

Indicators of freeze-damaged sugarcane varieties which can predict processing problems

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Abstract

In raw sugar manufacture, the quality of the sugarcane supply to the factory plays the most important role in production costs. Sugarcane can be very susceptible to damage by freezes, and freeze-deteriorated sugarcane can cause problems in processing which sometimes leads to a factory shut-down. A reliable indicator of whether a certain shipment of sugarcane can be processed economically is still needed. This study was undertaken during the 2002/2003 harvest season to measure the cold-tolerance performance of eight commercial sugarcane varieties, and to establish indicators which can predict processing quality and problems. Varieties studied included CP 70-321, CP 79-318, LHo 83-153, LCP 85-384, HoCP 85-845, HoCP 91-555, HoCP 96-540 a newly released Louisiana variety, and TucCP 77-42, developed in Argentina. Freezing temperatures occurred between January 18–19 and 24–25, 2003. The minimum field temperature recorded was -5.1 °C on January 25. Freezing conditions prevailed for 10–14 h during each freeze. Serious tissue damage occurred within the stalks of all varieties following the January 24–25 freeze, including freeze cracks. Samples were taken one day before the first freeze (pre-freeze control) on January 17, and subsequently again 12, 18 and 26 days post-freeze. Marked changes for most indicators of freeze-deterioration, for all varieties, were observed, particularly 18 and 26 days post-freeze, and viscosity increased and percentage pol-filterability decreased on freeze-deterioration. Variety TucCP 77-42 had significantly ($P < 0.05$) the worst cold-tolerance, even after 12 days. Strong polynomial trends or quadratic fits existed between ASI-II dextran and titratable acidity ($r^2 = 0.916$) and pH ($r^2 = -0.883$). Deterioration effects became greater than varietal effects at threshold levels of ~ 2500 and ~ 2800 ppm/Brix dextran for titratable acidity and pH, respectively. Titratable acidity and pH may, therefore, only be useful in predicting problems caused by severe dextran concentrations. Reactions of *Leuconostoc mesenteroides* of importance to sugarcane deterioration, including the production of dextran, levan, and alternan polysaccharides are fully described. Mannitol, produced by mannitol dehydrogenase from *Leuconostoc*, was a slightly better predictor of viscosity ($r^2 = 0.838$) than both ASI-II ($r^2 = 0.802$) and Haze ($r^2 = 0.814$) dextran, because it can indicate all *Leuconostoc* polysaccharides. In comparison, ethanol ($r^2 = 0.676$), leucrose ($r^2 = 0.711$), and pH ($r^2 = -0.711$) were only moderately correlated with viscosity. Mannitol was also better than dextran at predicting percentage pol-filterability, although substances present in the undeteriorated cane juice and sucrose interfere with this processing parameter. Overall, mannitol was the best predictor of sugarcane deterioration which contributes to sucrose losses, dextran, viscosity, and to a lesser extent, filterability problems. Model mannitol degradation reactions, simulating industrial conditions, showed that no mannitol degradation occurred, even after 1 h at 99 °C, pH 5.4 and high or low Brix, which further supports the use of mannitol to indirectly measure dextran and/or deterioration in the factory.

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1. Introduction

In raw sugar manufacture, the quality of the sugarcane supply to the factory plays the most important role in production costs. It is well known that deteriorated

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sugarcane detrimentally affects processing in the factory, and a major source of deteriorated cane in the US is from freezes which can sometimes lead to a factory shut-down. Not only does deteriorated sugarcane cause reductions in profits because of sucrose losses, but also production costs often increase dramatically. The decrease in sugarcane quality during processing leads to increases in the demand for lime because of associated higher acidity, increases in the use of processing aids, such as expensive commercial dextranases and flocculant, retards the settling time in clarifiers and prevents satisfactory removal of suspended impurities, impedes filtration of muds, and often slows factory flow and crystallization rates. Higher levels of invert and other deterioration products raise the cumulative melassagenic effect in the process streams, causing an uneconomic rise in molasses purity and quantity. Falls in recoverable sugar occur, raw sugar yield and quality are reduced, and factories in the US can incur raw sugar dextran penalties from refineries.

Frequent winter freezes in Louisiana force the industry to adapt to a short growing season (7–9 months) and a short milling season (approximately 3 months). The nature and extent of damage to cane by freezes depends on the temperature 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 sugarcane 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, sugarcane damaged by a severe freeze produces juices of lower purity, higher acidity, and abnormal amounts of polysaccharides (Legendre, Tsang, & Clarke, 1985), particularly dextran (Eggleston & Legendre, 2003). Recent studies (Eggleston, 2002; Eggleston & Legendre, 2003; Eggleston, Legendre, & Richard, 2001; Godshall, Legendre, Richard, & Triche, 2000) have confirmed that the major contributor to sugarcane deterioration in Louisiana, where humid conditions prevail, is *Leuconostoc* lactic acid bacterial infection. 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 sugarcane 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 for factory staff to decide whether a certain shipment of sugarcane 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 programmes are highly reliant on quality criteria/indicators to allow proper selection and development of cold-tolerant varieties. Several physico-chemical criteria or indicators (mostly formed deterioration products) have been reported to measure sugarcane 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 that mannitol and isomaltotriose were more sensitive indicators of freeze-deteriorated sugarcane; palatinose and leucrose, by-products of dextransucrase the extracellular enzyme of *Leuconostoc* which catalyzes the formation of dextran, were useful as indicators of severe sugarcane 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 sugarcane is being processed. Moreover, the measure of deterioration should vary predictably as the quality of the deteriorated sugarcane changes. As a consequence, the major objective of this study was to assess freeze-deterioration indicators for their effects 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 sugarcane varieties subjected to freezes. Viscosity is known to increase with processing of deteriorated sugarcane, which has been mostly attributed to the formation of polysaccharides; it can cause a general slowing down of factory flow rates, and can detrimentally affect clarification, and reduce evaporation and crystallization rates. As clarification is a unit process operation, which is often detrimentally affected by deteriorated sugarcane, a filtration parameter was also used to assess the processing quality of sugarcane 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.

