NEW INSIGHTS ON FACTORY INDICATORS OF FREEZE DETERIORATED CANE

By

G. EGGLESTON¹, B. LEGENDRE²,³ and T. TEW²

¹SRRC-USDA-ARS, 1100 Robert E. Lee Boulevard, New Orleans, LA 70124, USA
E-mail: gillian@srrc.ars.usda.gov
²Sugarcane Research Unit, SRRC-USDA-ARS, Houma, LA 70361, USA
³LSU Ag. Center Research and Extension, St. Gabriel Research Station
St. Gabriel, LA 70776, USA

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Abstract

CURRENTLY in the USA, there is no reliable, rapid, easy-to-use, and inexpensive method to measure cane deterioration at the factory. As a consequence, factory staff do not know quickly whether a load of cane can be processed economically or if associated processing problems are expected. For the last four years, basic studies on cane deterioration have been conducted with a final goal to develop such a method. This paper emphasises a study on the freeze-deterioration of eight commercial cane varieties. The major source of cane deterioration in the U.S., particularly under Louisiana’s humid conditions, is from Leuconostoc bacteria. Up until now, the major focus of Leuconostoc cane deterioration has been the formation of dextran, but numerous other products are also formed by Leuconostoc, which are of importance in sugar manufacture, including levan and alternan polysaccharides, and mannitol. In a green, whole-stalk cane freeze-deterioration study, mannitol, produced by mannitol dehydrogenase from Leuconostoc and measured using ion chromatography, was a slightly better predictor of pressed juice viscosity (r² = 0.84) than both ASI-II (r² = 0.80) and Haze (r² = 0.81) dextran, because it can independently indicate all Leuconostoc polysaccharides. In comparison, ethanol (r² = 0.68), leucrose (r² = 0.71), and pH (r² = -0.71) were only moderately correlated with viscosity. Mannitol was also better than dextran at predicting percentage filterability of pol solutions, although substances present in the undeteriorated cane juice and sucrose interfere with this processing parameter. Strong polynomial trend or quadratic fits existed between ASI-II (enzyme method) dextran and titratable acidity (r² = 0.92) and pH (r² = -0.88). However, threshold levels exist where deterioration effects become greater than varietal effects: -2500 ppm and -2800 ppm/Brix dextran for titratable acidity and pH, respectively, which explains their unreliability at predicting a wide range of cane deterioration, although the measurement of a specific deterioration acid such as D or L lactic may be worthwhile. Overall, mannitol was the best predictor of cane deterioration which contributes to sucrose losses, dextran, viscosity and, to a lesser extent, filterability problems. Furthermore, mannitol does not degrade under industrial processing conditions, which gives support to the use of mannitol to indirectly measure dextran and/or cane deterioration at the factory. A reliable, rapid, easy and inexpensive enzymatic method to measure mannitol at the factory is now being investigated.

Introduction

It is well known that deteriorated sugarcane detrimentally affects processing in the factory, and a major source of deteriorated cane in the U.S. is from freezes which can sometimes lead to a factory shut-down. Not only does deteriorated cane cause reductions in profits because of sucrose losses, but also production costs often increase dramatically. The decrease in cane quality being processed leads to increases in the demand for lime because of associated higher acidity, increases in the use of processing
Frequent winter freezes in Louisiana force the industry to adapt to a short growing season (7 to 9 months) and a short milling season (approx. 3 months). The nature and extent of damage to cane by freezes depends on the temperatures and duration of the freeze, with damage being even more severe when the freeze is followed by warm, wet weather, which is ideal for microbial growth. Following freeze injury, dead and moribund cells become vulnerable to the invasion of microbes. The entry of microbes into cane tissue is facilitated by dead lateral buds –4.4°C (24°F) and by freeze cracks –5.6°C (22°F).

Irvine and Legendre (1985) proposed two mechanisms for deterioration: 1) susceptibility of tissue to freezing, and 2) susceptibility to microbe invasion and subsequent polysaccharide formation after the freeze. Generally, cane damaged by a severe freeze produces juices of lower purity, higher acidity, and abnormal amounts of polysaccharides (Legendre et al., 1985), particularly dextran (Eggleston and Legendre, 2003). Recent studies (Eggleston and Legendre, 2003; Eggleston et al., 2001; Eggleston, 2002; Godshall et al., 2000) have confirmed that the major contributor to cane deterioration in Louisiana, where humid conditions prevail, is from *Leuconostoc* lactic acid bacterial infections. Ambient temperatures above 25°C and rainy weather encourage *Leuconostoc* growth and the production of metabolites such as dextran and mannitol.

Currently, there are two lines of approach to combating freeze deterioration effects at the factory. The first is for the factory to manipulate processes and add processing aids to overcome the negative impact of the poor quality cane. This is obviously a very expensive and inefficient approach. Furthermore, the evaluation of cane quality arriving in transport loads at the factory, for grower payment, is currently only based on the determination of sugar content (using polarimetry and refractometry) and fibre content. There is no routine consideration of products present in freeze deteriorated sugarcane that can negatively impact processing, although a few Louisiana factories qualitatively test the juice for pH, titratable acidity or Rapid Haze dextran, which are not always reliable.

An indicator or reliable procedure to give evidence to the factory staff whether a certain shipment of cane can be processed economically, if at all, is urgently needed. The second approach, which is a more long-term solution, is to breed for freeze or cold tolerant sugarcane varieties. Because of the prevalence of damaging frosts in Louisiana, historically great emphasis has been placed on the second approach. Legendre et al. (1985) showed that there was a varietal effect on the level of dextrans and total polysaccharides in cane left in the field after freeze damage. However, such breeding programs are highly reliant on quality criteria/indicators to allow proper selection and development of cold tolerant varieties. A variety of physico-chemical criteria or indicators (mostly formed deterioration products) have been reported to measure cane deterioration after a freeze.

