (5 ppm; normalized to the original enzyme activity) of "concentrated" dextranase (50,500 DU/ml) hydrolyzed ~43% antibody dextran. Filtrate juice from the mud tank contained dextran often at higher levels than in juice where dextranase was applied. Factories should ensure filtrate is recirculated back to the juice before or where they apply dextranase, to maximize the use of dextranase. Differences in dextran hydrolysis were compared using both antibody and Haze methods: The Haze method underestimated hydrolysis compared to the antibody method. As dextranase application did not always alleviate all processing problems in factory boiling houses, because lower molecular weight dextrans and other major *Leuconostoc mesenteroides* deterioration products such as mannitol still exist which detrimentally affect processing, it can only be regarded as a secondary tool. It does not replace the primary tool of prevention of dextran formation by good cane management in the field, factory yard and mills.

Pruebas de fábrica para optimizar la aplicación de la dextranasa en la fabricación de azúcar crudo: Parte II

La aplicación de dextranasas comerciales para hidrolizar la dextrana en la fabricación de azúcar en los Estados Unidos aún no ha sido optimizada. Esto se debe, en parte, a que todavía existe incertidumbre acerca de cuál enzima utilizar y también acerca de cómo y cuándo agregar la enzima. En esta Parte II del estudio inicial sobre dextranasas que se llevó a cabo en 2004, se describen los ensayos de optimización realizados en una fábrica durante los cuales se aplicó dextranasa a un tanque de incubación con un mínimo tiempo de retención 17 minutos. Soluciones de trabajo de dextrana "concentrada" en agua fueron necesarias para mejorar el contacto entre la enzima y el substrato (dextranasa/dextrana) y resultaron más económicas que cuando se utilizó la adición de soluciones no diluidas de dextranasa "no concentrada". La fábrica poseía niveles de anticuerpos de dextrana relativamente bajos (<300 ppm/oBrix) comparado con aquellos descriptos para la fábrica en la Parte I de este estudio (Eggleston et al., 2006). La aplicación de la dextranasa fue más problemática debido al bajo contacto entre la dextranasa y la dextrana. La adición de una solución de trabajo cinco veces mayor (5 ppm; normalizada respecto a la actividad enzimática original) de dextranasa "concentrada" (50,500 DU/ml) hidrolizó aproximadamente un 43% de anticuerpos de dextrana. El filtrado de jugo del tanque de lodo contenía dextranas en un nivel frecuentemente más alto que en el jugo donde se había aplicado la dextranasa.

Werksversuche zur Optimierung der Anwendung von Dextranase bei der Rohzuckerherstellung: Teil II

Handelsübliche Dextranase wird in der US-amerikanischen Zuckerproduktion noch nicht optimal zur Hydrolisierung von Dextran eingesetzt – zum Teil aufgrund von Unsicherheit darüber, welches Enzym verwendet und wie und wo es hinzugefügt werden sollte. Dieser zweite Teil einer Dextranasestudie aus dem Jahr 2004 berichtet von Optimierungsversuchen in einer Fabrik, bei denen die Dextranase einem Inkubationstank mit 17-minütiger Verweilzeit hinzugegeben wurde. Arbeitslösungen von in Wasser aufgelöster „konzentrierter" Dextranase waren nötig, um den Kontakt zwischen Enzym und Substrat (Dextranase/Dextran) zu verbessern, und sind kosteneffektiver als die unverdünnte Beigabe einer „nicht-konzentrierten" Dextranase. Verglichen mit denen des Werks, über das im ersten Teil dieser Studie berichtet wurde (Eggleston et al., 2006), hatte die Fabrik relativ niedrige Anteile von Dextran-Antikörpern (<300 ppm/oBrix). Der Einsatz der Dextranasen war problematischer aufgrund des geringeren Kontakts zwischen Dextranase und Dextran. Die Beigabe einer fünffachen Arbeitslösung (5 ppm; normalisiert zur ursprünglichen Enzymaktivität) von „konzentrierter" Dextranase (50,500 DU/ml) hydrolisierte ~43 % der Dextran-Antikörper. Das Saftfiltrat aus dem Schlammbecken enthielt oft eine höhere Dextrankonzentration als der Saft, dem Dextranase beigegeben worden war. Fabriken sollten zur Maximierung der Dextranaseverwendung dafür sorgen, dass das Filtrat in den Saft zurückgeführt wird, bevor oder wo sie Dextranase

La fábrica debería asegurarse que el filtrado es recirculado de vuelta al jugo antes o donde se aplica la dextranasa para maximizar el uso de la misma. Se compararon las diferencias en la hidrólisis de la dextrana utilizando los métodos Haze y con anticuerpos: el método Haze subestimó la hidrólisis comparado con el método que utiliza anticuerpos. Teniendo en cuenta que la aplicación de dextranasa no siempre mitigó todos los problemas del procesamiento en las estaciones de calderas de la fábrica, debido a la persistencia de dextranas de peso molecular más bajo y la presencia de importantes productos de la deterioración de Leuconostoc mesenteroides, tales como el manitol, que afectan perjudicialmente el procesamiento, se concluye que esta herramienta sólo pueda ser considerada como de valor secundario. No puede reemplazar la herramienta principal para prevenir la formación de dextrana representada por el buen manejo de la caña tanto en el campo, como en el área de recepción de la caña, y en la fábrica.

beigeben. Unterschiede bei der Dextran-Hydrolyse wurden sowohl mittels der Antikörper- als auch der Haze-Methode verglichen: Im Vergleich zur Antikörpermethode unterschätzte die Haze-Methode die Hydrolyse. Da die Dextranase-Beigabe nicht immer alle Verarbeitungsprobleme in Fabrikskesselhäusern milderte, weil weiterhin Dextrane niedrigeren molekularen Gewichts und andere wichtige Leuconostoc mesenteroides Verschlechterungsprodukte wie beispielsweise Mannitol existieren und die Verarbeitung nachteilig beeinflussen, kann sie nur als ein sekundäres Mittel angesehen werden. Sie ersetzt nicht das primäre Mittel der Verhütung der Dextranbildung durch gutes Zuckerrohrmanagement auf dem Feld, in den Fabrikshöfen und -gebäuden.