2. Materials and methods

2.1. Field experiments, freezes during the 2002/2003 cane harvest, and sampling dates

Field experiments consisting of three-row plots are routinely planted at the Ardoyne farm of the USDA-ARS-SRRC Sugarcane Research Unit at Houma, Louisiana, for the estimation of stalk cold-tolerance of commercial and candidate sugarcane varieties. In this study, 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, which is categorized as having good cold-tolerance (Irvine & Legendre, 1985) and CP 79-318, which is known for its poor cold-tolerance (Eggleston & 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 has 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, Brown, & Gray, 2003). In comparison, TucCP 77-42 is currently a major commercial variety being grown in Tucumen, Argentina and is currently being tested for adaptability to Louisi-

ana conditions (Mariott, Levi, Duncelman, & Legendre, 1991).

Planting occurred on October 28, 2000, on raised ridge rows 1.8 m apart. Variety plots were 15.2 m long and 3 rows wide. The experimental design was a randomized complete block with four replications. Plots were cultivated and fertilized according to recommended practices; insecticides were applied as required, according to the economic threshold (Legendre, 2001). The sugarcane remained in the field until January 17, 2003, the day prior to the first freeze of the 2002/2003 crop (or grinding season). On January 17 (1 day pre-freeze-control) and on three subsequent dates, January 30 (12 days post-freeze) and February 5 (18 days post-freeze) and 13 (26 days post-freeze), 10-stalk samples were removed serially along the centre row of each plot. Sampling dates and weather conditions are illustrated in Fig. 1. 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 sufficient time for processing 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 analyzed.

The first freezes of the 2002/2003 harvest actually occurred on January 4 and 14, 2003; however, the extent and duration of these two initial freezes caused no

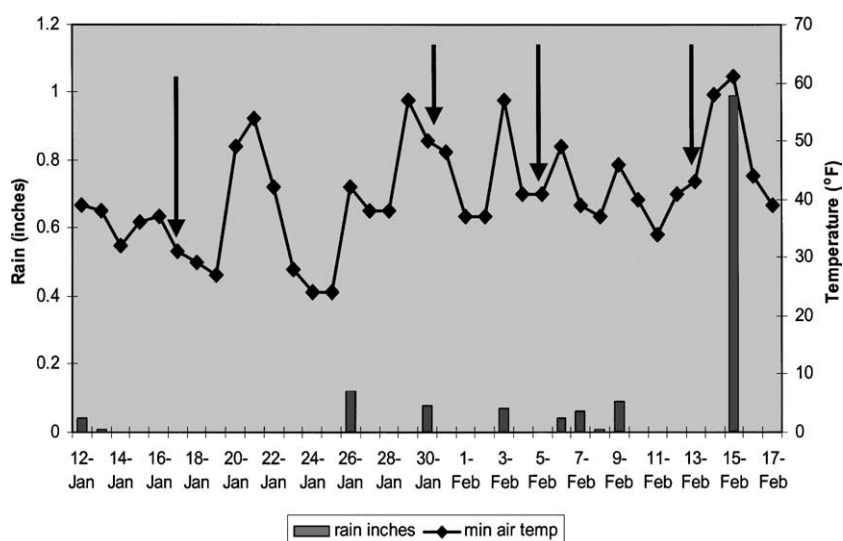


Fig. 1. 2003 weather data and sampling dates for USDA-ARS-SRRC Research Station, Houma, LA, USA. Sampling dates are noted by arrows.

Table 1
Freeze data

Freeze date (2003)	Minimum temperature		Duration of freeze in hours
	°F	°C	
18 January	25.5	−3.6	13
19 January	25.7	−3.5	10
24 January	23.1	−4.9	14
25 January	22.9	−5.1	14

noticeable damage to the apical meristem (bud) or stalk tissue. Freezing temperatures that affected the Louisiana sugar industry during the 2002/2003 harvest occurred on January 18–19 and 24–25, 2003, which is illustrated in Fig. 1. The actual minimum temperatures recorded and approximate duration of each freeze in the field at the Ardoyne Farm test site are listed in Table 1.

After the freezes of January 18 and 19, no freeze cracks were visible, although approximately one-third of the stalk tissue of all varieties appeared affected. In contrast, after the freezes of January 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 auxiliary buds. It was evident from field investigations that serious tissue damage had occurred within the stalks of all varieties following the January 24–25 freezes.

Freezing temperatures occurred on January 18 and 19, 2003 when the minimum field temperatures were between −1.7 and −2.8 °C, and again on January 24 and 25 when the lowest field temperatures recorded were at −4.4 °C.

2.2. Dextran

Each replicate was analyzed for dextran using the ASI (Audubon Sugar Institute)-II method (Sarkar & Day, 1986, Chap. 9) which uses dextranase. Average results of four replicates are reported.

2.3. pH and titratable acidity

Measurements of pH were at room temperature (~25 °C) on a Orion Model 720 pH-meter using an Orion Sure-flow Model 81-72BN electrode. At the start of each day the pH-meter was calibrated against two buffers of pH 4.01 and 7.00. Titratable acidity was determined on four replicates by quantifying the volume, in ml, of 0.1 N NaOH required to raise the pH of cane juice (50 ml) to a pH of 8.3, using a pH-meter to check the endpoint. The results were expressed in ml of 0.1 N NaOH per 10 ml of juice.

For the following quality parameters, composite (20 g of each replicate were combined) samples were analyzed.

2.4. °Brix

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

2.5. Sucrose, glucose and fructose by gas chromatography

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

2.6. Mannitol, ethanol, and leucrose by ion chromatography with integrated pulsed amperometric detection (IC-IPAD)

See Eggleston (2002) for method. Duplicate samples were diluted (1 g/25 ml), then filtered through 0.45 μm filters. All compounds analyzed were quantified in reference to standards in the linear range. Ethanol and mannitol were quantified in reference to ethanol (linear range 0–1000 ppm) and mannitol (0–15 ppm), leucrose in reference to isomaltose (0–50 ppm), and isomaltotriose in reference to isomaltotriose (0–15 ppm).

