ENZYMIC REDUCTION OF DEXTRAN IN PROCESS - LABORATORY EVALUATION OF DEXTRANASES

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Abstract

The dextran content of both mixed juice and very high pol sugar has shown an increase in recent seasons. Laboratory trials have been used to show that thermally stable dextranase could be used to reduce the dextran concentration in evaporator syrup when high levels of dextran are unavoidable. Dextranase treatment of diffuser juices is unsuitable because of the high temperature, low Brix conditions encountered in this part of process. Potential benefits probably include viscosity decreases leading to increased sugar recovery whilst the enzymic reduction of dextran in process will result in improved sugar quality.

Keywords: enzyme, dextranase, dextran, sugar quality

Introduction

Dextrans can be responsible for problems in sugar processing which reduce both the recovery of sucrose during sugar production and the final quality of the sugar. Dextrans can be formed by many microorganisms and are not well-defined substances with specific properties. Dextran is the name given to a large class of extra-cellular bacterial polysaccharides composed almost exclusively of glucose units linked predominantly by 1:6 bonds, but also containing 1:4, 1:3 and some 1:2 glucosyl linkages. Dextrans in the sugar industry are predominantly linear, but Edye *et al.* (1995) have shown that branching can be significant, particularly with the low molecular weight dextrans where 5 to 8% branching was indicated. Dextrans in sugar processing occur as a result of post-harvest delay and, infrequently, as a result of poor factory hygiene.

Physical separation methods such as ultrafiltration, dialysis and reverse osmosis are useful methods of polysaccharide removal on a small scale, but technologically are probably not yet sufficiently developed for economic application in sugar processes and are in limited commercial use.

Enzymic hydrolysis of dextran is carried out with the enzyme dextranase which breaks down the polysaccharide dextran to smaller oligosaccharide molecules and offers the possibility of practical control of dextran in sugar manufacturing. The use of dextranase enzyme to reduce the dextran content of factory streams is not new. The application of dextranase in the sugar industry to degrade dextrans into smaller molecules was suggested by Tilbury (1969, 1971), Tilbury and French (1974) and by Fulcher and Inkerman (1974). Inkerman was instrumental in pioneering and implementing the use of dextranase in the Australian industry and published an excellent review of its application in Australia (Inkerman, 1980). The use of dextranase has become a routine and viable procedure in Australian sugar factories during the limited periods of processing of deteriorated cane with reportedly about 3% of the cane supply being treated. The recommended point of application is just prior to clarification and only sufficient enzyme is used to reduce viscosity. Economic application is dependent on the availability of suitable enzymes with the degree of dextran removal being dependent on time, temperature and enzyme concentration. Initially application was dependent on in-house (CSR) preparations until commercial availability improved. The process has not been recommended for general use due to the high cost of the enzyme and the possible introduction of inefficient harvesting techniques (Atkins and McCowage, 1984).

Later Polack and Birkett (1978) demonstrated the technical feasibility of using dextranase in pilot plant trials at Audubon, but further commercial application in the USA was dependent on FDA approval of the enzyme. This motivated a search for enzymes which might more readily receive approval, *e.g.* Koenig and Day (1988) reported on a dextranase produced by *Lipomyces starkeyi*. However, these enzymes were extremely sensitive to high temperature and so had little potential application. In a series of laboratory trials DeStefano (1988) compared the relative merits of alternative points of application such as mixed juice, final evaporator effects and syrup storage tanks using several commercial dextranases. More recently Chavan *et al.* (2000) have published results of a factory application in India. In recent years the use of dextranase has become more widespread in the European beet industry (Stoppok and Buchholz, 1994; de Bruijn, 2000).

Hattori and Minato (1985) investigated the properties of *Chaetomium gracile* dextranase and found that heat stability increased markedly at high sucrose concentration. The enzyme was stable at 80°C for at least 60 minutes at 60° Bx. Limited factory trials using this enzyme in evaporator syrups have been described by Clarke *et al* (1997) and by Edye *et al.* (1997). Cuddihy and Day (1999) and Cuddihy *et al.* (2000) have discussed some of the financial implications associated with dextranase treatment.