Introduction

Dextran polysaccharides (α-(1→6)-α-D-glucans) are products of the deterioration of sugarcane, mainly by *Leuconostoc mesenteroides*, that deleteriously affect raw sugar processing. Dextrans are polydisperse by nature, i.e., they exist as a wide range of molecular weights. Dextrans isolated from sugarcane products possess a largely linear structure (Khalikova *et al*, 2005) composed of ~95% glucose units linked by (1→6) glycosidic bonds, but also containing ~5 % branching through (1→4), (1→3) and some (1→2) linkages (Fig. 1a). Dextranase (1→6 α-glucan hydrolases; EC 3.2.1.11) is applied in raw sugar factories to hydrolyze dextran mostly to improve boiling house operations. Dextranases catalyze the endohydrolysis of the α-(1→6)-D linkages in random sites of dextran (Khalikova *et al*, 2005). The hydrolysis of dextran by dextranase is not by an "all or nothing" mechanism. Instead, there is a gradual decrease in the average molecular weight of the various dextran fragments produced from the original high molecular weight dextran, and these fragments in turn are continuously hydrolyzed (Fig. 1b).

Although dextranase application was pioneered by Australians in the 1970s (see Inkerman, 1980), its application in U.S. sugar manufacture is still not optimized (Eggleston *et al*, 2005). As a consequence, at the request of the Louisiana (LA) raw sugar manufacturing industry, optimization studies were undertaken. First, a comprehensive laboratory study on the effect of dextranases on cane juices and syrups was completed (Eggleston and Monge, 2004, 2005). Second, factory trials on dextranase optimization were undertaken at two factories across the 2004 LA processing season. Part I of the factory trial study (Eggleston *et al*, 2006) occurred at a factory that had relatively high dextran levels (>1000 ppm/°Brix antibody dextran). This Part II of the study occurred at another LA factory where lower levels (<300 ppm/ °Brix antibody dextran) of dextran frequently occurred

The efficiency of dextranases in the factory depends on the pH, °Brix, temperature, retention time, agitation, and type, activity, and dosage of the dextranase applied. In Louisiana factories, there is often insufficient retention time, and wide variation in activity of available commercial dextranases has caused major confusion in how and where to apply them. In the initial laboratory study (Eggleston and Monge, 2005), it was observed that most commercial dextranases in the U.S. are from *Chaetomium gracile* or *erraticum* and generally recognized as safe (GRAS). They are available in "non-concentrated" (<25,000 DU/ml as measured by the factory titration method of Eggleston [2004] but usually occur at activities <6,000 DU/ml) or "concentrated" (25,000-58,000 DU/ml but often between 48,000-58,000 DU/ml) forms. Eggleston and Monge (2005) reported that in 2003 an approximate 8-10 fold difference in activity existed between the two forms, but by

Figure 1a. Basic chemical structure of dextran. Glucose molecules are linked by (1→6)-D-glycoside bonds. Approximately 5% branching occurs in cane dextrans, mostly through (1→3) and (1→4)-D-glycoside linkages. (1→3)-D-glycoside branching is shown in the figure