2.7. Haze dextran

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

2.8. Definition of processing quality

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

2.8.1. Viscosity

Viscosity was measured on a Brookfield (Middleboro, US) DV-II⁺ rotational viscometer at 25 °C. Because of the low viscosity of the juice samples, a special low viscosity ULA™ adaptor was used, to ensure torque was >10%. Temperature was maintained via a jacketed adaptor sample cell (16 ml) which was connected to a Neslab RTE-100™ waterbath accurate to ± 0.1 °C. The shear rate applied was 150 rpm. Viscosity, in centipoise (cP), was determined with Brookfield Wingather™ software and was calculated as % torque \times the spindle factor. Preliminary experiments were undertaken to ensure repeatability of the method applied. To remove Brix affects (Honig, 1959), juice viscosities were quoted on a Brix basis. A correlation of $r^2 = 0.91$ existed between the viscosity calculated on a Brix and non-Brix basis.

2.8.2. Filterability of juices

Two simple laboratory methods were developed to measure the differences in juice filterability amongst the samples.

2.8.2.1. Method (A). Undiluted juice was filtered under vacuum. The filtration apparatus consisted of a 55 mm Buchner funnel and 50 ml flask, attached to a constant laboratory vacuum line (22–23 in. Hg vac). All measurements were undertaken at room temperature ($\sim 25^\circ\text{C}$). Whatman qualitative filter paper (55 mm; #1 medium fast flow [$8\ \mu\text{m}$]) was added to the Buchner funnel. The filter paper was first pre-wetted with 8 drops of the sample to be measured. Sample (20 ml) was added to the funnel under vacuum and allowed to filter for 2.0 min. After 2.0 min, the filtrate volume was measured using a measuring (10–25 ml) cylinder; percentage filterability was calculated as: filtrate in ml/total juice in ml $\times 100/\text{l}$. Preliminary experiments were undertaken to ensure repeatability ($\sim \pm 10\%$).

2.8.2.2. Pol-filterability method (B). Another laboratory method was developed for juice filterability, based on the filtering of juices before pol measurements. Whatman 91 filter paper (185 mm; $10\ \mu\text{m}$) was folded into four quarters and placed in a funnel over a receiving measuring cylinder (100 ml). Juice 26.00 g/100 ml deionized 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 min; filtrate volume in millilitres was measured after 15 min (4 min was insufficient for the accurate differentiation of filterabilities among the juices). Percentage filterability was calculated as: filtrate in ml/total juice in ml $\times 100/\text{l}$. Repeatability was $\pm 5\%$.

The linear correlation between the two filterability methods was $r^2 = 0.42$; $P < 0.0005$.

2.9. 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.

2.10. Mannitol – model thermal degradation reactions

Mannitol was reacted at high temperature ($99 \pm 1.0^\circ\text{C}$) in low and high Brix sucrose solutions. For the low Brix (15.7) sucrose solution; 15.0 g sucrose was added to 85 ml, pH 5.4, sodium acetate buffer in a small conical flask. Mannitol (0.3 g) was added to the mixture and

stirred until total dissolution. For the high Brix (66.7) sucrose solution; 52 g sucrose was added to 28 ml sodium acetate buffer. The solution was slightly heated until all the sucrose dissolved. Mannitol (1.04 g) was added to the mixture and stirred until total dissolution. The conical flasks were covered with aluminium foil and placed in a water bath at $99 \pm 1.0^\circ\text{C}$ and 25 rpm. Aliquots (5 ml) were removed from each flask after 0, 5, 10, 30 and 60 min and cooled on ice before Brix and GC analysis (Eggleston, 2002). The high Brix solutions were diluted to ~ 15 Brix before GC analysis.

3. Results and discussion

3.1. Sucrose, glucose and fructose true purities

Any study on sugarcane deterioration is concerned with sucrose losses, not only because of the reduction in profits for farmers and processors, but also for the formation of sucrose degradation products, such as glucose, fructose and acids which can adversely effect processing by increasing lime consumption and raising losses of sucrose to molasses. True purities of sucrose and its primary degradation products, fructose and glucose (invert), were analyzed by gas chromatography (GC) and are listed in Table 2. Except for TucCP 77-42, which had markedly lower sucrose and higher invert levels, there was little variation among varieties in the sugar concentrations of the pre-freeze undeteriorated samples (Table 2). 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 stabilized well from 12 to 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 2).

In this study, fructose concentrations were usually greater than those of glucose (Table 2) which most likely indicates dextran formation because dextransucrase (EC 2.4.1.5), an enzyme secreted mainly by *Leuconostoc* bacteria, hydrolyzes 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 & Legendre, 2003).

Table 2
 Sucrose, glucose, and fructose true purities^a (composite samples)

Cane variety	1 day pre-freeze (control)	12 days post-freeze	18 days post-freeze	26 days post-freeze
<i>Sucrose true purity</i>				
CP 70-321	88.3	86.9	79.1	77.6
CP 79-318	87.6	83.3	77.5	70.4
LHo 83-153	87.1	87.1	79.6	74.4
LCP 85-384	89.3	90.3	84.3	79.4
HoCP 85-845	88.1	86.4	81.7	81.5
HoCP 91-555	87.0	84.8	72.5	66.7
HoCP 96-540	88.0	78.5	77.1	78.3
TucCP 77-42	84.0	70.9	59.1	42.3
<i>Fructose true purity</i>				
CP 70-321	1.11	2.09	2.99	4.53
CP 79-318	1.36	3.10	4.51	5.93
LHo 83-153	1.34	3.80	4.51	6.21
LCP 85-384	1.52	3.25	3.05	4.20
HoCP 85-845	1.25	1.76	2.37	2.38
HoCP 91-555	1.40	3.28	5.88	6.98
HoCP 96-540	1.48	4.71	4.24	4.15
TucCP 77-42	2.39	6.93	8.59	12.64
<i>Glucose true purity</i>				
CP 70-321	1.01	2.00	2.97	4.92
CP 79-318	0.99	2.60	4.31	6.71
LHo 83-153	1.10	3.46	4.72	6.90
LCP 85-384	1.30	2.95	3.56	4.92
HoCP 85-845	1.16	1.75	2.78	2.89
HoCP 91-555	1.26	2.97	5.34	7.21
HoCP 96-540	1.24	3.86	4.07	4.88
TucCP 77-42	2.06	5.23	6.88	12.54

^a True purity is % sugar measured by GC quoted on a Brix basis.