Over the years the characteristics of dextranases have been improved, particularly in respect of temperature stability. Nevertheless, dextranases are generally far less tolerant of typical processing conditions than are the more familiar amylases used for starch removal. Active enzymes are not readily available and they are generally considerably more expensive than the amylases with which the local industry is familiar. Economic constraints usually dictate that the most efficient use of dextranase is as an emergency shock dose when necessary. To-date its use has not been considered necessary in South Africa. However, evidence points to increasing levels of dextran in both mixed juice (MJ) and very high pol (VHP) sugar in recent years. The former has an impact on factory performance which is reflected in poor clarification, increased target purity differences (TPD) and inferior boiling house recoveries (BHR) whilst the latter affects downstream consumers. These concerns prompted an assessment of the feasibility of dextranase treatment under local conditions. This report covers the initial phase *viz.* laboratory trials.

Experimental

Three commercial enzyme preparations were evaluated *viz.* Genencor Dextranex L-4000 (*Chaetomium gracile*), Novo 50 L (*Penicillium lilacinum*) and Novo Plus L (*Chaetomium erraticum*). The enzyme activity was determined using the Novozyme procedure (Anon, 2001) so that comparable dosages could be used.

Test solutions consisted of factory mixed juice or syrup diluted to either 15°Bx or 60°Bx with water. Reaction was carried out in conical flasks (250 ml) held at constant temperature in a waterbath. Sample aliquots were transferred to centrifuge tubes containing alcohol (acidified with trichloroacetic acid). This step served the dual purpose of quenching the reaction by denaturing the enzyme and precipitating the unreacted dextran as the initial step in the dextran assay.

Dextran was measured using the SMRI enzymic-HPAEC procedure (Morel du Boil, 2000). Total dextran was measured after initial precipitation with 80% ethanol while the high molecular weight (HMW) portion was distinguished by precipitation with 50% ethanol.

Results and discussion

Sporadic incidents of high dextran sugars have always occurred. However, there is a perception that the situation has worsened in recent years. Data are available from the South African Sugar Terminal (SAST) for dextran (haze) analyses for the period 1982 to 1992 and from 2000 to date. The monthly average data for each supplying mill have been consolidated in Table 1. For the seven seasons available prior to 1992, a total of 56 mill-months contained more than 100 ppm dextran and only 29 mill-months exceeded the export specification of 150 ppm dextran (haze). During this period, 665 mill-month deliveries were received. By contrast for the two seasons (2000/01 and 2001/02) since the resumption of dextran (haze) analyses, there have been 85 mill-months with over 100 ppm dextran and 59 occasions (out of a total delivery of 210 mill-months) when the specification of 150 ppm has been exceeded. During the period 1985 to 1992 the seasonal *average* dextran content did not exceed 50 ppm (haze), whereas 115 ppm was recorded for the 2000/01 season and 95 ppm for the 2001/02 season. There has obviously been an increase in the dextran content of VHP being delivered to the Terminal.

Table 1. Dextran analyses on VHP delivered to SAST.

Season	Mill-month (total)	Mill-month (no. > 100 ppm)	Mill-month (no.>150 ppm)	Mill-month (max) (haze - ppm)	Season mean (weighted) (haze - ppm)
82/83	115	6	1	215	20
85/86	80	10	4	230	
86/87	93	1	-	110	
87/88	96	12	8	630	50
88/89	98	13	7	600	35
89/90	97	5	2	200	20
91/92	86	9	7	580	
	No haze data available 1992 to 1999				
00/01	109	43	32	1485	115
01/02	101	42	27	805	95

A mill-month represents the average (weighted) monthly data for a supplying mill for a particular month

Data accumulated for the past two seasons show conclusively that high dextran sugars result when juice high in dextran is processed (Figure 1). The partition ratio between dextran in VHP to that in mixed juice is approximately 20% (r^2 = 0.70 for 294 samples from the 2000/01 and 2001/02 seasons) *i.e.* to produce VHP within specification it is necessary to process juice containing less than 750 to 1 000 mg dextran/kg Bx. It is also evident from Figure 1 that a considerable proportion of the mixed juice supply exceeds this threshold.