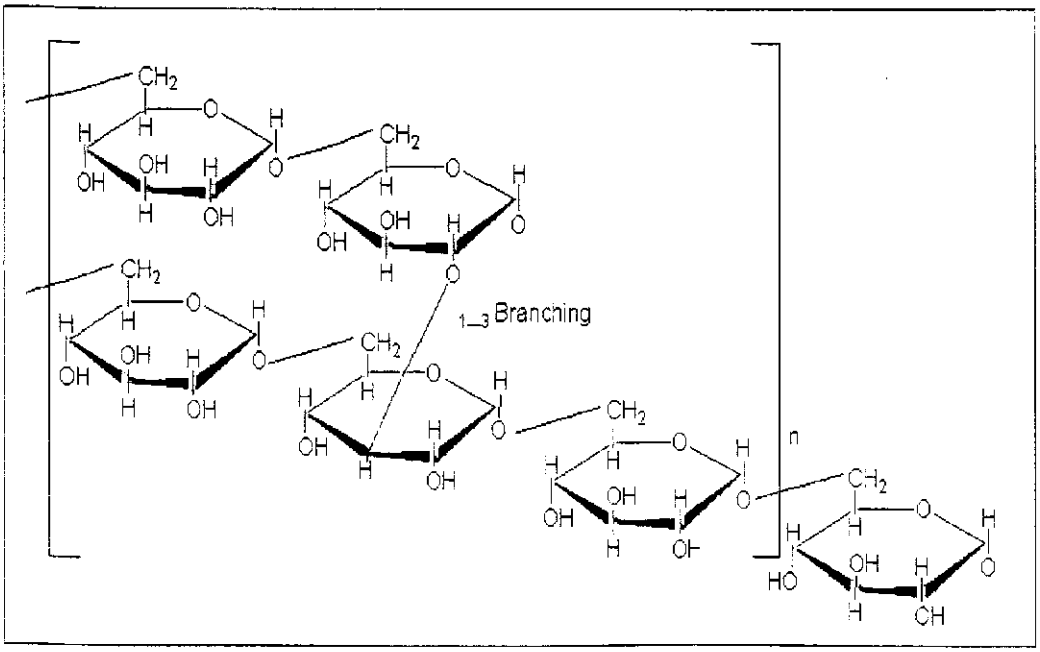
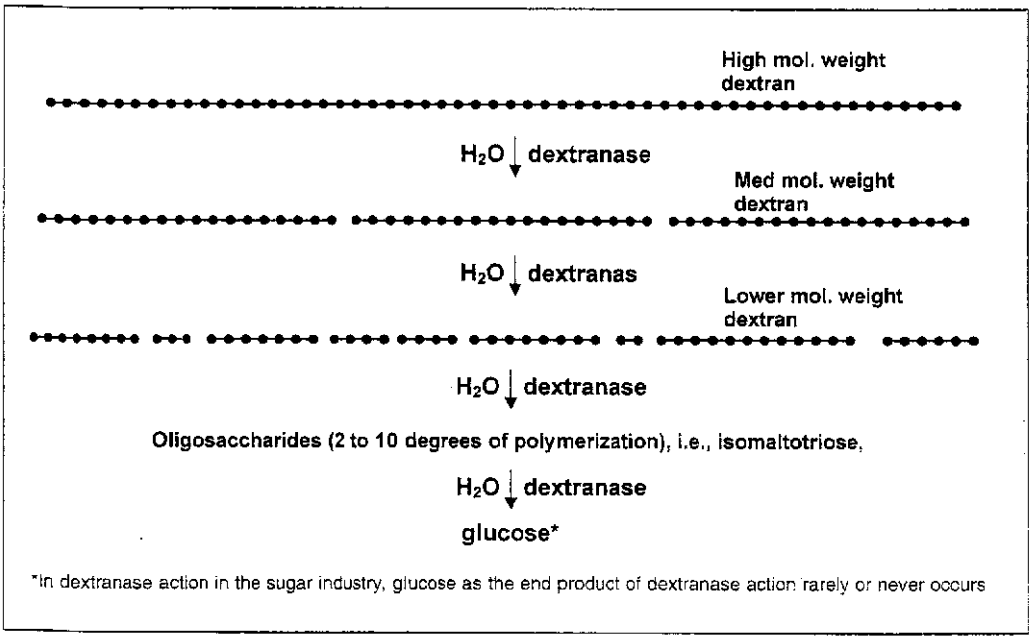


Figure 1b. Mode of endohydrolysis action of dextranase on (1→6)-D-glycoside linkages in random sites of high molecular weight dextran. Dots and connecting lines represent *chains* of glucose molecules linked by (1→6) glycosidic bonds in the dextran molecule. A small amount of (1→3) and (1→4) linkages exist in dextran but these are not hydrolyzed by the dextranase enzyme. Reaction time is not represented. Dextran molecular weight values are from Hidi and Staker (1975)



2004 this had changed to a 14-20 fold difference between the two forms (Eggleston *et al.* 2006). Factory storage characteristics of the two dextranase forms vary widely (Eggleston and Monge. 2005), with the "non-concentrated" form losing its activity much faster because there is extra water to unravel the protein conformation structure. Both "non-concentrated" and "concentrated" dextranases at juice pH of 5.4-5.8, showed similar maximum activity at ~120 °F (50 °C), and activities decreased after 25-30 °Brix (Eggleston and Monge, 2004). Up until 2004 only "non-concentrated" dextranases were applied in LA sugarcane factories to either last evaporator syrup or juice.

Although the initial laboratory study (Eggleston and Monge, 2005) gave a useful indication of the necessary dosages and conditions of adding dextranase to cane juice, results were gained under ideal conditions compared to those at factories, where dextranase is added to much larger volumes of juice in tanks and pipes with fluctuating flow rates and agitation. The laboratory study did, however, give a solid foundation from which to start the factory study. As it was unequivocally shown (Eggleston and Monge, 2005) that juice application was more efficient and economical than application to syrup, the factory trials reported in this paper were not undertaken on evaporator syrups, but on juices.

Materials and methods

Dextranase activity

The modified titration method of Eggleston (2004) was used to measure the activity of dextranases. One dextranase unit (DU/ml) is the amount of dextranase that hydrolyzes dextran T2000™ (2000 KDa) to produce reducing sugar corresponding to the reducing power of one micromole of sodium thiosulfate in one min at 99°F (37 °C) and pH 5.8. Averages of duplicates are reported.

Haze method for dextran

Haze dextran in juices was based on the modified method of Eggleston and Monge (2005). Termamyl™ (Novo, U.S.) α-amylase enzyme was added to hydrolyze interfering starch. Dextran T2000™ was the standard, and dextran was precipitated with 100 % absolute ethanol.

Monoclonal antibody method for dextran

The Rapid Dextran Test or SucroTest™ (Midland, U.S.) was used (Rauh *et al*, 1999; Anon, 2003). A conversion factor was calculated for each batch of antibody used. For massecuites, the samples were first diluted to ~14 °Brix before analysis.

