

A REVIEW OF SUGARCANE DETERIORATION IN THE UNITED STATES AND SOUTH AFRICA

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Abstract

A review of sugarcane deterioration that detrimentally affects processing in the United States (US) and South Africa (SA) is presented. Postharvest sugarcane deterioration products are dependent on sugarcane injury, environmental conditions, variety, cut-to-crush delays, and extent of adventitious infection. When compared to the US, particularly Louisiana, the South African sugar growing region is geographically and climatically varied and dispersed, with a much longer processing season and many regional differences. Sugarcane management and harvesting methods also differ. *Leuconostoc* infections (resulting in formation of dextran, mannitol and lactic acid and to a lesser extent acetic acid, ethanol and carbon dioxide) have been considered the main cause of factory processing difficulties when handling deteriorated sugarcane. The high humidity and temperatures early in the three month sugarcane processing season in Louisiana, as well as late season winter freezes, are conducive to the formation of this viscous glucopolysaccharide. Dextran was seldom present in South African raw sugar. In the past 10 to 15 years, cane with higher dextran concentrations has been entering some factories, especially in the KwaZulu-Natal Midlands region, and has detrimentally impacted sugar quality. Unfortunately, current methods to determine dextran at the factory all have drawbacks. The effects on processing of other major degradation products that have been monitored (e.g. mannitol, lactic acid, kestoses and ethanol) will be discussed in this review.

Keywords: sugarcane deterioration, dextran, mannitol, kestoses, ethanol, lactic acid

Introduction

Deterioration of sugarcane occurs any time sugarcane is injured. This can occur in the field through the actions of pests or animals, freezes, burning and harvesting (cutting), and is exacerbated by storage and environmental conditions (Figure 1). The delivery of consignments of deteriorated sugarcane to factories can detrimentally affect multiple process units, and occasionally leads to a factory shut-down. Most countries, including the United States (US) and South Africa (SA) do not incorporate a deterioration quality parameter in their sugarcane payment formulae, although refineries do penalise factories for excessively high contents of dextran in raw sugar. Furthermore, with the worldwide emphasis on delivering high quality sugarcane to the factory, sugarcane payment formulae incorporating a deterioration quality parameter may serve as a deterrent against the delivery of overly deteriorated sugarcane, and may improve processing and encourage better sugarcane management. For the sake of clarity, this review paper will mainly discuss the deterioration of sugarcane in the US and SA separately, and will focus only on studies undertaken in the past 40 years.

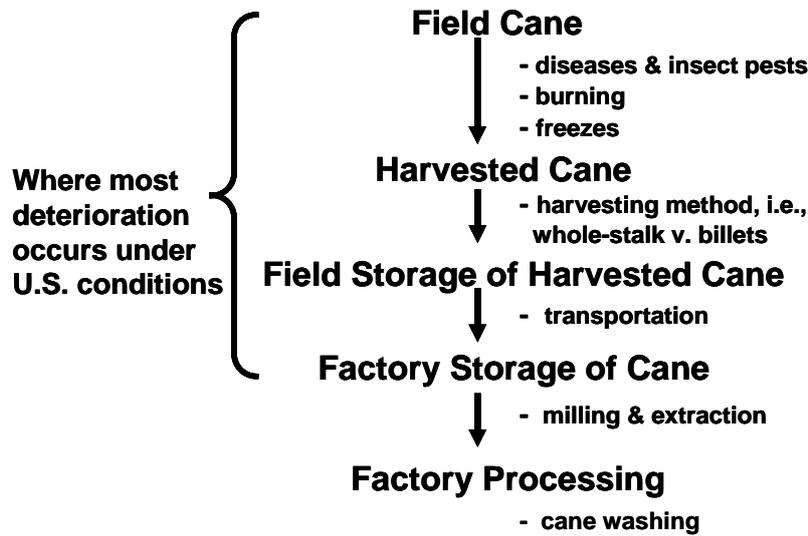


Figure 1. Where sugarcane deterioration generally occurs (from Eggleston and Grisham, 2003).

Sugarcane deterioration in the United States

In the US, sugarcane is currently grown mostly in the states of Louisiana and Florida, with considerably lower amounts in Texas and Hawaii. Environmental conditions vary across these four states from tropical to sub-tropical and from humid to arid conditions. However, depending on the harvesting, storage and environmental conditions, sugarcane in all four states sometimes suffers from *Leuconostoc* sugarcane deterioration and the formation of dextran (glucose polysaccharide). Research findings presented here will concentrate on Louisiana because the possibility of sugarcane deterioration is greater for the following reasons. Louisiana has a semi-tropical climate and an approximate three month (October to December) harvesting/processing season to minimise the effects of later freezes. When harvest begins the cane is immature and humidity and temperature values are high, and these decrease later in the season. Sometimes freezes occur in late November and December, causing sugarcane freeze deterioration that can be devastating when severe (Irvine and Legendre, 1985). In Louisiana and throughout the US there was no validated, reliable, rapid, easy and inexpensive method to measure sugarcane deterioration at the factory. This meant that factory personnel were not able to screen individual consignments of sugarcane and did not know which consignments would detrimentally affect processing and were thus unable to reject unsuitable consignments.