3.2. Sugarcane *Leuconostoc* deterioration

Recent studies (Eggleston, 2002; Eggleston & Legendre, 2003; Eggleston et al., 2001; Godshall et al., 2000) have confirmed that, in general, the major contributor to sugarcane deterioration in Louisiana, where humid conditions prevail, is *Leuconostoc* lactic acid bacterial infections. *Leuconostoc dextranicum* and *Leuconostoc mesenteroides* species produce an extracellular enzyme, dextransucrase, which catalyzes the production of dextran (a mainly α -(1 \rightarrow 6) linked glucose polysaccharide) from sucrose, but *Leuconostoc mesenteroides* is considered the more prolific producer in sugarcane (Lillehoj, Clarke, & Tsang, 1984) (see Fig. 2). 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 crystallization rates, raises losses of sucrose to molasses and distorts factory pol readings. Worse still, the factory is penalized by refineries on dextran in the raw sugar. Although clarification processes remove some dextran (Eggleston, Monge, & Ogier, 2003), commercial dextransucrase is often used in sugarcane factories to breakdown the dextran. In some Louisiana factories dextran concentrations ≥ 1000 ppm/Brix in

mixed juice cause the staff to add dextransucrase (Adrian Monge, personal communication), whilst other factories just add it when factory processes are obviously suffering (Tony Parris, personal communication).

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, D-lactic and acetic acids, and ethanol. However, not all these *Leuconostoc* products are formed from the same enzymic pathway and formation varies with conditions, which are illustrated in Fig. 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 [Robytt & Walseth, 1979]) and alternan (an alternating α -(1 \rightarrow 6)- and α -(1 \rightarrow 3)-linked glucose polysaccharide [Côté & Robytt, 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 attributed to *Bacillus* bacteria (Imrie & Tilbury, 1972). Mannitol, formed by the action of mannitol dehydrogenase on fructose (Fig. 2), has been observed to reduce sucrose recovery on processing

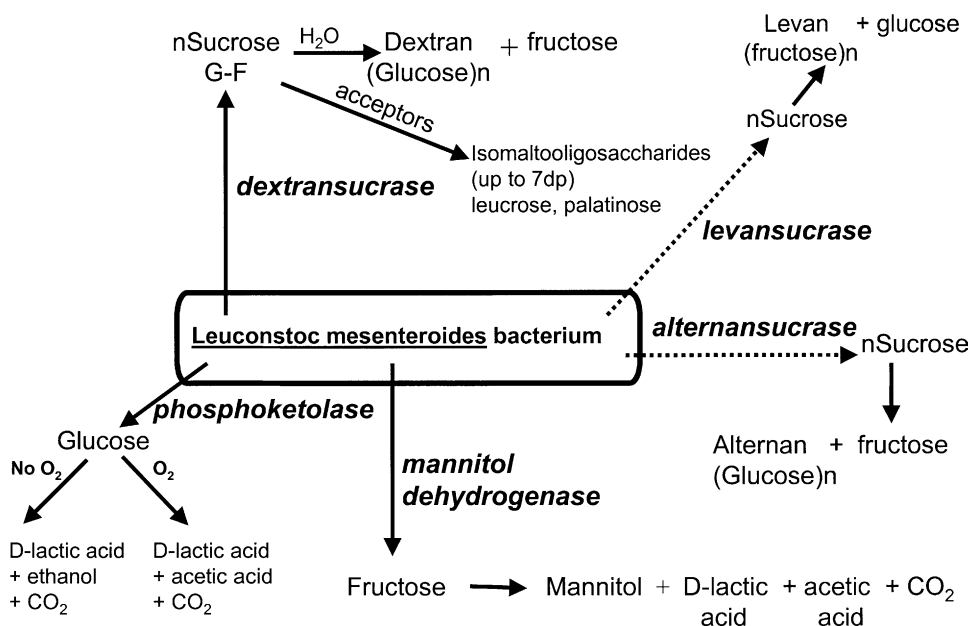


Fig. 2. Metabolic products and enzymic pathways of heterofermentative, lactic acid *Leuconostoc mesenteroides* bacteria of interest of sugarcane deterioration. Dashed arrows indicate enzymic pathways and products. From Robyt and Walseth (1979), Côté and Robyt (1982), Vandamme, Raemaekers, Vekemans, and Soetart (1996), Grobben et al. (2001) and Erten (1998).

(Bliss, 1975). It was also strongly correlated with frost-damaged sugarbeets (Steinmetz, Buczys, & Bucholz, 1998), 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 quantified and can be used as indirect measures or indicators of dextran deterioration (Eggleston et al., 2003; Thielecke, 2002).

Many *Leuconostoc* products were formed on freeze-deterioration in the sugarcane varieties in this study. Dextran formation varied markedly with sugarcane variety (Table 3), and Haze dextran was highly correlated with ASI-II dextran ($r^2 = 0.851$, $P < 0.0001$) 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 ($P < 0.05$) different 1 day pre-freeze (Table 3). 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 2). 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,

Table 3
Changes in dextran (ASI-II method) concentrations

Sugarcane variety	Av. ASI-II dextran (ppm/Brix)			
	1 day pre-freeze (control)	12 days post-freeze	18 days post-freeze	26 days post-freeze
CP 70-321	112 AB, ba	995 B, b	1942 C, b	4561 C, a
CP 79-318	135 AB, b	2573 B, ab	5297 C, a	4848 C, a
LHo 83-153	83 B, b	598 B, ab	1267 C, ab	1644 C, a
LCP 85-384	155 AB, a	2172 B, a	1817 C, a	3479 C, a
HoCP 85-845	133 AB, b	397 B, b	1248 C, ab	1760 C, a
HoCP 91-555	126 AB, b	928 B, b	8862 AB, a	3537 B, a
HoCP 96-540	195 A, b	2417 B, b	7048 BC, a	3102 C, b
TucCP 77-42	131 AB, b	11272 A, a	12673 A, a	15869 A, a

Upper case letters represent statistical differences ($P < 0.05$) amongst the varieties on the date studied. Lower case letters represent statistical differences ($P < 0.05$) between the four dates studied for each variety.