°Brix

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

Mannitol

The rapid enzymatic method of Eggleston and Harper (2005) was used. Massecuite samples were measured in duplicate.

Ion chomatography with Integrated Pulsed Amperometric Detection (IC-IPAD) profiles

See Eggleston and Grisham (2003) for full method. Ion chromatograms of massecuite samples were analyzed. In order to directly compare chromatograms, samples were °Brix adjusted to 13.34 °Brix by adding de-ionized water, diluted 1 g/25 ml, and filtered through a 0.45 µm filter.

General factory sampling

Juices before and after the application of dextranase in the factories, were carefully stored to prevent further degradation reactions and/or microbial growth. Each sample was collected in a container (250 ml) containing 5 drops of biocide (Bussan 881™, Buckman Labs., U.S.). The juices were immediately placed in dry ice before transportation to the Southern Regional Research Center laboratory in New Orleans, LA, then stored in a -80 °C laboratory freezer subsequent to analyses.

Factory conditions

The factory applied dextranase to an incubator tank that had its capacity increased from 10 to 17 min retention time to improve application. Of the juice entering the tank, 20% was filtrate juice (~ 150 °F or 66 °C) from the mud filter station. The filtrate juice increased the tank juice pH from ~ 5.40 to 5.54. Flow rate was 580 short tons/h and 2300 gallons/min. 100% billeted cane was processed, of which 2% was burnt. Dextranase was added using a diaphragm pump (Model B111-925, Milton Roy, U.S.) of 38 gallons per day capacity.

Factory sampling

Factory trials: Initial application of "non-concentrated" and "concentrated" dextranases

The factory applied a variety of "non-concentrated" and "concentrated" dextranases from 15 Oct. to 1 Nov. 2004. From 15-18 Oct., samples were collected of mixed juice entering and exiting the tank taking into account the 17 min retention time. Composite sample collection occurred every min over a 15 min period. From 22 Oct. – 1 Nov., filtrate juice entering the tank was also collected as a composite. A "concentrated" dextranase (50,500 DU/ml) was applied as a 5-fold (1:4) diluted working solution prepared in distilled water, and added at a dosage of 2 ppm (normalized to the originally enzyme activity) on volume of juice. Dextran content in the total juice entering the tank was calculated as 80 % juice dextran + 20 % filtrate dextran.

Factory trial on the effect of applying a working solution of "concentrated" dextranase

This trial occurred on 23 Nov. 2004. A working solution of "concentrated" dextranase (50,500 DU/ml) was first prepared by diluting 5-fold in distilled water, and applied at a dosage of 5 ppm (normalized to the originally enzyme activity) to the tank. The retention time of the tank was reduced from 17 to 10 min because the factory switched to intermediate liming to repair the hot lime system. Samples were collected of mixed and filtrate juice entering and exiting the tank taking into account the 10 min retention time. Sample collection was repeated seven times every 10 min. Juice temperature was ~ 125 °F (52 °C).

Massecuite collection

Random samples of massecuites produced from dextranase treated juice were collected.

Results and discussion

Early factory trials: Application of "non-concentrated" and "concentrated" dextranases and the effect of filtrate juice

The factory in this study is the only one in Louisiana to have an incubator tank. It was installed to allow natural cane diastase to hydrolyze starch in juice; the tank is also used for dextranase applications. Australian factories add dextranase in similar holding tanks with minimum retention times of 15 min (Inkerman, 1980). In recent years, however, staff at the factory have questioned the efficiency and economics of dextranase applications, and decided to improve the application of dextranase by increasing the capacity of the incubation tank from 10 to 17 min retention time. Like the factory in Part I of this study (Eggleston *et al*, 2006), this factory had traditionally applied "non-concentrated" dextranases. However, unlike the factory staff Part I of this study who use a monoclonal antibody method to measure dextran, this factory used a Rapid Haze method based on spectrophometry, which measures the formation of turbid "Haze" after absolute ethanol is added. Fig. 2 shows typical dextran hydrolysis results obtained by the factory after applying various "non-concentrated" and "concentrated" dextranases (activities ranged from 2,750 – 50,500 DU/ml) in the early 2004 season. The initial application of 5-10 ppm (Fig. 2) of "non-concentrated" dextranase A (3,500 DU/ml) gave disappointing dextran hydrolysis (11 to 13%). Applying 17.5 ppm of another "non-concentrated" dextranase B (3,000 DU/ml) only improved hydrolysis to 26% (Fig. 2). These results are similar to previous laboratory studies (Eggleston and Monge, 2005) where 4 ppm of a "non-concentrated" dextranase (5,999 DU/ml) applied to cane juice, only hydrolyzed 12% Haze dextran after 10 min at 90 °F (32°C), and increased to only ~27% at 20 ppm. These disappointing results were further investigated. Not only was Haze dextran measured in the juice entering the tank, but also in the filtrate juice added to the same tank on the opposite side. Filtrate juice (recirculated from the bottom of the mud tank) contained dextran usually at levels higher than the juice entering the tank. Therefore, the initial concentration of dextran in the tank was being under-measured. Concomitantly, the hydrolysis of dextran by dextranase was being under-estimated. This is an important result for factories. Factory staff should ensure that

Figure 2. Factory measurements of dextran hydrolysis at the factory in October and early November, 2004 using various "non-concentrated" and "concentrated" dextranases. Conditions: ~105-130 °F (40.6-54.4 °C); composite samples. Factory ppm additions are noted directly on the graph. *The dextran content in filtrate recycled into the tank was used in the calculations