Internationally, a variety of deterioration indicators to predict processing problems at the factory have been proposed. Such indicators need to be measurable and to be related to one or more processing difficulties to be useful. In 1985, Legendre and co-workers showed that freeze deteriorated sugarcane in Louisiana produced juices of lower purity, higher acidity and abnormal amounts of polysaccharides, especially dextran. There was also a varietal effect on the level of dextrans and total polysaccharides in cane left in the field after freeze damage. These authors additionally reported that changes in juice pH and titratable acidity were useful criteria of sugarcane freeze deterioration (Legendre *et al.*, 1985). However, a later study by Eggleston *et al.*, (2004), using the Audubon Sugar Institute (ASI-II) enzymic method for dextran, observed that titratable acidity and pH were only useful in predicting problems caused by severe dextran concentrations, i.e. $> \sim 2\ 500$ and $\sim 2\ 800$ mg/kg Bx (or ppm/%Bx or dissolved solids), respectively (Figure 2). Although pH is easy to determine it is typically

unreliable and not considered to be a sensitive measure of deterioration because the buffering capacity of the juice reduces the pH change on deterioration. Titratable acidity values in juice usually increase on deterioration, but the absolute value of titratable acidity alone is not considered a sensitive measure of deterioration. This is because titratable acidity in fresh, undeteriorated sugarcane juice varies markedly by variety, soil type and environment. Nevertheless, the existence of strong correlations between pH or titratable acidity and dextran (Eggleston *et al.*, 2004) suggest that an acid such as D-lactic acid (a *Leuconostoc* metabolite) may be worth considering. Enzymic analytical techniques have been used in beet sugar factories for lactic acid analysis (Samaraweera *et al.*, 1995), but rapid, immobilised enzyme analytical techniques may be even more suitable for factory applications.