HoCP 96-540, showed tolerance to dextran formation similar to the existing major commercial variety LCP 85-384, with no statistical differences across the freeze dates studied (Table 3).

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 Fig. 3. The formation of mannitol is further illustrated in Fig. 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 ($r^2 = 0.854$, $P < 0.0001$) and Haze dextran ($r^2 = 0.797$, $P < 0.0001$), re-confirming previous reports (Eggleston & 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 dextransucrase, which forms dextran (see Fig. 2), mannitol can be considered an independent indicator of *Leuconostoc* activity and capable not only of indicating dextran but other *Leuconostoc* polymers as well, such as levan or fructan (Fig. 2), and this may explain why the correlation with dextran was not higher. Furthermore, mannitol was also more highly negatively correlated with sucrose concentration ($r^2 = -0.882$, $P < 0.0001$) than ASI-II dextran ($r^2 = -0.835$, $P < 0.0001$), which suggests that mannitol can also indicate deterioration that contributes to expensive sucrose losses.

Leucrose is also formed by *Leuconostoc* on sugarcane deterioration. Dextransucrase is capable of catalyzing

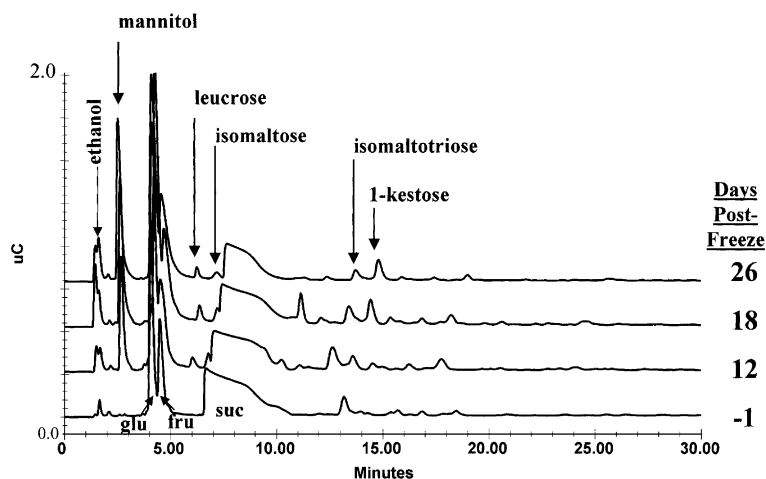


Fig. 3. Changes in IC-IPAD chromatograms for variety TucCP 77-42. Brixes were not standardized.

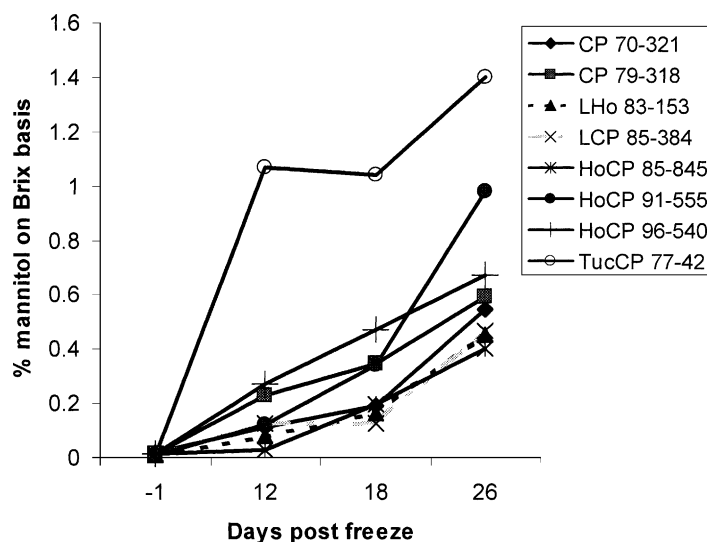


Fig. 4. Varietal changes in mannitol concentration with days after freeze.

the transfer of glucose from sucrose to other carbohydrate acceptors present in sugarcane juice (Fig. 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 (Fig. 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 Fig. 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 (Fig. 5) and dextran (Table 3). The correlation between leucrose and mannitol was very high ($r^2 = 0.889$, $P < 0.0001$) because both are formed from fructose. However, the concentrations of leucrose were much lower than those of mannitol (compare Fig. 5 with Fig. 4), which is most likely because leucrose is only a by-product of dextransucrase action, whereas mannitol is the major product of mannitol dehydrogenase action. Leucrose was also slightly less correlated with ASI-dextran ($r^2 = 0.781$, $P < 0.0001$) than mannitol ($r^2 = 0.854$, $P < 0.0001$), further suggesting that mannitol is a more sensitive indicator of dextran.

Leuconostoc forms other products, when grown on injured sugarcane, such as D-lactic acid, acetic acid and ethanol, which are also shown in Fig. 2. Acetic acid is produced more than ethanol when aerobic conditions prevail (Fig. 2). However, unlike dextran, mannitol, leucrose and isomaltooligosaccharides, which are more specific to *Leuconostoc*, lactic and acetic acids and eth-

anol are also products of the growth and activity of numerous other microbes. The amount formed depends on the type of microbe, as well as microbial growth parameters, including temperature and humidity. Ethanol, advocated as a sugarcane deterioration indicator in burnt whole-stalk cane (Lionnet & 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 (Fig. 2). Eggleston (2002) observed that ethanol was not always a direct indicator of sugarcane 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 & Legendre, 2003) ethanol, in this study, was only moderately correlated to ASI ($r^2 = 0.634$) and Haze ($r^2 = 0.618$) dextran, and mannitol ($r^2 = 0.645$), and even more weakly with leucrose ($r^2 = 0.543$). This confirms that ethanol is not sensitive enough as a predictor of dextran-related processing problems, although ethanol is formed on sugarcane deterioration (results not shown), most likely from multiple microbial reactions.