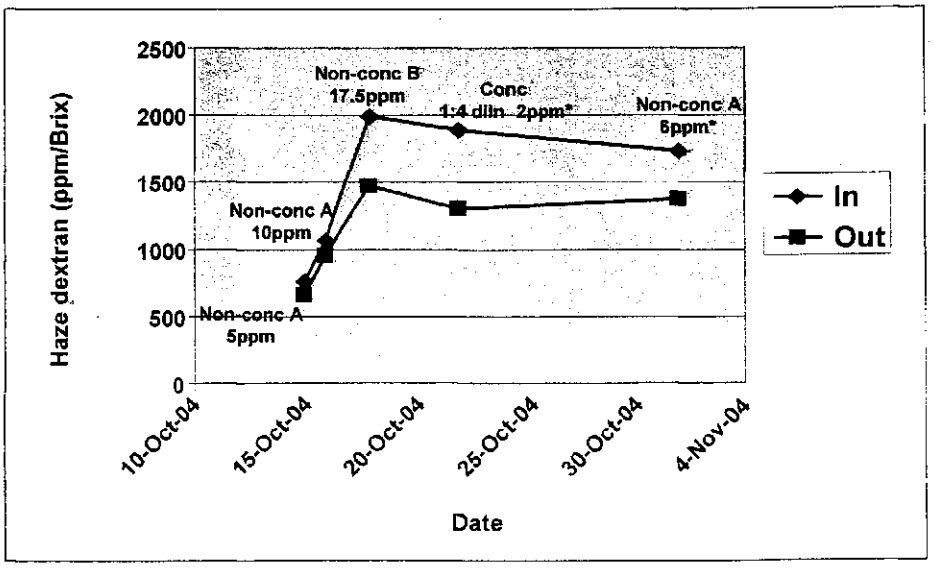


Figure 3a. Measurement of the dextran hydrolysis at the factory on 23 Nov, 2004 by "concentrated" dextranase (50,500 DU/ml) using Haze dextran measurements. Conditions: 5 ppm; 5-fold working solution; 125 °F (52.0 °C); R_t=12 min

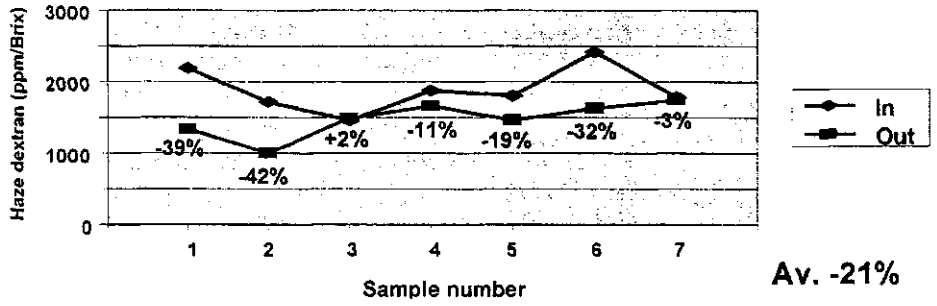
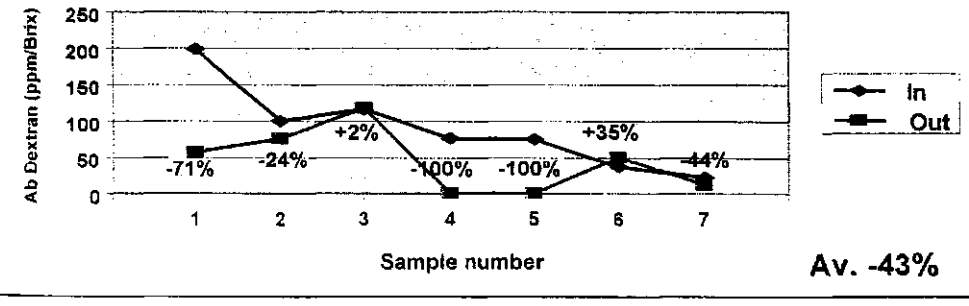


Figure 3b. Measurement of dextran hydrolysis at the factory 2 on 23 Nov, 2004 by "concentrated" dextranase (50,500 DU/ml) using antibody dextran measurements. Same conditions as Fig. 3a.



filtrate is recirculated back to the juice before or where they add dextranase, to maximize the use of the enzyme. As 20% of the total juice entering the tank was filtrate juice, this was subsequently taken into account in future calculations of dextran hydrolysis by dextranase across the tank (Fig. 2).

The factory staff also tried to apply 2 ppm of a 5-fold working solution (dosage normalized to the original enzyme activity) of "concentrated" dextranase (50,500 DU/ml), as previous laboratory studies (Eggleston and Monge, 2005) indicated that this may be a viable dosage to apply to cane juice, especially if the temperature was optimum ~120 °F (50 °C). As seen in Fig. 2, the low 2 ppm dosage gave a slightly higher dextran hydrolysis at 31%. This, and the subsequent addition of 6 ppm of "non-concentrated" dextranase A (Fig. 2), are most likely attributed to the accounting for dextran in filtrate juice. For all these initial applications of dextranase in this factory, dextran reductions caused very little changes in factory performance, including boiling house operation.