Conventional processing technologies are generally inefficient in terms of dextran removal. When high incoming dextran levels are unavoidable it is technically feasible to alleviate some of the adverse processing effects and to improve sugar quality by using dextranase enzyme to reduce the level of dextran. Laboratory trials using artificial juice and syrup (sucrose plus reagent dextran) and factory mixed juice and syrup have been carried out to establish favourable reaction conditions. Only the latter are reported here.

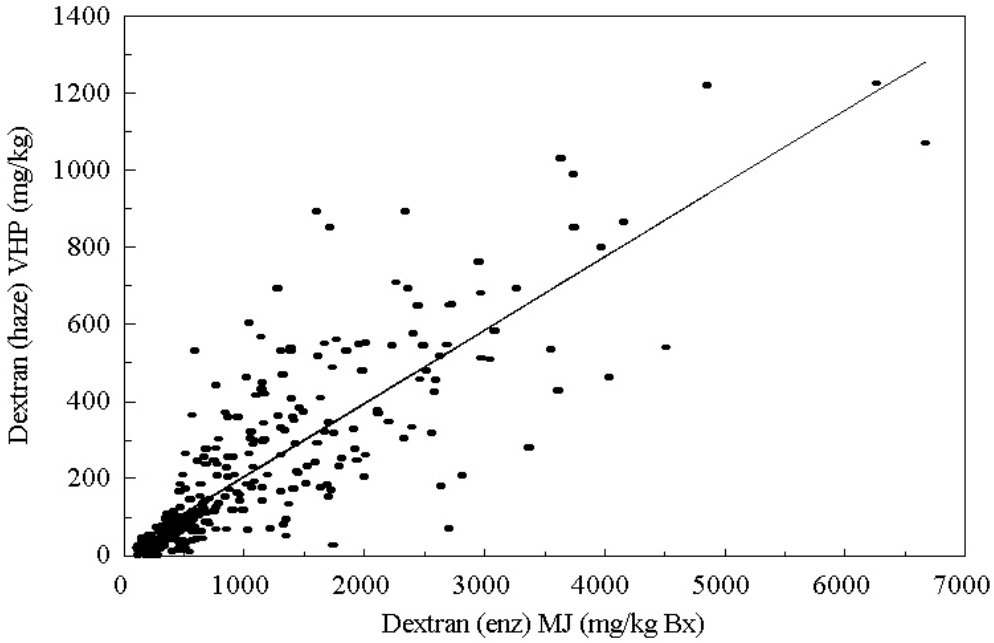

Figure 1. Effect of mixed juice dextran (enz) concentration on VHP dextran (haze) concentration (294 samples from 2000/01 and 2001/02 seasons).

The activities of the dextranases are summarised in Table 2. Trial dosages were adjusted so that equivalent activities were used. Dosages were also adjusted to compensate for Brix and temperature inactivation based on the supplier's data sheets (Anon, 1996; 1999a; 1999b). There are other suppliers of similar dextranases.

Table 2. Dextranase activity.