Changes in juice pol, titratable acidity and dextran content were reported by Legendre et al. (1985) to be useful criteria. Eggleston and Legendre (2003) recently reported mannitol and isomaltotriose were more sensitive indicators of freeze deteriorated cane; palatinose and leucrose, by-products of dextran-sucrase, the extracellular enzyme of *Leuconostoc* which catalyses the formation of dextran, were useful as indicators of severe cane dextran deterioration (>1500 ppm/Brix in mixed juice).

An indicator/predictor of freeze deterioration will only be useful if it can be directly related to one or more processing problems in the factory that are known to occur when low quality, deteriorated cane is being processed. Moreover, the measure of deterioration should vary predictably as the quality of the deteriorated cane changes. As a consequence, the major objective of this study was to assess freeze deterioration indicators for their effect on juice processing parameters and their associated ability to predict processing problems.

Viscosity and filterability parameters were chosen to determine the processing quality of juices from eight cane varieties subjected to freezes. Viscosity is known to increase with processing of deteriorated cane, which has been mostly attributed to the formation of polysaccharides, and can cause a general slowing down of factory flow rates, as well as detrimentally affect clarification, and reduce evaporation and crystallisation rates. As clarification is a unit process operation which is often detri-
mentally affected by deteriorated cane, a filtration parameter was also used to assess the processing quality of cane juices extracted from freeze deteriorated sugarcane varieties. Another objective of this study was to delineate the freeze deterioration characteristics of commercial sugarcane varieties, to aid breeders in their selection process.

Experimental

Field experiments, freezes during the 2002-2003 cane harvest, and sampling dates

Eight commercial and candidate sugarcane varieties were studied. Two commercial varieties of known stalk cold tolerance were grown as controls and included CP 70-321 (good cold tolerance [Irvine and Legendre, 1985]) and CP 79-318 (poor cold tolerance [Eggleston and Legendre, 2003]). Other varieties studied included four commercial ones: LHo 83-153, LCP 85-384 which is the most widely planted variety in Louisiana and very high yielding but with a tendency to lodge easily, HoCP 85-845 and HoCP 91-555, and two candidate varieties, TucCP 77-42 and HoCP 96-540. HoCP 96-540 was a candidate for commercial release that was subsequently released in June, 2003 and in trials yielded more sucrose/acre than LCP 85-384 in plant cane and stubble crops, and does not lodge as easily (Knipling et al., 2003). In comparison, TucCP 77-42 is currently a major commercial variety being grown in Tucuman, Argentina and is currently being tested for adaptability to Louisiana conditions (Marriott et al., 1991).

Planting on raised ridge rows 1.8 m apart occurred on October 28, 2000 at the Ardoyne farm of the USDA-ARS-SRRC Sugarcane Research Unit at Houma, Louisiana. Variety plots were 15.2 m long and 3 rows wide. The experimental design was a randomised complete block with four replications, and plots were cultivated and fertilised according to recommended practices. Sampling dates and weather conditions are illustrated in Figure 1.

The cane remained in the field until Jan 17, 2003, the day prior to the first freeze of the 2002-2003 crop (or grinding season). On Jan 17 (1 day pre-freeze-control) and on three subsequent dates, Jan 30 (12 days post-freeze), Feb 5 (18 days post-freeze) and Feb 13 (26 days post-freeze), 10-stalk samples were removed serially along the centre row of each plot. Samples were processed for the first sampling date on the date of sampling; however, for subsequent sampling dates, the samples were processed on the day following sampling to allow for sufficient time to process the samples. Each sample consisted of 10 stalks...
cut at the ground by hand but not stripped of leaves or tops. The 10-stalk sample was passed once through a pre-breaker and core press. A sub-sample of milled juice was taken immediately after extraction for dextran (ASI-II method), pH and acid titration analysis. The remainder of the juice was treated with the biocide Bussan 881 (Buckman Labs.), frozen and then transported to the analytical laboratory at the Southern Regional Research Center in New Orleans, LA, where it was stored in a –40°C freezer until analysed.

Freezing temperatures that affected the Louisiana sugar industry during the 2002/2003 harvest occurred on Jan 18–19 and 24–25, 2003 (Figure 1). The min. field temperatures for 18 and 19 Jan, 2003 were between –1.7 to –2.8°C, and on 24–25 Jan the lowest field temperatures recorded were at –4.4°C. After the freezes of Jan 18–19, no freeze cracks were visible, although approx. one-third of the stalk tissue of all varieties appeared affected.

In contrast, after the freezes of Jan 24–25, freeze cracks were observed in all the varieties, with the most in variety TucCP 77-42. Furthermore, most stalk tissue showed visual signs of freeze damage with leakage of juice occurring from the axillary buds.

**Dextran**

Each replicate was analysed for dextran using the ASI (Audubon Sugar Insitute) II method (Sarkar and Day, 1986). Average results of four replicates are reported.

**Titratble acidity and pH**


For the following quality parameters, composites (20 g of each replicate were combined) were analysed:

*Brix*

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

Sucrose, glucose and fructose by gas chromatography

See Eggleston (2002) for method. Results are averages of duplicates.

Mannitol, ethanol and leucrose by ion chromatography with pulsed amperometric detection

See Eggleston et al. (2004) for method. Duplicate samples were diluted (1 g/25 mL) then filtered through 0.45 μm filters. All compounds analysed were quantitated in reference to standards in the linear range.

Haze dextran

Haze dextran in sugarcane juices was determined following the modified alcohol method (ICUMSA GS1-15 [1994]). Juice (30 mL) was added to 30 mL of deionised water initially.