Comparison of the use of antibody or Haze dextran methods to measure the efficiency of "concentrated" dextranase to hydrolyze dextran

A trial was undertaken on 23 Nov. to evaluate the application of a 5-fold working solution of a "concentrated" dextranase (50,500 DU/ml) to the incubator tank. On the trial day the factory had to change its clarification process from hot to intermediate liming, and the third tank of the incubation tank complex was needed for lime addition, which reduced the retention time from 17 to 10 min for the trial. Tank juice temperature was maintained at ~125 °F (52 °C), slightly above the optimum temperature of 120 °F (50 °C) (Eggleston and Monge, 2005). Dextran hydrolysis results, determined by the Haze method, are shown in Fig. 3a. Hydrolysis of Haze dextran ranged from +2 to -42 %, with an average of only -21% (Fig. 3a). This was investigated further, by measuring dextran hydrolysis in the same samples using the antibody method, which is more specific for the dextran polysaccharide (see Fig. 3b). In general, % antibody dextran hydrolysis was considerably higher than when measured with Haze dextran, with an av. hydrolysis of 43% (Fig. 3b). This indicated that the working solution of the "concentrated" dextranase was performing better than first considered. Returning the retention time back to 17 min would most likely further improve dextranase hydrolysis, as more time would be provided for dextranase action. The discrepancy in dextran measurements between the antibody and Haze methods is explained in Fig. 4. Antibody dextran measures high molecular weight (HMW) dextran (D. Day, personal communication) that contributes mostly to viscosity problems in the boiler house (Hidi and Staker, 1975). In comparison, Haze dextran measures high (>1000 KDa) and medium (~100-100 KDa) MW dextran, medium MW starch (if a-amylase is applied to hydrolyze the high MW starch), indigenous cane polysaccharide (Blake and Clarke, 1984), and other compounds that create "Haze" such as bagasse microparticles. If trichloracetic acid is not used in the Haze method, then protein will also be present that can contribute to Haze formation. Furthermore, *L. mesenteroides* is able to form other, minor microbial polysaccharides including levan and alternan (Eggleston *et al*, 2004), and if other types of microbial deterioration have occurred then other microbial polysaccharides may be present that can contribute to the Haze. These reasons also explain why there is always a background Haze measurement in fresh cane of approximately 500-700 ppm/°Brix. There was no significant correlation between juice dextran measured by the two methods, which further suggests that chemical parameters other than dextran most likely interfere in one or both methods.

The levels of antibody dextran in juice measured at this factory were consistently much lower than those measured at the factory in Part I of this study (compare Fig. 3b with Figs. 1 and 4 in Eggleston *et al*, 2006). It was unequivocally shown in Part I of this study that it is more difficult to hydrolyze dextran by dextranase when low dextran levels are present because of low contact between the enzyme and substrate. The 5-fold working solution may, therefore, not be of substantial volume for the relatively low levels of dextran and higher dilutions may be required. The larger tank capacity may also have mitigated contact. Improved agitation using serpentine pipes will improve the contact between the dextran and substrate (as well as increase retention time in a relatively small space),

Figure 4. Differences in the measurement of categories of molecules by the antibody and Haze dextran methods. Dextran molecular weight values are from Hidi and Staker (1975)

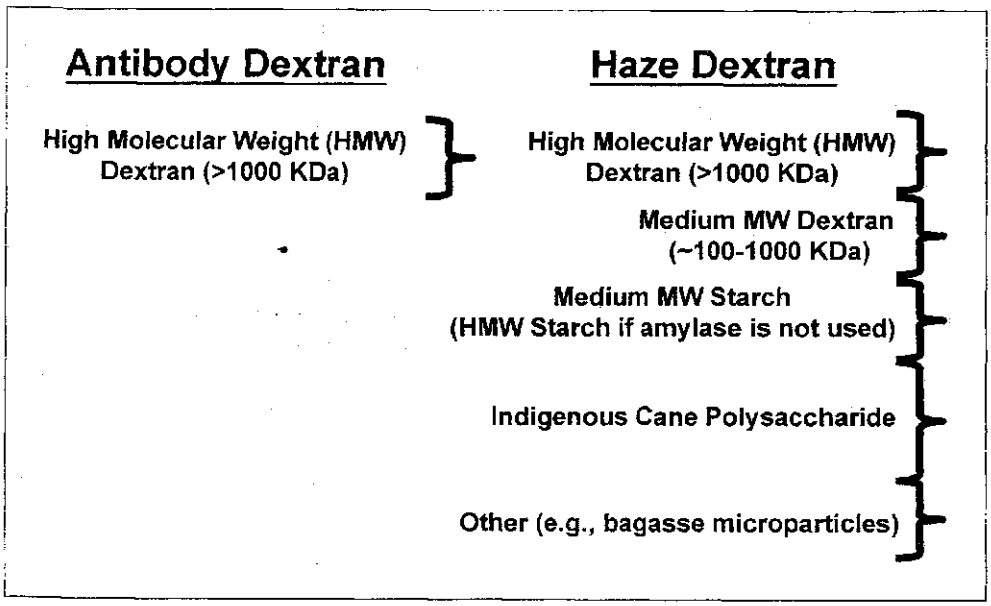


Figure 5. IC-Chromatogram of normal and mixed crystals in massecuites from the factory