3.3. Changes in pH and titratable acidity after freezes

Changes in pH and titratable acidity among the varieties and on freeze deterioration are listed in Table 4. Changes in the titratable acidity and pH value indicate

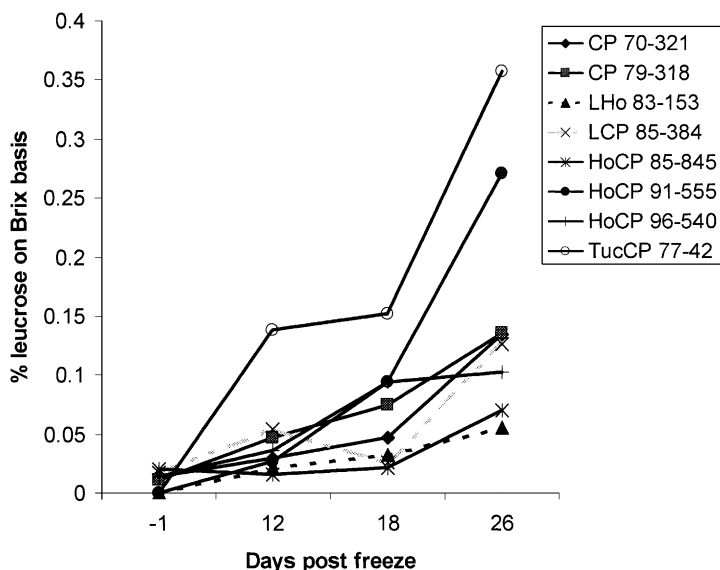


Fig. 5. Varietal changes in leucrose concentration with days after freeze.

Table 4
The effect of freezes on varietal variations in pH and titratable acidity^a

Cane Variety	1 day pre-freeze (control)	12 days post-freeze	18 days post-freeze	26 days post-freeze
<i>Average pH</i>				
CP 70-321	5.50 A, aa	5.46 AB, a	5.28 AB, a	4.86 BDC, b
CP 79-318	5.40 BC, a	5.18 B, ab	4.78 C, bc	4.52 D, c
LHo 83-153	5.46 AB, ab	5.51 A, a	5.31 AB, bc	5.14 AB, c
LCP 85-384	5.47 AB, a	5.47 AB, a	5.26 AB, a	5.16 AB, a
HoCP 85-845	5.47 AB, a	5.53 A, a	5.43 A, a	5.38 A, a
HoCP 91-555	5.49 A, a	5.51 A, a	4.85 BC, b	4.67 D, cb
HoCP 96-540	5.50 A, a	5.42 AB, a	4.98 ABC, b	5.06 BAC, b
TucCP 77-42	5.33 C, a	4.52 C, b	4.28 D, bc	3.99 E, c
<i>Average titratable acidity (mL of 0.1 N NaOH)</i>				
CP 70-321	2.44 B, b	2.46 BC, b	2.81 B, cb	3.83 BC, a
CP 79-318	2.64 B, b	3.08 B, b	4.23 B, ab	4.85 B, a
LHo 83-153	2.61 B, b	3.36 BC, b	2.79 BC, ab	3.12 BC, a
LCP 85-384	1.81 C, a	3.14 C, a	1.91 C, a	2.24 C, a
HoCP 85-845	2.69 B, a	2.73 BC, a	2.70 BC, a	2.59 C, a
HoCP 91-555	2.41 B, b	2.59 BC, b	4.22 B, a	4.92 B, a
HoCP 96-540	2.10 C, b	2.15 BC, b	3.00 BC, a	2.58 C, ab
TucCP 77-42	3.11 A, c	6.39 A, b	8.07 A, b	11.55 A, a

^a Upper case letters represent statistical differences ($P < 0.05$) amongst the varieties on the date studied. Lower case letters represent statistical differences ($P < 0.05$) between the four dates studied for each variety.

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 shown 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 4). Nevertheless, in many varieties there was a significant ($P < 0.05$) drop in pH, particularly 18 and 26 days post-freeze (Table 4), with the greatest drop occurring in TucCP 77-42. Moreover, TucCP 77-42 was the only variety where pH and titratable acidity were statistically ($P < 0.05$) lower and higher than the control, respectively, after just 12 days post-freeze.

pH was strongly linearly correlated ($r^2 = -0.79$, $P < 0.0001$) with titratable acidity, although the correlation was lower than in previous deterioration studies (Eggleston & 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 shown 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 $\Delta 8.44$ for TucCP 77-42 which also had the greatest pH drop (Table 4).

The relationships between pH and titratable acidity with dextran (ASI-II) are illustrated in Fig. 6. Polynomial trends or quadratic fits were better than linear fits, especially for titratable acidity. In Fig. 6(b) it is clear that 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 (Fig. 6(a)). 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 affects start at ~ 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.

3.4. Evaluation of sugarcane deterioration indicators as predictors of processing problems

3.4.1. General

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.

3.4.2. Viscosity

The practical importance of viscosity in manufacturing operations is related to its effect on fluid flow. It is

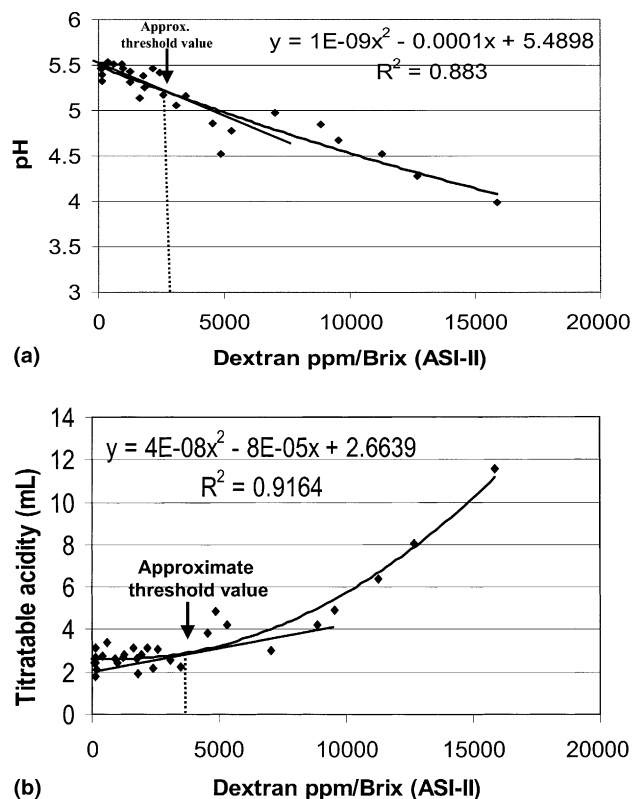


Fig. 6. (a) Relationship of pH with ASI-II dextran in freeze-deteriorated sugarcane. (The short line represents the initial linear relationship.) (b) Relationship of titratable acidity with ASI-II dextran in freeze-deteriorated sugarcane. (The short line represents the initial linear relationship.)