**Definition of processing quality**

The processing quality of the sugarcane juice was evaluated by measuring two physical parameters of viscosity and filtration in the composite samples:

*Juice viscosity*

Viscosity was measured on a Brookfield (Middleboro, U.S.) DV-II+ rotational viscometer at 25°C with a special low viscosity ULA™ adaptor. See Eggleston et al., 2004 for full method. As Brix affects viscosity (Honig, 1953), the juice viscosities were quoted on a Brix basis. A correlation of $r^2 = .91$ existed between the viscosity calculated on a Brix and non-Brix basis.

*Juice filterability*

A method was developed for juice filterability, based on the filtering of juices before pol measurements. Whatman 91 filter paper (185 mm; 10 cm) was folded into four quarters and placed in a funnel over a receiving measuring cylinder (100 mL). Juice 26.00 g/100 mL de-ionised water was prepared in a volumetric flask and shaken thoroughly before filtration. One level teaspoon of celite was added to the filter paper in the funnel. The juice solution (100 mL) was poured into the funnel and allowed to filter for 15 mins; filtrate volume in mLs was measured after 15 mins (4 mins was insufficient for the accurate differentiation of filterabilities among the juices). Percentage filterability was calculated as: filtrate in mL/total juice in mL X 100/1. Repeatability was 5%.
Statistical correlations

Pearson correlation coefficients were calculated to investigate relationships among the various deterioration criteria (N = 32) using PC-SAS 6.12 (SAS Institute, Cary, NC). Means comparisons were undertaken using Duncan’s New Multiple Range Test following ANOVA.

Results and discussion

Sucrose, glucose, and fructose true purities

Table 1—Sucrose, glucose, and fructose true purities\(^a\) (composite samples).

<table>
<thead>
<tr>
<th>Cane variety</th>
<th>1 day pre-freeze (control)</th>
<th>12 days post-freeze</th>
<th>18 days post-freeze</th>
<th>26 days post-freeze</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sucrose True Purity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP 70-321</td>
<td>88.28</td>
<td>86.90</td>
<td>79.05</td>
<td>77.57</td>
</tr>
<tr>
<td>CP 79-318</td>
<td>87.58</td>
<td>83.26</td>
<td>77.48</td>
<td>70.42</td>
</tr>
<tr>
<td>LHo 83-153</td>
<td>87.05</td>
<td>87.14(^b)</td>
<td>79.56</td>
<td>74.39</td>
</tr>
<tr>
<td>LCP 85-384</td>
<td>89.33</td>
<td>90.25(^b)</td>
<td>84.27</td>
<td>79.39</td>
</tr>
<tr>
<td>HoCP 85-845</td>
<td>88.07</td>
<td>86.36</td>
<td>81.67</td>
<td>81.52</td>
</tr>
<tr>
<td>HoCP 91-555</td>
<td>86.99</td>
<td>84.76</td>
<td>72.54</td>
<td>66.74</td>
</tr>
<tr>
<td>HoCP 96-540</td>
<td>88.02</td>
<td>78.52</td>
<td>77.10</td>
<td>78.29</td>
</tr>
<tr>
<td>TucCP 77-42</td>
<td>84.04</td>
<td>70.86</td>
<td>59.07</td>
<td>42.33</td>
</tr>
<tr>
<td></td>
<td>Fructose True Purity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP 70-321</td>
<td>1.11</td>
<td>2.09</td>
<td>2.99</td>
<td>4.53</td>
</tr>
<tr>
<td>CP 79-318</td>
<td>1.36</td>
<td>3.10</td>
<td>4.51</td>
<td>5.93</td>
</tr>
<tr>
<td>LHo 83-153</td>
<td>1.34</td>
<td>3.80</td>
<td>4.51</td>
<td>6.21</td>
</tr>
<tr>
<td>LCP 85-384</td>
<td>1.52</td>
<td>3.25</td>
<td>3.05</td>
<td>4.20</td>
</tr>
<tr>
<td>HoCP 85-845</td>
<td>1.25</td>
<td>1.76</td>
<td>2.37</td>
<td>2.38</td>
</tr>
<tr>
<td>HoCP 91-555</td>
<td>1.40</td>
<td>3.28</td>
<td>5.88</td>
<td>6.98</td>
</tr>
<tr>
<td>HoCP 96-540</td>
<td>1.48</td>
<td>4.71</td>
<td>4.24</td>
<td>4.15</td>
</tr>
<tr>
<td>TucCP 77-42</td>
<td>2.39</td>
<td>6.93</td>
<td>8.59</td>
<td>12.64</td>
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<td></td>
<td>Glucose True Purity</td>
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</tr>
<tr>
<td>CP 70-321</td>
<td>1.01</td>
<td>2.00</td>
<td>2.97</td>
<td>4.92</td>
</tr>
<tr>
<td>CP 79-318</td>
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<td>2.60</td>
<td>4.31</td>
<td>6.71</td>
</tr>
<tr>
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<td>3.46</td>
<td>4.72</td>
<td>6.90</td>
</tr>
<tr>
<td>LCP 85-384</td>
<td>1.30</td>
<td>2.95</td>
<td>3.56</td>
<td>4.92</td>
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<tr>
<td>HoCP 85-845</td>
<td>1.16</td>
<td>1.75</td>
<td>2.78</td>
<td>2.89</td>
</tr>
<tr>
<td>HoCP 91-555</td>
<td>1.26</td>
<td>2.97</td>
<td>5.34</td>
<td>7.21</td>
</tr>
<tr>
<td>HoCP 96-540</td>
<td>1.24</td>
<td>3.86</td>
<td>4.07</td>
<td>4.88</td>
</tr>
<tr>
<td>TucCP 77-42</td>
<td>2.06</td>
<td>5.23</td>
<td>6.88</td>
<td>12.54</td>
</tr>
</tbody>
</table>

\(^a\) True purity is % sugar measured by gas chromatography quoted on a Brix basis.