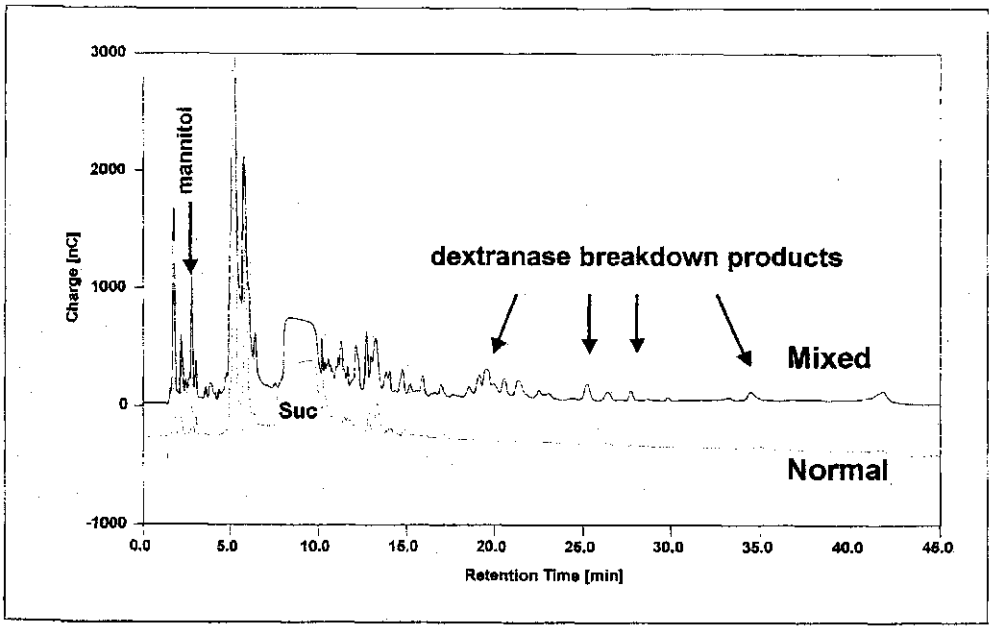


Table 1. Average dextran and mannitol in factory massecuites

Crystal Shapes	Av. Antibody Dextran (ppm/Brix)	Av. Mannitol (ppm/Brix)
Normal	327	1240
Mixed[a]	201	7365

[a] Contained normal and elongated sucrose crystals

and the factory intends to undertake future studies on this.

The impact of dextranase on the crystallization of massecuites

Factory staff noted that although ~30-45% antibody dextran was hydrolyzed by application of dextranases (particularly working solutions of "concentrated" dextranase), molasses purities were alleviated, viscosity of boiling house syrups reduced (allowing boiling to occur in less elevated time), and the dextran content of raw sugar slightly lowered, that elongated crystals were still observed. When dextran occurred at the factory, sometimes the massecuite crystals contained a majority of elongated crystals, and sometimes crystals of mixed shapes, which included elongated crystals. This is further highlighted by the comparative analyses of example massecuite samples that contained either mixed or normal shaped crystals, shown in Table 1. The normal massecuite contained a higher amount (327 ppm/°Brix) of antibody dextran than the mixed massecuite (201 ppm/°Brix). HMW dextran, such as measured by the antibody method, has been recognized not to be responsible for crystal elongation (Hidi and Staker, 1975), although it does slow crystal growth. However, the mixed massecuite contained approximately 6-fold more mannitol than the massecuite with normal crystals (Table 1). Mannitol is a major deterioration product of *L. mesenteroides* deterioration of sugarcane (Eggleston *et al*, 2004; Eggleston and Harper, 2006) and its presence at such concentrations in the mixed massecuite, indicates that the massecuite was at least partially formed from deteriorated cane containing other deterioration products that could affect crystal shape.

We further investigated this phenomenon by analyzing the normal and mixed massecuites with ion chromatography (IC) with a 45 min NaOH/NaOAc gradient profile. As seen in Fig. 5, IC confirmed the markedly higher mannitol levels measured by an enzymatic method (Table 1) in the mixed massecuites than the normal massecuites. Moreover, compounds of 3 to 12 degrees of polymerization were also present in the mixed massecuite IC chromatograms (Fig. 5), which are most likely dextran hydrolysis products (Eggleston and Monge, 2005), and indicate that the sample contained a very wide range of intermediate MW dextran hyrdolysis products. Although high MW dextrans (≥ 1000 KDa) have little effect on elongation, dextrans as low as 40 KDa have been reported (Hidi and Staker, 1975) to be strong inhibitors of crystal

growth along the B-axis, thus causing elongation across the C-axis. Furthermore, partially hydrolyzed HMW dextran does not reduce viscosity significantly but is a powerful inhibitor of crystallization (Hidi and Staker, 1975). Thus, although dextranase can alleviate viscosity related problems from dextran in the factory, particularly the boiling house, it cannot alleviate all dextran related processing problems, especially crystal elongation. Furthermore, dextranase cannot hydrolyze or transform mannitol or other deterioration products also formed by *L. mesenteroides* which are known to detrimentally affect processing (Eggleston *et al*, 2004). For these reasons, plus the relative high expensive use of such a processing aid, the use of commercial dextranases to manage dextran in sugarcane processing should only be viewed as a secondary tool to manage dextran. It does not replace good cane management in the field and factory yard.