Enzyme	Source	Activity (KDU/g)
Novo 50 L	*Penicillium lilacinum*	35
Genencor - Dextranex L-4000	*Chaetomium gracile*	65
Novo Plus L	*Chaetomium erraticum*	100

1 KDU = amount of enzyme producing reducing sugars equivalent to 1 g maltose per hour from dextran under standard assay conditions

Commercially available dextranases react by randomly cleaving 1:6 bonds and so the initial products are smaller dextrans, followed by oligosaccharides, disaccharides and monosaccharides. Inkerman and James (1976) have indicated that it is only necessary to degrade dextrans to smaller dextrans in order to improve processability. The comparison between the removal of total and HMW dextran using Novo 50 L at 60°Bx in factory syrup (6 000 mg HMW dextran/kg Bx) at 55°C with 6 KDU/kg Bx is shown in Figure 2. The HMW dextrans (mainly > $2.5 * 10^5$ Da) are broken down rapidly, whereas further breakdown to polysaccharides with molecular weight < $1.5 * 10^4$ takes considerably longer or requires much higher enzyme dosages. Further enzyme assessment was confined to measuring the reduction of HMW dextrans.

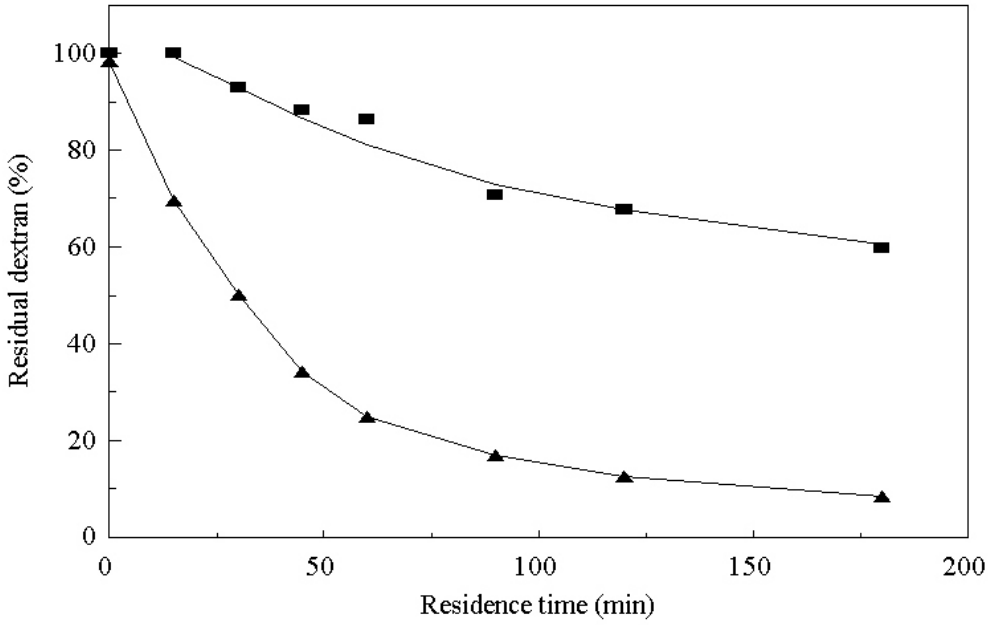

Figure 2. Dextranase reduction of total (■) *vs* HMW (▲) dextran (Novo 50 L at 5.8 KDU/kg Bx; 60° Bx; 55° C).

During initial work samples were buffered to about pH 5 to 6, but in subsequent runs with factory samples the Brix was adjusted with water and reaction was carried out at natural pH. The behaviour of Novo 50 L (*Penicillium sp*) and Novo Plus L (*Chaetomium sp*) at both 15°Bx and 60°Bx at 55°C is shown in Figure 3. The reaction rate is faster for both the low and high Brix application using Novo Plus L. Similar faster reaction with *Chaetomium sp* has been reported previously (Brown and Inkerman, 1992). During the course of this investigation 50 L was withdrawn from the market and replaced with the more efficient Plus L. Both enzymes sourced from *Chaetomium sp* (Novo Plus L and Dextranex 4000 L) showed similar efficiencies in both high and low Brix material (Figure 4).