\(^b\) The rise in sucrose from 1 day post-freeze for these two varieties is due to experimental error.

Any study on sugarcane deterioration is concerned with sucrose losses not only because of the reduction in profits for growers and processors, but also for the formation of sucrose degradation products such as glucose, fructose and acids which can adversely affect processing, i.e., by increasing lime consumption and raising losses of sucrose to molasses. Except for TucCP 77-42 which had markedly lower sucrose and higher invert levels, there was little variation among varieties in the sugar concentrations in the pre-freeze undeteriorated samples (Table 1). After 12 days post-freeze, only two varieties, HoCP 96-540 and TucCP 77-42, had marked sucrose losses with associated invert increases, although HoCP 96-540 stabilised well from 12-26 days post-freeze in comparison to TucCP 77-42 where extensive sucrose losses and deterioration occurred. Overall 18-26 days post-freeze, all varieties suffered sucrose losses with HoCP 85-845 suffering the least, and LCP 85-384 also being quite tolerant against extensive sucrose losses (Table 1).

In this study, fructose concentrations were usually greater than those of glucose (Table 1) which most likely indicates dextran formation because dextransucrase (EC 2.4.1.5), an enzyme secreted mainly by Leuconostoc bacteria, hydrolyses glucose from the sucrose molecule to form dextran, leaving fructose...
(from the sucrose) as a secondary product. However, for most varieties, glucose/fructose ratios generally increased with post-freeze days. The formation of mannitol from fructose by mannitol dehydrogenase (EC 1.1.1.67) also secreted by Leuconostoc may have contributed to this, but it also suggests that Leuconostoc deterioration was not solely responsible for all the freeze deterioration and that other microbial, enzymic and chemical reactions were also occurring (Eggleston et al., 2003).

**Sugarcane Leuconostoc deterioration**

The major contributor to sugarcane deterioration in Louisiana, where humid conditions prevail, is from Leuconostoc lactic acid bacterial infections (Eggleston and Legendre, 2003; Eggleston et al., 2001; Eggleston, 2002; Godshall et al., 2000). Leuconostoc dextranicum and mesenteroides species produce an extracellular enzyme dextranucrase which catalyses the production of dextran (a mainly α-(1→6) linked glucose polysaccharide) from sucrose, but Leuconostoc mesenteroides is considered (Lillehoj et al., 1984) the more prolific producer in sugarcane (see Figure 2).

Moderate and severe dextran 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 crystallisation rates, raises losses of sucrose to molasses and distorts factory pol readings. Worse still, in the U.S., the factory is penalised by refineries on dextran in the raw sugar. Although clarification processes remove some dextran (Eggleston et al., 2003), commercial dextranase is often used in sugarcane factories to break down the dextran. In some Louisiana factories, dextran concentrations, 1000 ppm/Brix in mixed juice cause the staff to add dextranase (Adrian Monge, personal communication), while other factories just add it when factory processes are obviously suffering (Tony Parris, pers. comm.).

Numerous products other than dextran are formed by Leuconostoc mesenteroides bacteria, which are of importance in sugar manufacture, including mannitol, leucrose, palatinose, a series of isomaltooligosaccharides, lactic and acetic acids, and ethanol (Figure 2). However, not all these Leuconostoc products are formed from the same enzymic pathway and formation varies with conditions, which are also illustrated in Figure 2. Although dextran is considered to be the most detrimental product to the factory because it is a
high viscosity polymer, *Leuconostoc mesenteroides* is also capable of producing other polymers in lower concentrations: levan (a fructose polysaccharide or fructan [Robyt and Walseth, 1979] and alternan (an alternating \(\alpha-(1\rightarrow6)-\) and \(\alpha-(1\rightarrow3)-\) linked glucose polysaccharide [Cote and Robyt, 1982]), and their formation may have been under-estimated or under-appreciated by sugar manufacturers as contributors to impeding high viscosity problems at the factory.

Furthermore, the presence of levans in sugarcane products had previously been only attributed to *Bacillus* bacteria (Irvine and Tilbury, 1972).

Mannitol, formed by the action of mannitol dehydrogenase on fructose (Bliss, 1975), was also reported by Steinmetz et al. (1998) to be strongly correlated with frost-damaged sugarbeets, and Eggleston (2002) observed it to be a more sensitive indicator of *Leuconostoc* activity than dextran on sugarcane deterioration.

Moreover, products such as mannitol, isomaltooligosaccharides, and leucrose are present in relatively lower concentrations than dextran and are, therefore, more easily measured and quantitated and can be used as indirect measures or indicators of dextran deterioration (Thielecke, 2002; Eggleston, 2003).

Many *Leuconostoc* products were formed on freeze deterioration in the sugarcane varieties in this study. Dextran formation varied markedly with sugarcane variety (Table 2), and Haze dextran was highly correlated with ASI-II dextran \((r^2 = 0.85, P<.0001)\), although the correlation was low in the pre-freeze samples, which reflects the difficulty of measuring very low amounts of dextran. Only HoCP 96-540 and LHo 83-153 were statistically \((P<.05)\) different 1 day pre-freeze (Table 2).

### Table 2—Changes in dextran (ASI-II method) concentrations.

<table>
<thead>
<tr>
<th>Sugarcane variety</th>
<th>Av. ASI-II Dextran (ppm/Brix)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day pre-freeze</td>
</tr>
<tr>
<td>CP 70-321</td>
<td>112 AB, ba</td>
</tr>
<tr>
<td>CP 79-318</td>
<td>135 AB, b</td>
</tr>
<tr>
<td>LHo 83-153</td>
<td>83 B, b</td>
</tr>
<tr>
<td>LCP 85-854</td>
<td>155 AB, a</td>
</tr>
<tr>
<td>HoCP 85-845</td>
<td>133 AB, b</td>
</tr>
<tr>
<td>HoCP 91-555</td>
<td>132 AB, b</td>
</tr>
<tr>
<td>HoCP 96-540</td>
<td>195 A, b</td>
</tr>
<tr>
<td>TucCP 77-42</td>
<td>131 AB, b</td>
</tr>
</tbody>
</table>

* Upper case letters represent statist. differences \((P<.05)\) among the varieties on the date studied. Lower case letters represent statist. differences \((P<.05)\) between the four dates studied for each variety.