Conclusions

Factory trials for dextranase optimization were conducted in the 2004 Louisiana processing season. As previous laboratory studies (Eggleston and Monge, 2005) had shown dextranase applications to syrup were relatively uneconomical, only juice applications were studied. The normalized application of working solutions of "concentrated" dextranases, prepared using distilled or tap water (Eggleston *et al*, 2006), may improve the contact between the dextranase and dextran. Moreover, they are more cost-effective than adding "non-concentrated" dextranases. At the factory in Part I of this study (Eggleston *et al*, 2006), which had relatively high antibody dextran levels (>1000 ppm/°Brix), the addition of 6 ppm of 2- or 5-fold working solutions of "concentrated" dextranase (52,000 DU/ml) were successful in hydrolyzing 70-94% antibody dextran. However, at this factory with relatively low antibody dextran levels (<300 ppm/°Brix), addition of 5 ppm of 5-fold working solutions of "concentrated" dextranase (50,500 DU/ml) was more problematic because of lower contact between the dextranase and dextran, i.e., high enzyme/substrate ratio. Average dextran hdyrolysis was ~43%, as measured with the antibody dextran method. Further studies are now required to study dextranase/dextran ratios with different commercial dextranases. Marked differences existed in the measurement of dextran hydrolysis when the use of antibody or Haze dextran methods was compared. The antibody method measures HMW dextran (>1000 KDa). The Haze method underestimated hydrolysis compared to the antibody method, because it also measures medium MW dextran and other compounds contributing to the "Haze" such as other levan and alteman (*L. mesenteroides* polysaccharides) and indigenous cane polysaccharide. Filtrate juice from the mud tank was found to contain dextran often at higher levels than in juice where dextranase was applied. Therefore, staff should ensure that filtrate juice is recirculated back to the juice before or where dextranase is applied, to maximize dextranase use.

Dextranase did not alleviate all factory problems related to dextran, particularly crystal elongation. This is because 1) dextranase cannot hydrolyze or transform mannitol or other major deterioration products also formed by *L. mesenteroides* which are now known to detrimentally affect processing (Eggleston *et al*. 2004), and 2) low MW (<45 KDa) intermediate dextran hydrolysis products have a powerful inhibitor effect on crystallization (Hidi and Staker, 1975). For these reasons, as well as the relatively high cost of dextranase application, the use of commercial dextranases should only be viewed as a secondary tool to manage dextran in processing. The primary tool has to remain the prevention of dextran formation. Thus, dextranase application does not replace good cane management in the field, factory yard, and factory mills. Major aspects of good cane management are the coordination of harvest schedules with factory deliveries and crushing, and ceasing of cane burning when ambient temperatures are >65 °F (~18 °C). Currently in Louisiana, 12 h cane delivery schedules are operated from 6:00 a.m. to 6:00 p.m. Greater than 12 h cane delivery times, such as 18 or 24 h, also need to be considered. Dextranase does remain an essential tool, however, for sugar technologists when acts of nature cause emergency dextran occurrences.

Acknowledgements

The authors are grateful for funding from the American Sugar Cane League, and excellent technical assistance from Mr. Eldwin St. Cyr. The authors thank Mario Acevedo, Roddy Hullett, and MaryAn Godshall for useful discussions. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

References

Anon. (2003). Analytical procedure using antibody for rapid dextran test. Midland Research Laboratories, Kansas, *US. Publication M9022.*

Blake, J. D. and Clarke, M.L. (1984). Observations on the structure of I.S.P. *International Sugar Journal*, 86: 295-299.

Eggleston, G. and Grisham, M. (2003). Oligosaccharides in Cane and Their Formation on Cane Deterioration. *ACS Symposium Series* 849. Eds: Eggleston, G. and Cote G., Oxford Univ. Press, Chapter 16, p. 211-232.

Eggleston, G. (2004). Easy and Uniform Measurement of the Activity of Dextranase at the Sugarcane Factory or Refinery. *Sugar Journal*, 67(1): 32-33.

Eggleston, G. and Monge, A. (2004). Optimization of factory applications of dextranases in the U.S. *Proceedings of the Sugar Processing Research Conference*, Atlanta, U.S.A., p. 371-394.

Eggleston, G., Legendre, B. L. and Tew, T. (2004). Indicators of freeze-damaged sugarcane varieties which can predict processing problems, *Food Chemistry*, 87: 119-133.

Eggleston, G. and Monge, A. (2005). Optimization of sugarcane factory application of commercial dextranases. *Process Biochemistry*, 40(5): 1881-1894.

Eggleston, G. and Harper, W. (2006). Determination of sugarcane deterioration at the factory: Development of a rapid, easy and inexpensive enzymatic method to determine mannitol. *Food Chemistry*, 98: 366-372.

Eggleston, G., Monge, A., Montes, B. and Stewart, D. (2006). Optimization of industrial dextranase application in raw sugar manufacture: Factory Trials Part I. *International Sugar Journal*. 108: 528-537

Hidi, P. and Staker, R. (1975). Enzymic hydrolysis of dextran in mills. Part I. *Proceedings of the Queensland Society of Sugar Cane Technologists*, 42nd conf, p. 331-344.

Inkerman, P. A. (1980). An appraisal of the use of dextranase. *Proceedings of the International Society of Sugar Cane Technologists*, 17: 2411-2427.

Khalikova, E., Susi, P. and Korpela, T. (2005). Microbial dextran-hydrolyzing enzymes: Fundamentals and applications. *Microbiology and Molecular Biology Reviews*, 69(2): 306-324.

Rauh, J.S., Cuddihy, Jr., J. A. and Falgout, R. N. (1999). Analyzing dextran in the sugar industry: A review of dextran in the factories and a new analytical technique. *Proceedings of Sugar Industry Technologists*, 58th Annual meeting, Portugal, p.17-27.