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 Fig. 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 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 also had substantial increases (Fig. 7).

In fresh sugarcane juices, viscosities are expected to increase with increasing sucrose concentration. However, in this study, sucrose concentration was negatively linearly correlated with viscosity ($r^2 = -0.797$, $P < 0.0001$). 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 ($r^2 = 0.789$) and ASI-II ($r^2 = 0.746$) dextrans, as well as the primary products of sucrose degradation, fructose ($r^2 = 0.729$) and glucose ($r^2 = 0.645$), 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, and these are illustrated in Fig. 8. Furthermore, mannitol was more

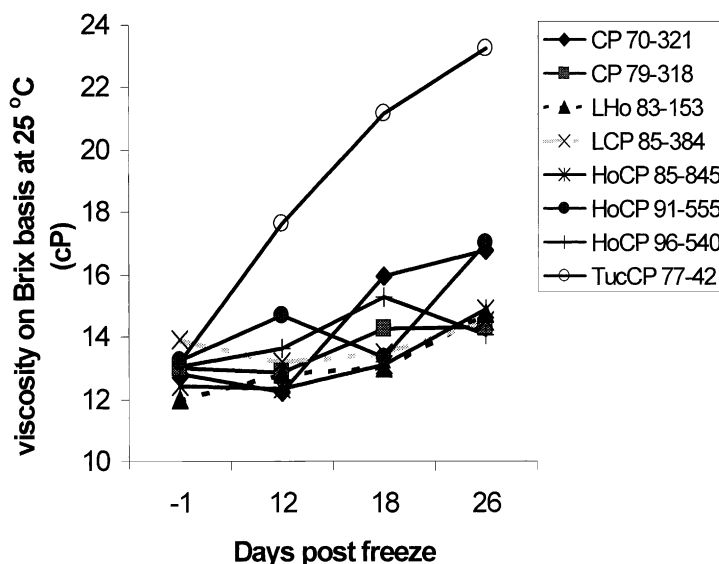


Fig. 7. Changes in viscosity with days after freeze.

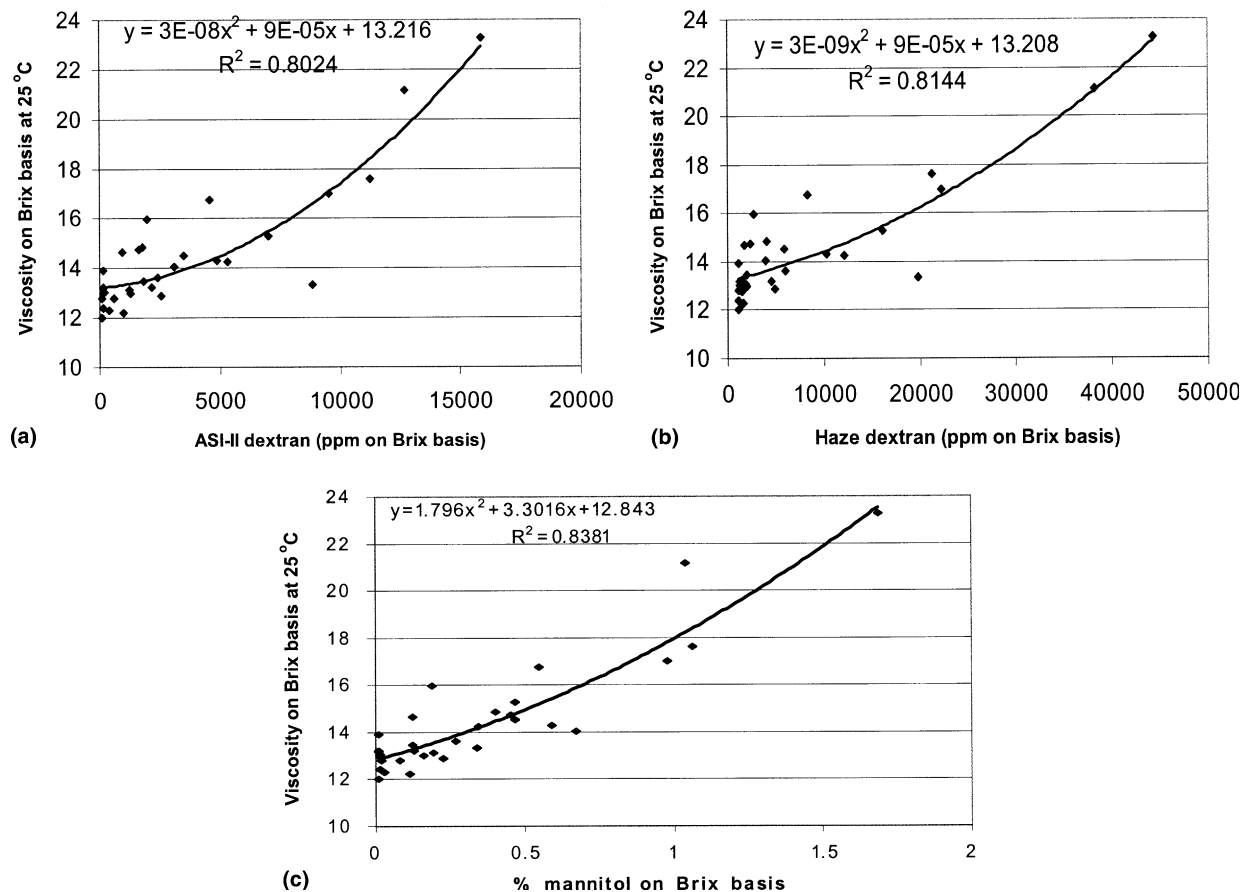


Fig. 8. Polynomial trend or quadratic fits of: (a) ASI-II dextran, (b) Haze dextran and (c) mannitol with viscosity in freeze-deteriorated samples.

strongly fitted ($r^2 = 0.838$) with viscosity than both Haze ($r^2 = 0.814$) and ASI-II ($r^2 = 0.802$) dextran at $P < 0.01$. 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 (Fig. 2), which also explains why the correlation with Haze dextran was higher than with ASI-II dextran. In comparison with dextran and mannitol, leucrose ($r^2 = 0.711$), ethanol ($r^2 = 0.676$), and pH ($r^2 = -0.711$) were only moderately correlated with viscosity at $P < 0.001$.