After 12 days post-freeze, little dextran formed in CP 70-321, LHo 83-153, HoCP 91-555 and HoCP 85-845, with only TucCP 77-42 showing a statistical increase over the control (1 day pre-freeze). However, more variation existed 18 and 26 days post-freeze, with generally more being measured after 26 days.

Extremely poor tolerance to dextran formation was observed in variety TucCP 77-42 even 12 days post-freeze, and the high dextran levels at least minimally explain the corresponding very low sucrose levels (Table 1).

Overall, LHo 83-153 and HoCP 85-845 showed marked and stable tolerance to dextran formation over the dates studied. Except for the anomalous 18 days post-freeze, the newly released commercial variety HoCP 96-540 showed similar tolerance to dextran formation as the existing major commercial variety LCP 85-384, with no statistical differences across the freeze dates studied (Table 2).

Eggleston (2002) previously showed that IC-IPAD could be used to simultaneously measure low molecular weight *Leuconostoc* products including ethanol and mannitol, and typical chromatograms of juice from variety TucCP 77-42 over the dates studied are shown in Figure 3. The formation of mannitol is further illustrated in Figure 4.
Like dextran, small amounts of mannitol were present in the pre-freeze samples confirming *Leuconostoc* presence but not exponential growth. Mannitol was strongly correlated with both ASI-II dextran ($r^2 = 0.85$, $P<.0001$) and Haze dextran ($r^2 = 0.80$, $P<.0001$) re-confirming previous results (Eggleston and Legendre, 2003) that it can be used to detect sugarcane freeze deterioration. The larger correlation with ASI-II dextran was not surprising because it is a more specific method for dextran, whereas Haze dextran can detect other polymers contributing to the ‘haze’ as well.

As mannitol is formed by mannitol dehydrogenase rather than dextranulose which forms dextran (see Figure 2), mannitol can be considered an independent indicator of *Leuconostoc* activity and
capable of not only indicating dextran but other *Leuconostoc* polymers as well, such as levan or fructan (Figure 2), and this may explain why the correlation with dextran was not higher. Furthermore, mannitol was also more highly negatively correlated with sucrose concentration \( r^2 = -0.88, P < 0.0001 \) than ASI-II dextran \( r^2 = -0.84, P < 0.0001 \), which suggests that mannitol can also indicate deterioration that contributes to expensive sucrose losses.

Leucrose is also formed by *Leuconostoc* on sugarcane deterioration. Dextranases are capable of catalysing the transfer of glucose from sucrose to other carbohydrate acceptors present in sugarcane juice (Figure 2). D-Fructose, present in sugarcane juice as a product of inversion reactions and a by-product of dextran formation, acts as an acceptor to form leucrose (Figure 2).

Eggleston (2002) showed that the formation of leucrose in sugarcane juice on deterioration, was slower than for mannitol and isomaltotriose, and Eggleston and Legendre (2003) observed it was a more useful indicator of severe dextran formation. It can be seen in Figure 5 that there was very little or even no leucrose in the pre-freeze undeteriorated varieties.

Leucrose formation occurred on freeze deterioration particularly 18–26 days post-freeze. However, in varieties HoCP 85-845, CP 70-321 and LHo 83-153, little formation occurred until 26 days confirming the slow formation of leucrose compared to mannitol (Figure 5) and dextran (Table 2).

The correlation between leucrose and mannitol was very high \( r^2 = 0.89, P < 0.0001 \) because both are formed from fructose. However, the concentrations of leucrose were much lower than for mannitol (compare Figure 5 with Figure 4), which is most likely because leucrose is only a by-product of dextranases action, whereas mannitol is the major product of mannitol dehydrogenase action. Leucrose was also slightly less correlated with ASI-dextran \( r^2 = 0.78, P < 0.0001 \) than mannitol \( r^2 = 0.85, P < 0.0001 \), further suggesting mannitol is a more sensitive indicator of dextran.

Leucrose was also slightly less correlated with ASI-dextran \( r^2 = 0.78, P < 0.0001 \) than mannitol \( r^2 = 0.85, P < 0.0001 \), further suggesting mannitol is a more sensitive indicator of dextran.

![Fig. 5—Varietal changes in leucrose concentrations with days after freeze.](image)

*Leuconostoc* forms other products when grown on injured cane, such as D-lactic acid, acetic acid and ethanol, which are also shown in Figure 2. However, unlike dextran, mannitol, leucrose and isomaltooligosaccharides which are more specific to *Leuconostoc*, lactic and acetic acids and ethanol are also major products of the growth and activity of numerous other microbes, including yeasts (mannitol has been reported as a minor product of some fungi and yeasts [Aarnikunnas et al., 2003]).

The amount formed depends on the type of microbe, as well as microbial growth parameters including temperature and humidity. Ethanol, advocated as a cane deterioration indicator in burnt wholestalk cane (Lionnet and Pillay, 1987) in South Africa where conditions are predominantly dry, is a major by-product of yeast fermentation reactions. Yeasts convert sucrose into ethanol and carbon dioxide particularly under dry and anaerobic conditions.
In comparison, Lillehoj et al. (1984) reported that ethanol, lactic acid, and carbon dioxide are formed by *Leuconostoc*, if glucose, not sucrose, is the carbohydrate carbon source (Figure 2).