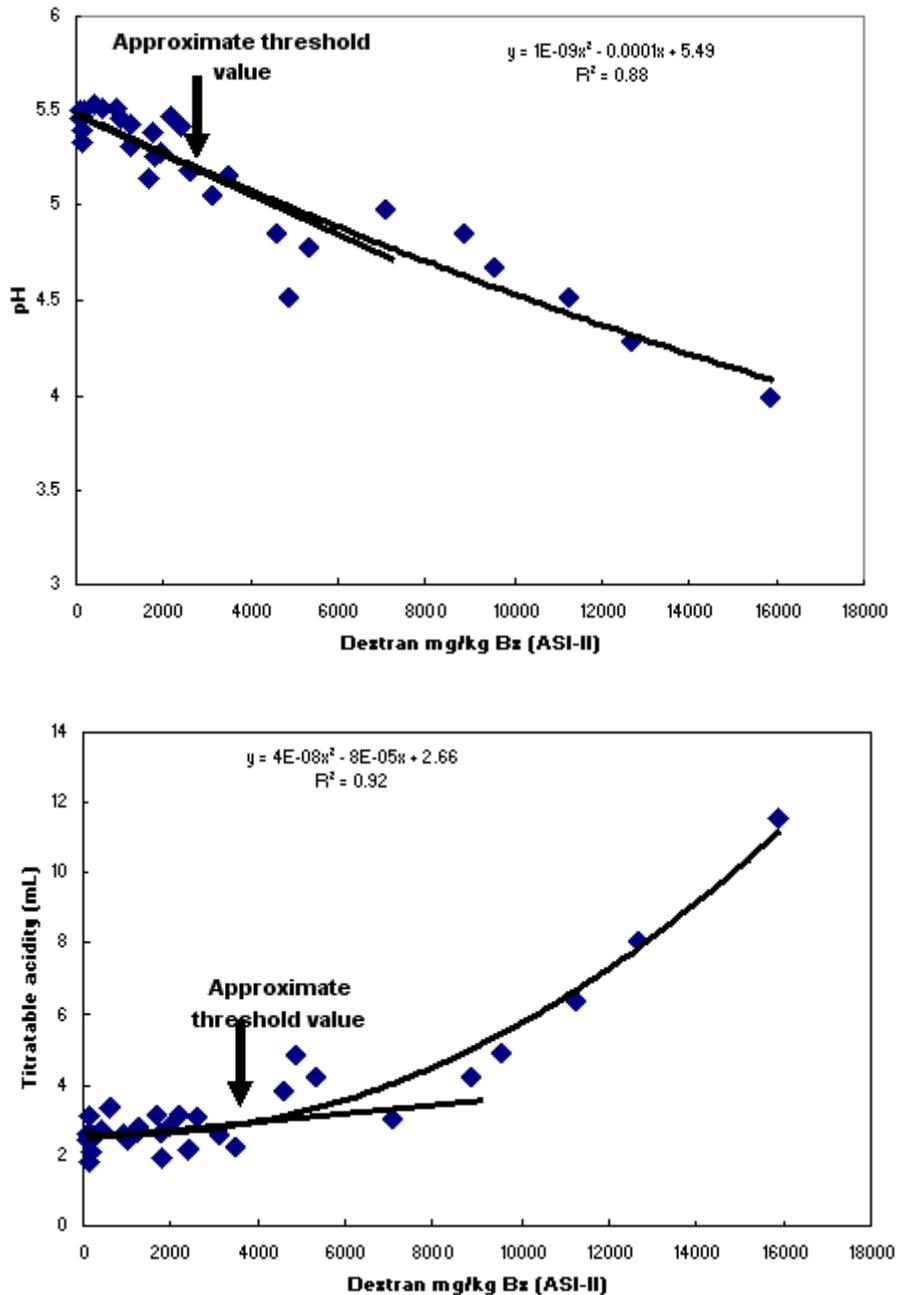


Figure 2. The relationship between dextran and pH (above), and titratable acidity (below) in freeze deteriorated sugarcane. Only equations for the polynomial curve fit are shown (from Eggleston *et al.*, 2004).

In the early 1990s, sugarcane deterioration in the field, factory storage pile, or during factory processing, became a topic of major concern in the US because mechanical harvesting of billeted cane (~23 cm stalk pieces) increased dramatically. At this present time in the US no sugarcane is hand cut and field cane is either mechanically harvested as whole-stalks by a soldier harvester or as billeted cane by a combine harvester. In 2006, approximately 60% of the billeted sugarcane was harvested green in Louisiana. Oligosaccharides that form during deterioration (Morel du Boil, 1991, 1995, 1998, 2003; Ravelo *et al.*, 1991a,b) have been used to verify the extent of deterioration by different harvest practices (Eggleston *et al.*, 2001b; Eggleston and Grisham, 2003). Oligosaccharides formed included kestoses (see Figure 6 and Morel du Boil, 1991) and those formed as products from unwanted dextran formation by dextransucrase from *Leuconostoc* bacteria, e.g. isomaltotriose, isomaltotetraose, leucrose and palatinose. Isomaltotriose is the most sensitive oligosaccharide indicator of cane freeze deterioration, and leucrose and palatinose can be used to confirm severe dextran formation (Eggleston and Legendre, 2002) (Figure 3).