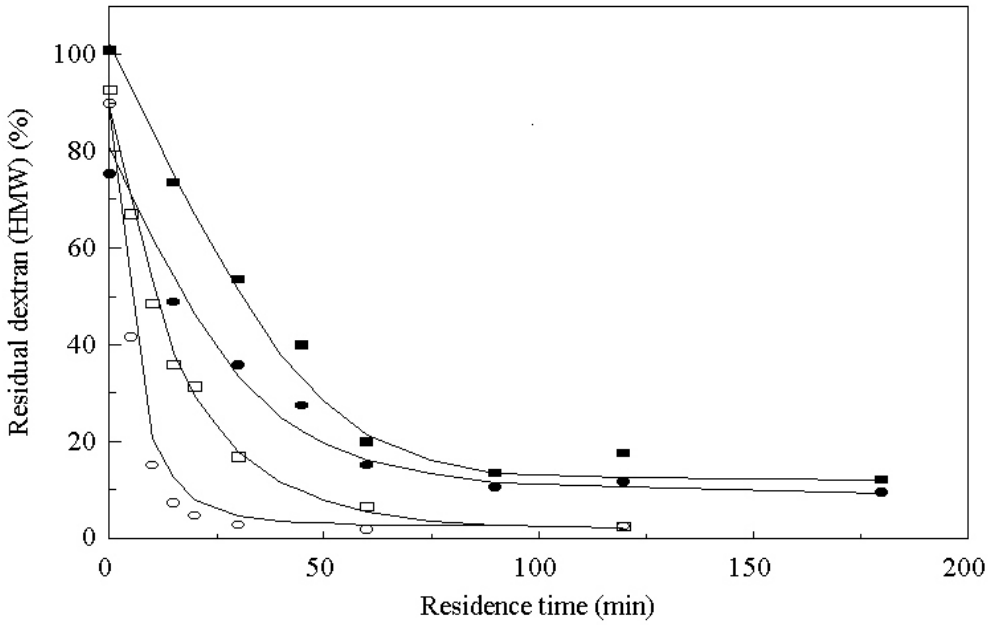

Figure 3. Effect of Brix at 55°C (mean of 3 runs)
■ 60°Bx, 50 L at 5.7 KDU/kgBx; ● 60°Bx, Plus L at 5.4 KDU/kgBx
□ 15°Bx, 50 L at 2.2 KDU/kgBx; ○ 15°Bx, Plus L at 2.0 KDU/kgBx