Eggleston (2002) observed that ethanol was not always a direct indicator of cane dextran deterioration, and Eggleston and Legendre (2003), in a field study of freeze deteriorated cane, observed that ethanol was only weakly correlated with dextran ($r^2 = 0.55$) and ‘did not always predict cane dextran deterioration’.

Similar to these recent studies (Eggleston, 2002; Eggleston and Legendre, 2003), ethanol, in this study, was only moderately correlated to ASI ($r^2 = 0.63$) and Haze ($r^2 = 0.62$) dextran, and mannitol ($r^2 = 0.65$), and even more weakly with leucrose ($r^2 = 0.54$).

This confirms that ethanol is not sensitive enough as a predictor of dextran related processing problems, although ethanol is formed on cane deterioration (results not shown) most likely from multiple microbial reactions.

**Changes in pH after freezes**

Changes in the pH and titratable acidity value indicate the formation of acids on deterioration. Acids, particularly D-lactic acid, are produced during sugarcane deterioration mostly from the microbial degradation of sugars and cause the reduction in pH.

The advantage of pH is that it is a parameter which is easy to determine. The disadvantage is that pH is usually unreliable and considered not to be a sensitive measure of deterioration because the buffering capacity of the juice reduces this pH change on deterioration.

This is evidenced by there being no statistical differences for LCP 85-384 and HoCP 85-845 across all four freeze dates in this study, even though average pH decreased moderately (Table 3).

Nevertheless, in many varieties there was a significant ($P<.05$) drop in pH, particularly 18 and 26 days post-freeze (Table 3), with the greatest drop occurring in TucCP 77-42. Moreover, TucCP 77-42 was the only variety where pH and titratable acidity were statistically ($P<.05$) lower and higher than the control, respectively, after just 12 days post-freeze.

**Changes in titratable acidity after freezes**

Values in pH were strongly linearly correlated ($r^2 = -0.79$, $P<.0001$) with titratable acidity, although the correlation was lower than in previous deterioration studies (Eggleston and Legendre, 2003; Eggleston et al., 2001).

Titratable acidity values in cane juice usually increase on deterioration. However, the absolute value of titratable acidity in sugarcane juice alone is not considered as sensitive a deterioration criterion as pH, because the titratable acidity of fresh, undeteriorated juice varies markedly by variety (1.81–3.11 mL in this study), soil type, and environment.

This is further evidenced by the improvement of numerous correlations of titratable acidity with other deterioration indicators, including dextran, viscosity, and filterability, when the pre-freeze undeteriorated data were removed.

Therefore, the change in titratable acidity is more meaningful for deterioration detection than absolute values, but this is not practical at the factory. As expected, the greatest change on post-freeze was .8.44 for TucCP 77-42 which had the greatest pH drop (Table 3).

**Relationship between pH and titratable acidity with dextran**

The relationships between pH and titratable acidity with dextran (ASI-II) are shown in Figure 6; polynomial trend or quadratic fits were better than linear fits, especially for titratable acidity. In graph 6b there is a threshold level where the deterioration effect on titratable acidity becomes greater than the varietal effect, and this corresponds to ~2500 ppm/Brix dextran.

Similarly, deterioration effects on pH become predominant ~2800 ppm/Brix dextran (Figure 6a). Consequently, it is still unclear how practical the use of pH and titratable acidity are as measures of deterioration at the factory where detrimental dextran effects start ~ 1000 ppm/Brix in mixed juice, although the measurement of a specific deterioration acid such as D-lactic acid may be worthwhile considering the strong overall correlations observed with dextran.
Table 3 — The effect of freezes on varietal variations in pH and titratable acidity*.  

<table>
<thead>
<tr>
<th>Cane Variety</th>
<th>1 day pre-freeze (control)</th>
<th>12 days post-freeze</th>
<th>18 days post-freeze</th>
<th>28 days post-freeze</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP 70-321</td>
<td>5.50 A, aa</td>
<td>5.46 AB, a</td>
<td>5.28 AB, a</td>
<td>4.86 BCD, b</td>
</tr>
<tr>
<td>CP 79-318</td>
<td>5.40 BC, a</td>
<td>5.18 B, ab</td>
<td>4.78 C, bc</td>
<td>4.52 D, c</td>
</tr>
<tr>
<td>LHo 83-153</td>
<td>5.46 AB, ab</td>
<td>5.51 A, a</td>
<td>5.31 AB, bc</td>
<td>5.14 AB, c</td>
</tr>
<tr>
<td>LCP 85-384</td>
<td>5.47 AB, a</td>
<td>5.47 AB, a</td>
<td>5.26 AB, a</td>
<td>5.16 AB, a</td>
</tr>
<tr>
<td>HoCP 85-845</td>
<td>5.47 AB, a</td>
<td>5.53 A, a</td>
<td>4.53 A, a</td>
<td>5.38 A, a</td>
</tr>
<tr>
<td>HoCP 91-555</td>
<td>5.49 A, a</td>
<td>5.51 A, a</td>
<td>4.85 BC, b</td>
<td>4.67 D, cb</td>
</tr>
<tr>
<td>HoCP 96-540</td>
<td>5.50 A, a</td>
<td>5.42 AB, a</td>
<td>4.98 ABC, b</td>
<td>5.06 BAC, b</td>
</tr>
<tr>
<td>TucCP 77-42</td>
<td>5.33 C, a</td>
<td>4.52 C, b</td>
<td>4.28 D, bc</td>
<td>3.99 E, c</td>
</tr>
</tbody>
</table>

Average titratable acidity (mLs of 0.1N NaOH)  