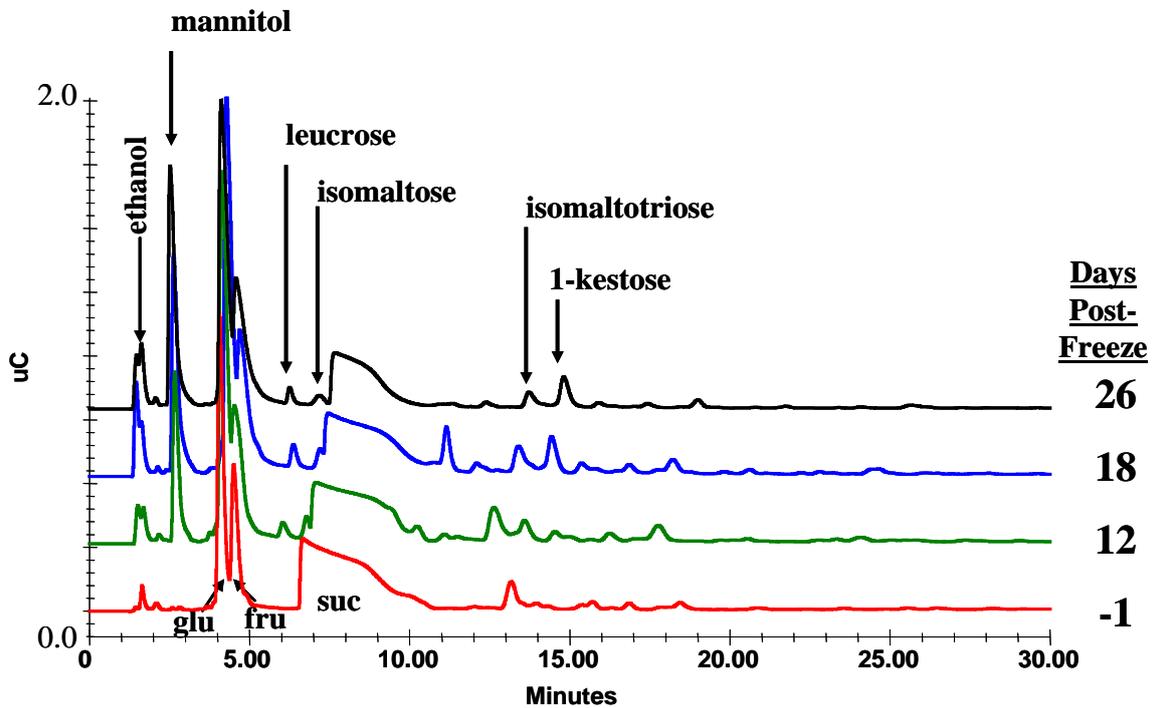


Figure 3. Changes in IC-IPAD (HPAE) chromatograms for sugarcane variety TucCP 77-42 that is susceptible to freeze deterioration in Louisiana, US. Brix values were not standardised. (From Eggleston *et al.*, 2004.)

By developing an ion chromatography method with integrated pulsed amperometric detection (IC-IPAD, aka high performance anion exchange chromatography (HPAEC)) using a strong NaOAc/NaOH gradient to separate oligosaccharides (up to 12 degrees of polymerisation) in juices, Eggleston *et al.* (2001a,b), in a controlled field study, unequivocally showed growers and processors that deterioration was greater and more rapid in billeted than whole-stalk cane and was more rapid and extensive in burnt than green (non-burnt) billeted cane. Also, when field cane is cut, freshness is more important than harvest method; if billeted cane can be delivered and processed at the factory in less than 14 hours no deterioration products should detrimentally affect processing (Eggleston *et al.*, 2001b). This important result was reinforced by a further sugarcane deterioration study at a Louisiana factory (Eggleston and Grisham, 2003). This and other research (Godshall *et al.*, 2000) resulted in optimum postharvest handling conditions being formulated for cane grower and factory processors to minimise

deterioration losses (Eggleston *et al.*, 2001a,b) and has led to better management of sugarcane in Louisiana. However, the use of oligosaccharide quantitative IC-IPAD ‘fingerprint’ profiles is only a research tool and not readily applicable for use at the factory, as expensive equipment, technical operators with troubleshooting expertise, and staff who can interpret the profiles, are required. IC-IPAD profiles have also been used to analyse simultaneously for the deterioration products ethanol, mannitol and oligosaccharides in sugarcane juice (Eggleston, 2002). It has been repeatedly demonstrated that ethanol is not very useful as an indicator of *Leuconostoc* sugarcane deterioration which occurs under humid Louisiana conditions (Eggleston, 2002; Eggleston and Legendre, 2002). However, ethanol has been advocated as a useful deterioration indicator in South Africa (Lionnet and Pillay, 1987, 1988). It has been suggested that the drier conditions in some regions of SA are more conducive to yeast or other microbial infections and, therefore, ethanol production. (Clarke and Legendre, 1996).

Although not necessarily applicable to sugarcane deterioration in the field or caneyard, laboratory trials on a factory cane juice showed that 93% of deterioration in a sugarcane juice was microbial, 6% enzymic and 1% chemical (acidic) (Eggleston, 2002). These results may extrapolate to juice deterioration across tandem mills where mill sanitisation is not adequate.

Limitations of current factory dextran methods

The major (but not sole) contributor to sugarcane deterioration in the US, particularly in Louisiana where humid conditions prevail, is infection by *Leuconostoc* lactic acid bacteria. Dextran, a high viscosity glucopolysaccharide, has historically been considered the major deterioration product of a *Leuconostoc* infection. Factors affecting *Leuconostoc* infection are:

- Ambient temperature and humidity
- Level of rainfall and mud
- Length of sugarcane billet
- Degree of burning
- Billet damage
- Delays between burning and cutting and subsequent processing
- Mill hygiene.

High concentrations of dextran (>1 000 mg/kg Bx) can reduce evaporation and crystallisation rates, and US factories are penalised by refineries for excessive dextran in the raw sugar. Current factory methods to determine dextran in juice, however, are either too time consuming, too complicated (ASI-II enzymic method; Sarkar and Day, 1986), not specific enough (haze method; Clarke *et al.*, 1987), too expensive (monoclonal antibody (MCA) method; Rauh *et al.*, 2001; Anon, 2003) or too difficult in the interpretation of results (haze method). An enzymic-HPAEC method is also available to measure dextran but is considered a research tool rather than a factory method (Morel du Boil, 2000).