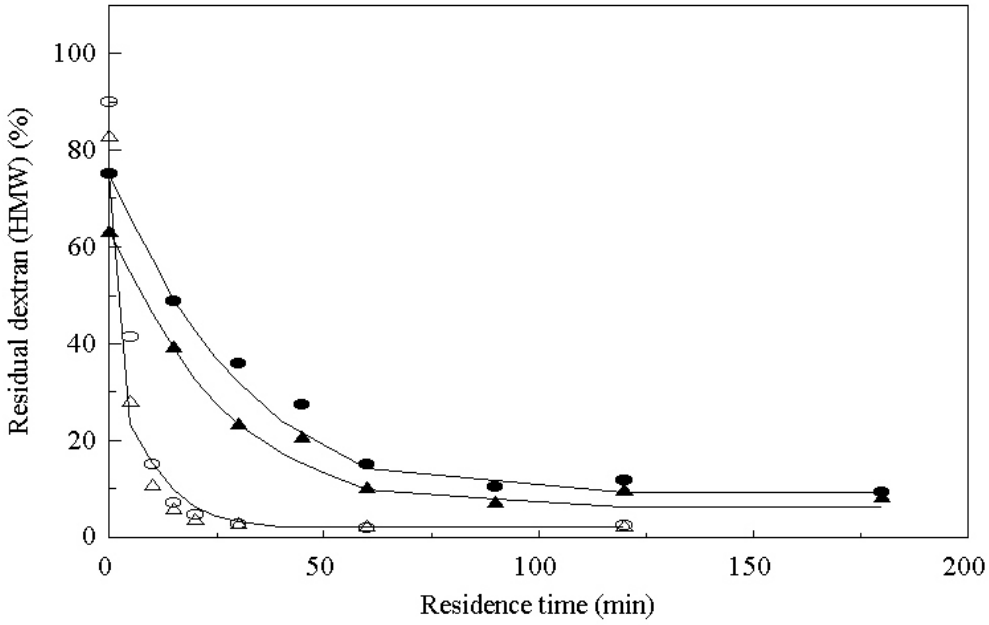

Figure 4. Effect of Brix at 55°C (mean of 3 runs) for *Chaetomium* dextranases
● 60°Bx, Plus L at 5.4 KDU/kg Bx; ▲ 60°Bx, Dextranex L-4000 at 6.9 KDU/kg Bx
○ 15°Bx, Plus L at 2.0 KDU/kg Bx; Δ 15°Bx, Dextranex L-4000 at 2.7 KDU/kg Bx

The brix-temperature interdependence was monitored at 15°Bx and 60°Bx using Novo Plus L as representative of dextranases from *Chaetomium sp*. The higher dosages used at the higher Brix and higher temperatures were based on the supplier's recommendations (Anon, 1996; 1999a; 1999b). Results are illustrated in Figures 5 and 6.

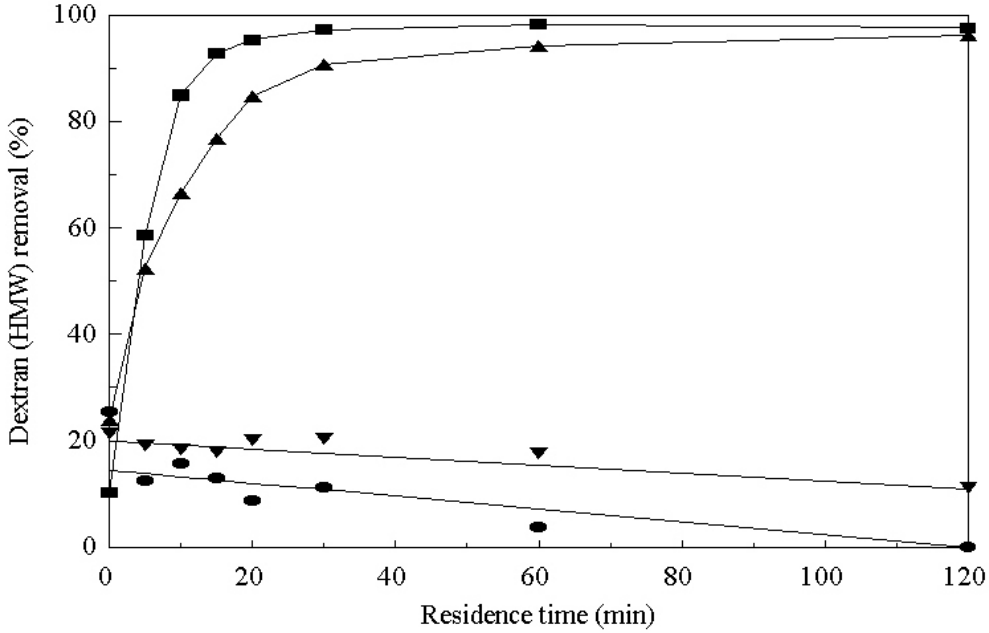

Figure 5. Effect of temperature at 15° Bx using Novo Plus L
■ 55° C; 2 KDU/kg Bx (20 mg/kg Bx);
▲ 65° C; 3 KDU/kg Bx (30 mg/kg Bx)
● 70° C; 5 KDU/kg Bx (50 mg/kg Bx);
▼ 75° C; 10 KDU/kg Bx (100 mg/kg Bx)