<table>
<thead>
<tr>
<th>Cane Variety</th>
<th>pH</th>
<th>Titratble acidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP 70-321</td>
<td>2.44 B, b</td>
<td>2.6 BC, b</td>
</tr>
<tr>
<td>CP 79-318</td>
<td>2.64 B, b</td>
<td>3.08 B, b</td>
</tr>
<tr>
<td>LHo 83-153</td>
<td>2.61 B, b</td>
<td>3.36 BC, b</td>
</tr>
<tr>
<td>LCP 85-384</td>
<td>2.18 C, a</td>
<td>3.14 C, a</td>
</tr>
<tr>
<td>HoCP 85-845</td>
<td>2.69 B, a</td>
<td>2.73 BC, a</td>
</tr>
<tr>
<td>HoCP 91-555</td>
<td>2.41 B, b</td>
<td>2.50 BC, b</td>
</tr>
<tr>
<td>HoCP 96-540</td>
<td>2.10 C, b</td>
<td>2.15 BC, b</td>
</tr>
<tr>
<td>TucCP 77-42</td>
<td>3.11 A, c</td>
<td>6.39 A, b</td>
</tr>
</tbody>
</table>

*Upper case letters represent statistic differences (P<.05) among the varieties on the date studied. Lower case letters represent statistic differences (P<.05) between the four dates studied for each variety.

Evaluation of sugarcane deterioration indicators as predictors of processing problems

The sugarcane varieties, subjected to freeze deterioration, were evaluated for processing quality to further ascertain the degree of deterioration and relationships with sugarcane deterioration indicators.
Viscosity

The practical importance of viscosity in manufacturing operations is related to its effect on fluid flow. It is dramatically affected by changes in temperature and density, decreasing rapidly with rising temperature and increasing with concentration.

The rate of juice flow through vacuum filters and the molasses through the sugar wall in centrifugals (A, B and C) is limited by the viscosity of the stream. Furthermore, viscosity has a profound effect on heat transfer (Payne, 1953) in evaporators and vacuum pans. The presence of dextran and other high molecular weight polymers causes significant increases in juice and syrup viscosities (Ness, 1984), and the viscosity of juice is expected to be related to syrup and molasses viscosities.

Changes in viscosities are shown in Figure 7 and, in general, for all varieties viscosity increased on freeze deterioration. The range of juice viscosities in the pre-freeze juices was small, 12.00-13.23 cP, but the range increased markedly on freeze deterioration achieving a varietal range of 14.05-23.25 cP after 26 days post-freeze.

The viscosity of juices from TucCP 77-42 increased dramatically post-freeze, and varieties CP 70-321 and HoCP 91-555 had substantial increases also (Figure 7).

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In fresh juices from undeteriorated sugarcane, viscosities are expected to increase with increasing sucrose concentration. However, in this study, sucrose concentration was negatively linearly correlated with viscosity ($r^2 = -0.80$, $P < .0001$).

The opposite occurred in these deteriorated samples because the decrease in sucrose was mostly because of microbial degradation with associated formation of polymers which caused the increase in viscosity.

This is confirmed with both Haze ($r^2 = 0.79$) and ASI-II ($r^2 = 0.75$) dextrans, as well as the primary products of sucrose degradation fructose ($r^2 = 0.73$) and glucose ($r^2 = 0.65$) being positively linearly correlated with viscosity at the 0.1% probability level.

As stated, ASI-II dextran and Haze dextran were linearly correlated with viscosity, but we found that quadratic fits were slightly better (Figure 8).

Furthermore, mannitol was more strongly fitted ($r^2 = 0.84$) with viscosity than both Haze ($r^2 = 0.81$) and ASI-II ($r^2 = 0.80$) dextran at $P < .01$. This suggests that, even though mannitol is an indirect indicator of viscosity, it can predict viscosity related processing problems equally as well if not better than dextran.

A simple explanation is that mannitol, as an independent indicator of Leuconostoc activity can indicate dextran and other Leuconostoc polymers such as levan (Figure 2), which also explains why the correlation with Haze dextran was higher than with ASI-II dextran. In comparison to dextran and mannitol, leucrose ($r^2 = 0.71$), ethanol ($r^2 = 0.68$), and pH (r = -0.71) were only moderately correlated with viscosity at $P < .001$. 

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Filterability

Filterability of raw sugars is important to refiners and the economic importance was early recognised (Chen, 1993). Starch, soluble phosphates, wax, dextran, silica and other sugarcane associated polysaccharides have been stated to be filtration-impeding substances (Chen, 1993), although Shafig and Samaniego (1974) found that silica had little or no effect, starch and waxes have some, and the gum and phosphate contents had a high correlation/effect on raw sugar filterability.

Fig. 8—Polynomial trend or quadratic fits of ASI-II dextran, and mannitol with viscosity.

James (1972) reported the depressing effect of deteriorated sugarcane on raw sugar filterability. Filterability of sugarcane juices can also be considered complex as many of the substances affecting raw sugar filterability are from the field cane and present in the juice; however, very little has been reported on this subject. Generally, in this study, filterability decreased with post-freeze deterioration, particularly 18–26 days post-freeze.
A filterability test was developed based on the filterability of diluted (26 g/100 mL) juices for pol readings, which are routinely undertaken at factories on core press juices. The ‘pol filterability’ of deteriorated juices have been often noticed to be more difficult and slow to filter and some sugarcane loads have even been rejected on this criterion in Costa Rica (Adrian Monge, pers. comm.).

As shown in Figure 9, there was a moderate quadratic fit between % pol filterability and mannitol ($r^2 = -0.59$), which was better than the fits for ASI-II ($r^2 = -0.47$) and Haze ($r^2 = -0.37$) dextran This further confirms that mannitol can be used to predict processing problems equally as well if not better than dextran, although it predicted viscosity better than filterability. However, as pH ($r^2 = 0.594$) and ethanol ($r^2 = -0.521$) were moderately fitted with % pol filterability, this is further evidence that deteriorated cane contributes to reduced filterability.