Mannitol

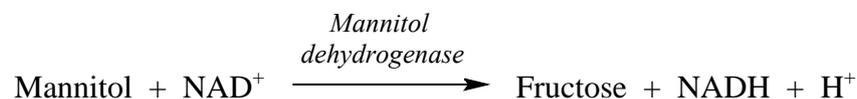
Mannitol, a sugar alcohol, is now known to be a major degradation product of *Leuconostoc* sugarcane deterioration, sugarbeet deterioration (Steinmetz *et al.*, 1998; Thielecke, 2002), and the bacterial contamination of fuel ethanol produced from sugarcane (Eggleston *et al.*, 2007). Eggleston and co-workers used laboratory, field and factory studies to confirm that mannitol is also a major degradation product of *Leuconostoc* sugarcane deterioration (Eggleston, 2002; Eggleston and Legendre, 2002; Eggleston *et al.*, 2004; Eggleston *et al.*, 2008). Mannitol was found to be a more sensitive indicator of sugarcane deterioration than dextran. Mannitol is also produced by *Lactobacillus* bacteria and a few fungi, although *Leuconostoc* is the greatest producer (Eggleston *et al.*, 2007). Mannitol can be used to predict sucrose losses and dextran

related problems such as viscosity and, to a lesser extent, filterability problems (Eggleston *et al.*, 2004). Moreover, mannitol can occur in large amounts in factory syrups and massecuites processed from deteriorated sugarcane, does not degrade under processing conditions (Eggleston *et al.*, 2004) and directly affects processing by reducing sugar recovery (Bliss, 1975) and evaporation rates (unpublished data¹). Recently, Eggleston and Antoine (2008) showed that mannitol was, at the least, a contributor to reduced heat transfer through 'hard or slow to boil' massecuites and molasses.

Mannitol enzymic method

As chromatographic techniques are too sophisticated for use at the US factories and a high level of expertise is required by the operator, Eggleston and Harper (2006) developed an enzymic method to measure mannitol in consignment juices from core samples at the factory. This method is rapid, easy, inexpensive and reliable and has been successfully evaluated at a Louisiana factory across a processing season (Eggleston *et al.*, 2008).

The enzymic method utilises mannitol dehydrogenase (MDH) to convert mannitol to fructose in the presence of co-enzyme NAD⁺. The NADH formed can be easily measured spectrophotometrically at 340 nm:



The consignment juice analysis time is around seven minutes at room temperature and within four minutes if a 40°C waterbath is used to incubate the juice. The method is accurate, precise, highly specific for mannitol, and is not affected by the presence of sucrose, glucose, fructose or dextran. The current cost per analysis for a juice sample is ~60 US cents, with the largest cost being NAD at 45 cents per analysis (Eggleston and Harper, 2006).

A strong polynomial relationship ($R^2=0.912$) existed between mannitol (enzymic method) and dextran (haze or MCA) in factory juices obtained across a processing season (Eggleston *et al.*, 2008) (Figure 4). Mannitol concentrations were usually higher than concentrations of haze and MCA dextran, confirming that mannitol was more sensitive and useful than dextran for predicting sugarcane deterioration from *Leuconostoc* and other *Lactobacillus* bacteria. Downstream processing difficulties were predicted when mannitol exceeded ~2 500 mg/kg Bx in juice. There has been considerable international interest in the enzymic mannitol method. It has been used to measure deteriorated cane in, for example, Guatemala and Argentina (personal communications²).

Sugarcane deterioration in South Africa

When compared to the US, particularly Louisiana, the South African sugar growing region is geographically and climatically varied and dispersed, with a much longer processing season (March to December) and many regional differences. Sugarcane management and harvesting methods also differ. Most cane in SA is burnt and hand cut as whole-stalks. However, in

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recent years there has been a small introduction (about 15% of the crop) of mechanically harvested billeted cane. Deterioration trials in SA have usually been conducted under controlled conditions, rather than in-field. Field trials have generally been in the form of surveys for a particular compound, including ethanol.