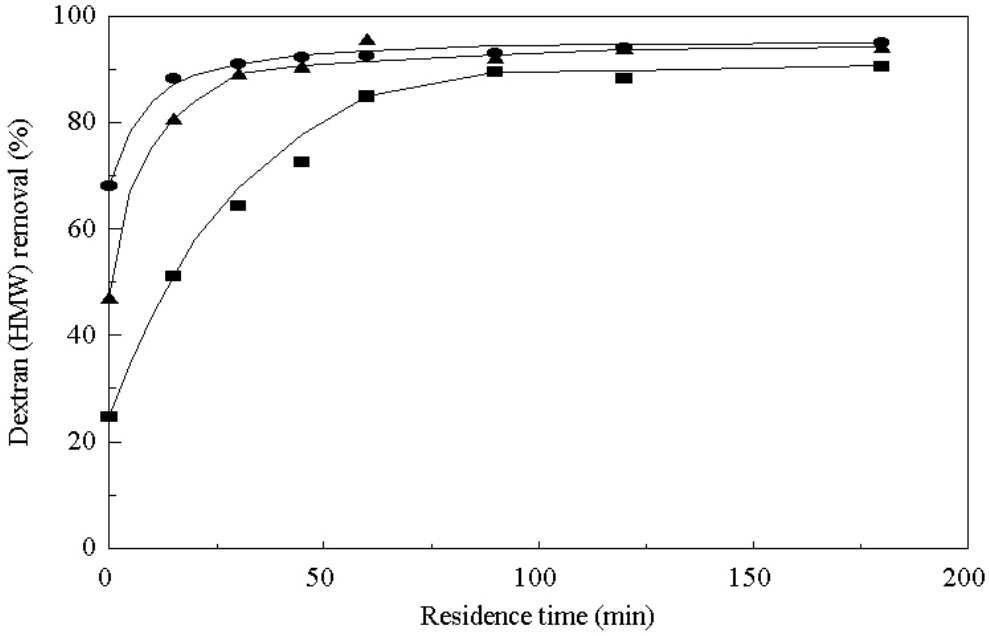

Figure 6. Effect of temperature at 60° Bx using Novo Plus L
■ 55° C; 5.4 KDU/kg Bx (55 mg/kg Bx);
▲ 65° C; 7.7 KDU/kg Bx (77 mg/kg Bx)
● 70° C; 13.4 KDU/kg Bx (135 mg/kg Bx)

The following comments can be made:

- at 15°Bx the enzyme is rapidly inactivated above 65°C, with no reaction at 70°C. Diffuser application is thus not possible.
- at 60°Bx reduction in HMW dextran can be achieved at temperatures between 55° and 70°C. However, increasing the temperature from 65°C to 70°C necessitates doubling the enzyme dosage. The dosage adjustment for the effect of temperature was based on the data sheet for 50 L. Clearly the dosage has been overcompensated, reinforcing the fact that dextranases from *Chaetomium sp* have better thermal stability.

It is thus technically feasible to reduce the dextran content of evaporator syrup. For example, to reduce the dextran content of a 60°Bx syrup from 3 000 mg/kg Bx to 1 000 mg/kg Bx with a residence time of about 30 minutes at 55°C requires 55 mg enzyme (Plus L)/kg Bx. Assuming a MJ purity of 86 and BHR of 89 this means that 62 g of enzyme is needed per ton of sugar produced. At the current (Dec, 2001) price of R1500/kg dextranase it would cost about R93 per ton of sugar produced under these conditions. This can be reduced considerably if the residence time can be increased. Of necessity, this costing undoubtedly errs on the high side at this stage and can probably be considerably reduced in practice. Any clarification benefits will not be realised with dextranase treatment this late in process and thus the main benefit will be seen in improved viscosities.