Overall, correlations between filterability and deterioration criteria, including dextran, mannitol, viscosity, sucrose, pH and titratable acidity, improved when the pre-freeze undeteriorated data were removed. In the undeteriorated, pre-freeze juice, many of these deterioration criteria were not present in levels above a threshold which would start affecting processing parameters.

This strongly suggests that other substances contribute to the filterability of fresh, non-deteriorated sugarcane juices, such as starch, phosphates and wax (Chen, 1993), and most likely interfere with the filterability of deteriorated juices as well.

Conclusions

For all varieties, marked changes for most indicators of freeze deterioration were observed, particularly 18 and 26 days post-freeze and, generally, the processing parameters viscosity and % pol filterability, increased and decreased, respectively, on freeze deterioration.

Strong polynomial trend or quadratic fits existed between ASI-II dextran and titratable acidity ($r^2 = 0.92$) and pH ($r^2 = -0.88$). Deterioration effects on titratable acidity became greater than varietal effects at a threshold level of ~2500 ppm/Brix dextran; deterioration effects on pH become predominant ~2800 ppm/Brix dextran.

Titratable acidity and pH may, therefore, only be useful in predicting problems caused by severe dextran concentrations. As detrimental dextran effects at the factory start ~1000 ppm/Brix in mixed juice, it is, therefore, unclear how practical the use of pH and titratable acidity are as indicators of deterioration at the factory, although the measurement of a specific deterioration acid such as D or L lactic acid may be worthwhile considering the strong overall correlations observed with dextran.
Mannitol, produced by mannitol dehydrogenase an extracellular enzyme of *Leuconostoc*, better predicted viscosity ($r^2 = 0.84$) than both ASI-II ($r^2 = 0.80$) and hazy ($r^2 = 0.81$) dextran, because it can independently indicate all *Leuconostoc* polysaccharides including dextran, levan, and alternan. In comparison, ethanol ($r^2 = 0.68$), leucrose ($r^2 = 0.71$), and pH ($r^2 = -0.71$) were only moderately correlated with viscosity.

Mannitol was also better than dextran at predicting % pol filterability, although substances present in the undeteriorated cane juice and sucrose interfere with this processing parameter. Overall, mannitol was the best predictor of cane deterioration which contributes to sucrose losses, dextran related problems, viscosity problems, and to a lesser extent filterability problems. Furthermore, because it is a low molecular weight (MW) compound, it is much easier to detect than the high MW polysaccharide, dextran. Eggleston *et al.* (2004) using model reactions recently showed that mannitol does not degrade under industrial processing conditions, and is present in juices and syrups across the factory.

This gives support to the use of mannitol to indirectly measure dextran and/or sugarcane deterioration in the factory, in press or core juices. A reliable, rapid, easy and inexpensive enzymatic method to measure mannitol at the factory is now being investigated.

Using the sensitive measures of deterioration and processing quality in this study, general ranking was, from best to worst: LHo 83-153 = HoCP 85-845 > LCP 85-384 = HoCP 96-540 > CP 70-321 > CP 79-318 > HoCP 91-555 >>> TucCP 77-42. Variety TucCP 77-42 had significantly ($P<.05$) the worst cold tolerance, with deterioration occurring even after 12 days. Furthermore, all indicators in this study showed that TucCP 77-42 had extremely poor cold tolerance, as it was selected in Argentina where freezes seldom occur.

REFERENCES


PARAMETRES POUR DES CANNES AFFECTEES PAR LE GEL

G. EGGLESTON1, B. LEGENDRE2,3 et T. TEW2
1SRRC-USDA-ARS, 1100 Robert E. Lee Boulevard, New Orleans, LA 70124, USA
Tel: 504-286-4446, Fax: 504-286-4367
E-mail: gillian@srrc.ars.usda.gov
2Sugarcane Research Unit, SRRC-USDA-ARS, Houma, LA 70361, USA
3LSU Ag. Center Research and Extension, St. Gabriel Research Station
St. Gabriel, LA 70776, USA

MOTS CLEFS: Gel, Détérioration, Cannes, Leuconostoc, Mannitol.

Résumé

Il n’existe pas aux États-Unis, une méthode rapide, simple et peu coûteuse pour mesurer le vieillissement de la canne quand elle arrive à la sucrerie. Le personnel ne sait donc pas si la canne va être facile à travailler ou si des problèmes vont surgir à la fabrication. On a étudié ce problème pendant les quatre dernières années avec le but de développer un système pour juger la détérioration. Ce papier donne des résultats pour huit variétés de cannes affectées par le gel. Aux États-Unis, particulièrement en Louisiane, la cause la plus importante de détérioration est la bactérie Leuconostoc. Jusqu’à présent on s’est concentré sur le dextran quand la détérioration est causée par le Leuconostoc; mais beaucoup d’autres produits sont formés par le Leuconostoc, par exemple le levan, le mannitol, et d’autres polysaccharides, tous étant importants à la fabrication. Avec des cannes vertes, affectées par le gel, nous avons suivi le mannitol, produit par le mannitol déhydrogénase, du Leuconostoc, et mesure par la chromatographie ionique; ce produit a donné une corrélation légèrement meilleure (r² = 0.84) avec la viscosité du jus que celles obtenues par ASI-II (r² = 0.80) et celle de la méthode du Haze pour le dextran (r² = 0.81), parce qu’il indique tous les polysaccharides du Leuconostoc. En comparaison, l’éthanol (r² = 0.68), le leucrose (r² = 0.71) et le pH (r² = 0.71) donnent des corrélations plus faibles avec la viscosité. Le mannitol est aussi un paramètre qui