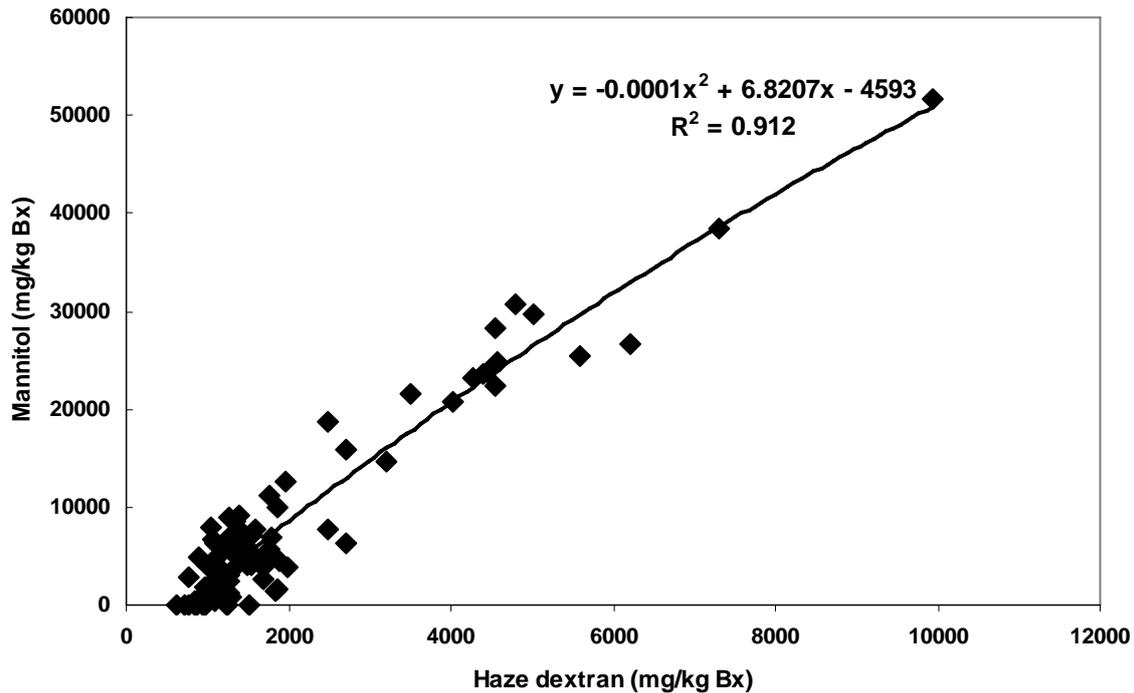


Figure 4. Relationship between mannitol and haze dextran in press and crusher juices collected across the 2004 processing season at a Louisiana, US factory (N=188) (from Eggleston *et al.*, 2008).

Several attempts have been made over the years, in SA and many other countries, to establish the rate and extent of sugarcane deterioration when harvested cane is stored under controlled conditions. One of the first such trials in SA was carried out on whole stalk cane of variety NCo376 stored in the open at the Sugar Milling Research Institute (SMRI) (Anon, 1964). The cane was sampled over a period of 21 days. The extracted juice was clarified and A-sugar produced in a laboratory vacuum pan. The gums (i.e. material soluble in water and precipitated in alcohol) content increased rapidly and this was reflected in the sugar produced. Sugar produced from freshly harvested cane contained 300 mg/kg Bx sugar gums, whereas that processed from cane which had been stored in the open for 15 days contained 600 mg/kg Bx sugar.

In the early 1970s, Wood and co-workers conducted a comprehensive and systematic series of trials on cane deterioration under controlled conditions. Factors such as burnt or not burnt, post-burn harvest or standing, variety, billet size and chemical ripening were investigated (Wood *et al.*, 1972; Wood, 1973a, 1973b, 1976; Clowes and Wood, 1978). The rates of deterioration varied considerably with the different post-harvest conditions.

- The loss in cane mass was caused by dehydration and was more severe for burnt than green cane
- This mass loss averaged ~1% per day
- Initially unburnt, whole-stalk cane deteriorated more rapidly than burnt whole-stalk cane – probably an enzymic effect

- After a lag period, burnt cane deteriorated rapidly – probably a result of microbial infection
- Deterioration products such as dextran formed more quickly in burnt cane
- Varietal rate differences were more pronounced for unburnt cane
- Deterioration is more rapid in billeted cane (particularly for short billets), while little difference was found in the rate of deterioration between green or burnt chopped cane.

Rates of deterioration are related to field and harvest conditions and are mainly the function of the degree of mechanical damage, cut to crush delay, environmental conditions, degree of burn and delay of harvest after burning, degree of freeze damage and combinations of these factors. Hot, humid conditions favour rapid accumulation of dextran, which is formed more rapidly in burnt than green cane, because burning damages cells and exposes them to infection. These factors have also been covered at length elsewhere and are universally acknowledged as contributing to post-harvest cane infection and deterioration (Egan, 1968; McNeil and Bond, 1980). Recently, Perry *et al* (2007) have investigated the metabolites of post-harvest microbial infestation of sugarcane.

Delays in delivering harvested cane to the factory lead to deterioration in the quality of the juice that can be extracted. This in turn impacts on the quality of the sugar produced. Generally, dextran is perceived by SA factory staff as being the main deterioration product. There are no rapid, routine analytical procedures for measuring dextran and, as already stated in this paper, there are numerous drawbacks to their use at the factory. Applied correctly, the haze procedure is probably still the best option (Urquhart *et al*, 1993).