Increased TPD at many factories has been associated with (but not necessarily caused by) high dextran levels in mixed juice (Figure 7). It has been estimated that the resultant increased molasses purities experienced in the Midlands in the 2000/01 season cost the industry about R5.2 million in lost sugar revenue ([1]personal communication). Sahadeo (1998) has demonstrated that the addition of industrial grade dextran to molasses led to increased viscosity and that the equilibrium molasses purity increased. Conversely, it can be expected that the use of dextranase will reduce the contribution from dextran, resulting in decreased viscosity and, presumably, better boiling house

[1]K Koster, Illovo Sugar

recoveries. However, several byproducts are associated with the formation of dextran. These include acetic acid, lactic acid, ethanol, mannitol and carbon dioxide as well as several oligosaccharides. Many of these products are melassegenic and will also contribute to decreased exhaustion. Their influence will be unaffected by dextranase treatment. An experimental programme is underway at the SMRI in an attempt to differentiate the two effects. Only then can the pros and cons of dextranase treatment at the syrup stage be evaluated.

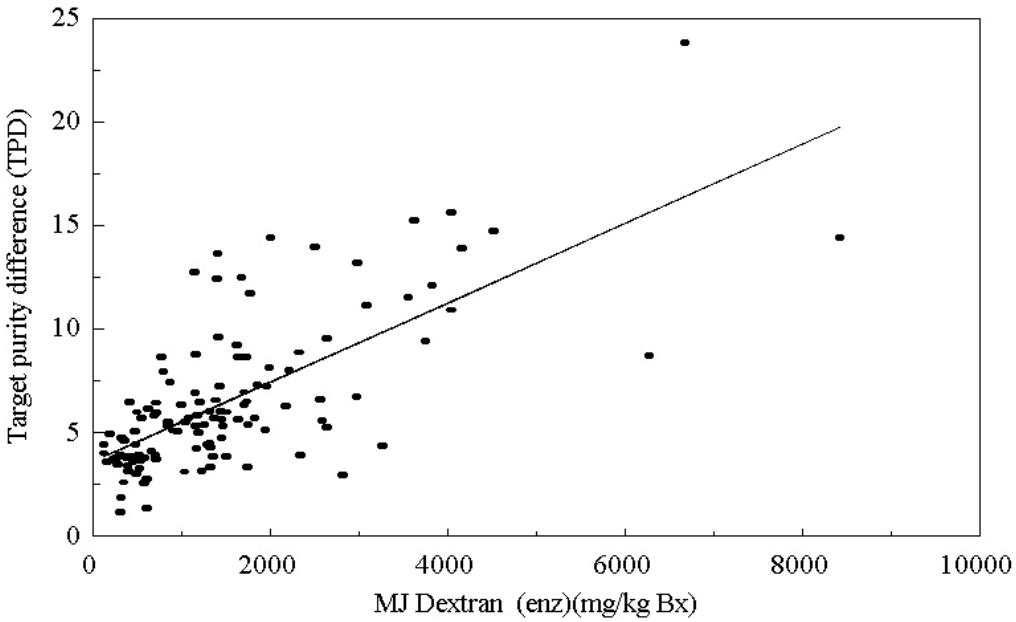

Figure 7. Target purity difference (TPD) increasing in sympathy with mixed juice (MJ) dextran concentration for Aug – Dec, 2000.

Conclusions

The increased dextran content evident in very high pol sugar in recent years has been linked to high mixed juice dextran levels. It has been shown that dextranase can be used to reduce the dextran concentration when high levels of dextran are unavoidable. Laboratory trials using factory liquors have been used to indicate that the application of enzyme treatment to diffuser juice would not be feasible. The enzyme is less active at the temperatures and Brixes encountered in evaporator syrups, but the stability improves (particularly for *Chaetomium sp*). The dosages need to be adjusted to compensate for Brix and temperature inactivation. The supplier's data sheets are suitable for this estimation. Dextran reduction at this stage of process is probably a viable option despite the relatively high dosages required. Potential benefits probably include viscosity decreases leading to increased sugar recovery. The enzymic reduction of dextran in process will result in improved sugar quality. Pilot plant or factory scale evaluations will need to be undertaken so that potential processing benefits can be offset against the costs incurred.

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