During the 1980s, Lionnet and colleagues tried to relate cane deterioration to the appearance of a readily detectable deterioration product. They reported that temperature was the dominant factor in the rate of deterioration and that large quantities of ethanol and lactic acid were produced. They focused their attention on measuring ethanol in direct analysis of cane (DAC) extracts. They developed an empirical model under controlled laboratory conditions relating ethanol concentrations (measured with gas chromatography) in extracts from deteriorating cane to a function of time, temperature and, to a lesser extent, variety for both burnt and green whole-stalk cane. They concluded that ethanol gave a good measure of cut-to-crush delay (Lionnet, 1986). In a later paper they tested the model under industrial conditions and found a relationship between sucrose lost and ethanol formed as whole-stalk cane deteriorated (Lionnet and Pillay, 1988; de Robillard *et al*, 1990). In South Africa, under normal conditions, ethanol has been used as the indicator of cane delay, with 1 000 mg/kg Bx ethanol indicative of 2-3% loss of the original sucrose. This relationship was subsequently challenged and different rates of ethanol formation were found under different storage conditions (Cox and Sahadeo, 1992; Smith, 1993) (Table 1).

Table 1. Relationship between ethanol concentration and sucrose loss under South African conditions.

Whole-stalk cane supply	Sucrose loss represented by 1 000 mg/kg Bx ethanol detected	Reference
Burnt bundled	1%	Cox and Sahadeo, 1992.
Burnt windrowed	2%	de Robillard <i>et al.</i> , 1990
Unburnt windrowed	>>2%	Smith, 1993
Effect on overall factory recovery	0.1 to 0.2 units decrease	Cox and Sahadeo, 1992

Despite the shortcomings of ethanol monitoring, the simplicity of the approach appealed to the industry and its implementation served to highlight the problems associated with delays in delivering cane to the factory. Historically, dextran has not been regarded as a serious problem in South Africa and in 1984 it was considered unnecessary to include a dextran penalty in the contract for sugar delivered to the central refinery (Alexander and Ravno, 1984). However, the situation has changed considerably in the interim (Morel du Boil and Wienese, 2002), and by 2000 a specific enzymic-HPAEC analytical technique for the analysis of dextran was developed so that the observed levels of dextran in factory products could be confirmed (Morel du Boil, 2000). It has since become obvious that in some areas high levels of dextran are encountered every season (Morel du Boil, 2005). Although a relationship between lactic acid and dextran formation was found [lactic (mg/kg Bx) = 293 + 0.264*dextran (mg/kg Bx), n=196, R²=0.74], the samples were sourced primarily from factories in the South Coast region of KwaZulu-Natal and the relationship may not be applicable in a wider context. Since lactic acid was oxidised chemically and the resultant acetaldehyde analysed chromatographically, both D and L lactic acid were measured. The scatter plot is shown in Figure 5.

Ravno and Purchase (2005) recently reviewed the South African situation for dextran and speculated that factors contributing to the increased significance of dextran in South Africa (as a result of cane delays and deterioration) include:

- Increased mechanical harvesting
- Difficulties in co-ordinating harvesting and transport from small-scale growers
- Increasing environmental concerns leading to fewer, and hence larger, burns.

As a result of the increasing concern about current dextran levels in the South African industry, there is a move to introduce penalties into the sugar quality specification. This will necessitate the development of criteria that will allow severely deteriorated cane to be rejected. Apart from suitable analytical methodology, it will also be necessary to address reliable sub-sampling of consignments and methods to obtain a suitable juice sample for control applications. The SMRI is currently undertaking a study to establish the suitability of several deterioration indicators.

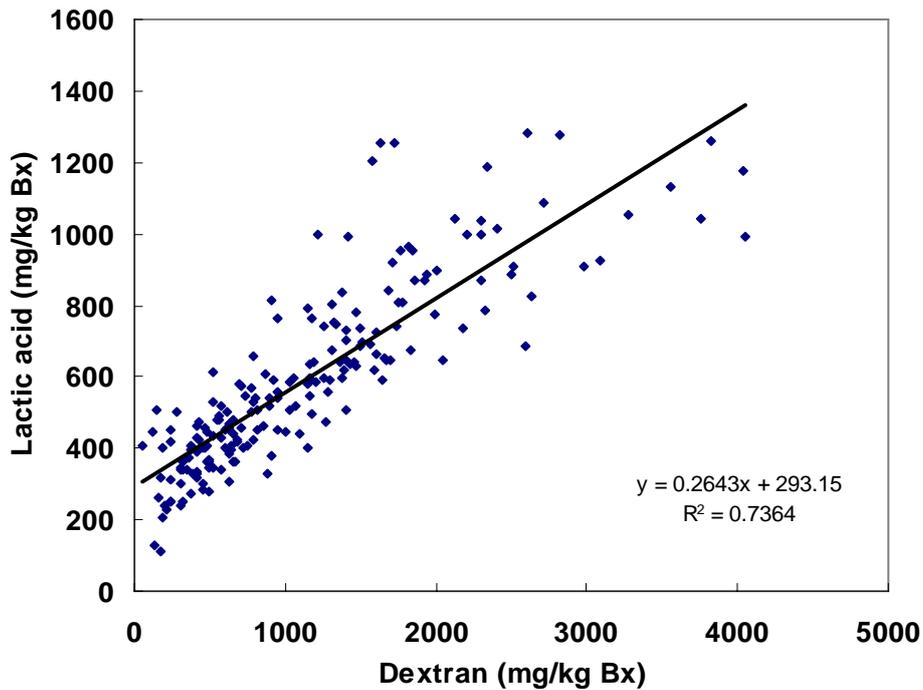


Figure 5. Scatter plot for lactic acid (chromatographic method) in mixed juice against dextran for 2000 to 2003 seasons in South Africa (from Morel du Boil, 2005).

During investigations into the cause of sucrose crystal habit modification it was established that oligosaccharides containing a sucrose moiety played a dominant role in this phenomenon and that these cause reduced crystallisation rates in the factory (Morel du Boil, 1991). Included in this group of sugars are the kestoses. These are trisaccharides and are essentially fructose-substituted sucrose molecules. The main oligosaccharides formed during cane deterioration are the kestoses (1-, 6- and neo-). The level is generally low in fresh cane, but too variable to have application as a useful monitor of post-harvest cut-to-crush delay. They accumulate rapidly with whole-stalk cane delay, particularly 1-kestose, while 6- and neo-kestose form more rapidly in burnt cane than in green cane (Morel du Boil, 1995). These last two compounds play a part in the occurrence of fence-post and triangular shaped crystals in the factory (Morel du Boil, 2003; Vaccari *et al.*, 1999). Typical trends with delay time are shown in Figure 6.

When unavoidable cane deterioration leads to high levels of dextran in juice, it has been shown that dextranase treatment can alleviate some of the processing difficulties encountered (Inkerman, 1980). However, with the predominance of diffuser factories in South Africa, although this option is technically feasible, it does not appear to be economically viable (Morel du Boil and Wienese, 2002).

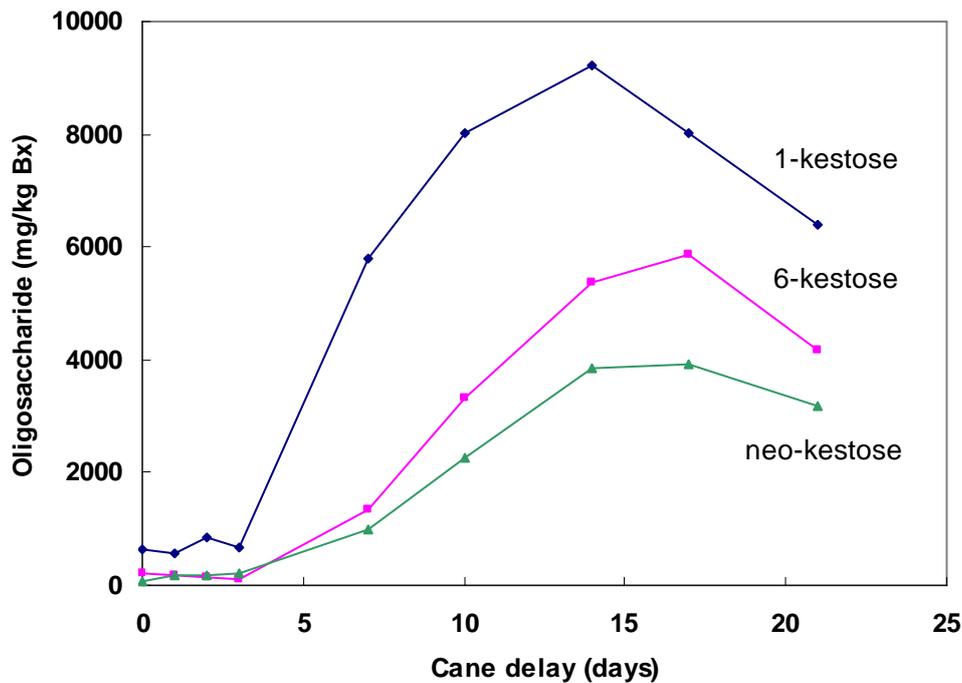


Figure 6: Rate of formation of kestose oligosaccharides as whole-stalk burnt cane deteriorates (from Morel du Boil, 1991).

Conclusions

A review of sugarcane deterioration in the US and SA has been presented. The role of environment is the predominant factor in post-harvest deterioration, so that control of sucrose loss becomes one of regional management. Dextran has previously been considered the major deterioration product of *Leuconostoc* infection. Localised studies in the US and other countries have shown that mannitol and D-lactic acid are also major deterioration products. Both of these show promise as deterioration indicators/predictors. Deterioration mechanisms are not fully understood and many different routes and pathways result in different end-products. The relationships of metabolites from *Leuconostoc* sugarcane deterioration with major indicators from other types of sugarcane deterioration such as ethanol and kestose oligosaccharides is not as clear and may vary from region to region as well as country to country, where environmental and other influences are vastly different.

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