3.4.3. Filterability

Filterability of raw sugars is important to refiners and the economic importance was early recognized (Chen, 1993). Starch, soluble phosphates, wax, dextran, silica and other sugarcane-associated polysaccharides have been stated to be filtration-impeding substances (Chen, 1993), although Shafiq 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. James (1972) re-

ported 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, as measured by both methods used, decreased with post-freeze deterioration, particularly 18–26 days post-freeze.

In the first filterability method we used, denoted method (A), the undiluted juice was filtered under vacuum. Linear correlations of percentage filterability were low but significant ($P < 0.05$), and quadratic fits were better. Percentage filterability was similarly, but only moderately fitted with Haze ($r^2 = -0.578$) and ASI-II dextran ($r^2 = -0.493$), and mannitol ($r^2 = -0.445$) at $P < 0.0001$. This indicated that factors other than *Leuconostoc* products affected filterability, or it may simply be a reflection of the simple filterability method used. This was further shown by the highest correlation being with Haze dextran, as Haze dextran detects the sum of all compounds contributing to the “haze”, and by sucrose being positively fitted with percentage filterability ($r^2 = 0.597$), i.e., the more sucrose in the juice the more it interfered with filterability. Furthermore, pH ($r^2 =$

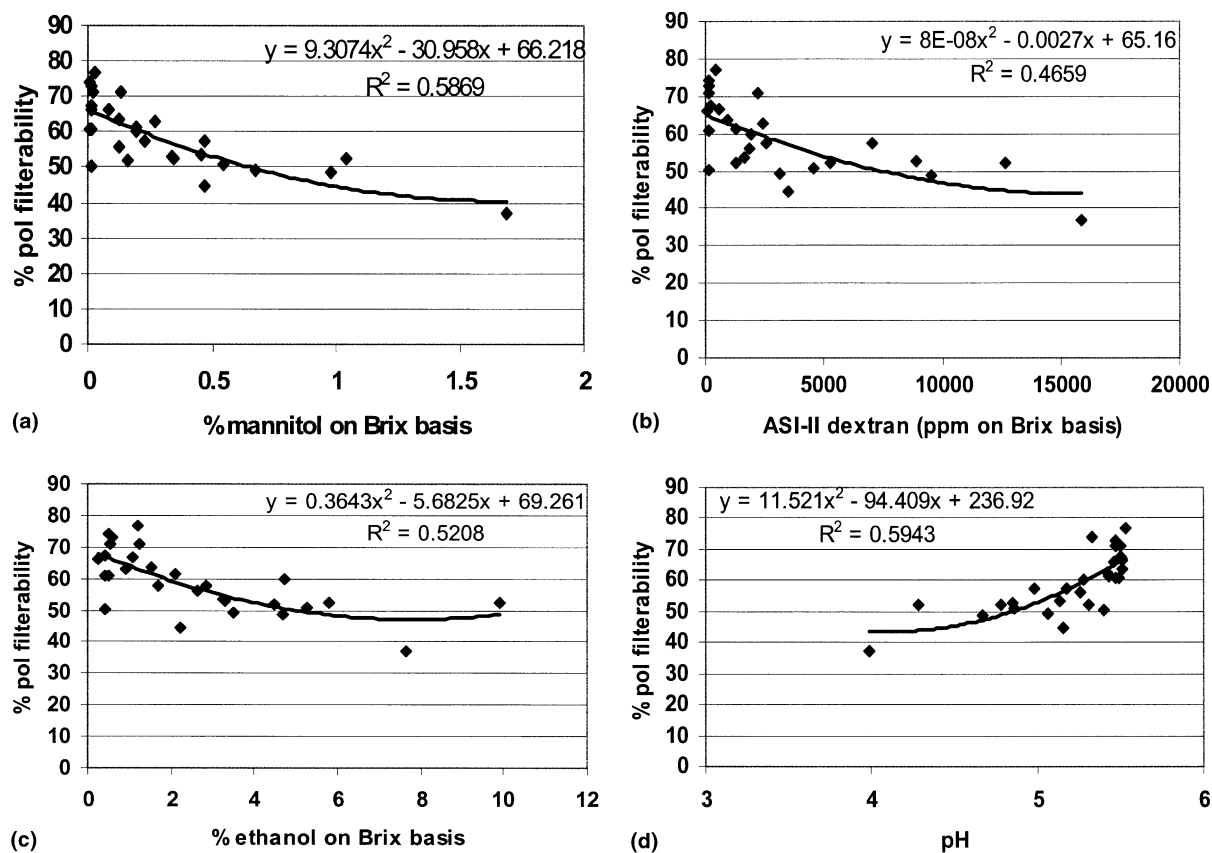


Fig. 9. Polynomial trend or quadratic fits of: (a) mannitol, (b) ASI-II dextran, (c) ethanol and (d) pH with the % pol-filterability of freeze-deteriorated sugarcane samples.

0.546) and ethanol ($r^2 = -0.559$) were also linear correlated with filterability (A) at $P < 0.0001$.

Because of the moderate correlations and suspecting that method (A) may have been too simple, another 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 has often been noticed to be more difficult and slow and some sugarcane loads have even been rejected on this criterion in Costa Rica (Adrian Monge, personal communication). Compared to the filterability method (A), juices in the pol-filterability method were pre-diluted and, therefore, percentage filtration levels were higher. As shown in Fig. 9, there was a moderate quadratic fit between percentage pol-filterability and mannitol ($r^2 = -0.587$), which was better than the fits for ASI-II ($r^2 = -0.466$) and Haze ($r^2 = -0.367$) 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 (Fig. 8) better than filterability (Fig. 9). At the present time, the authors have no simple explanation for the differences in the correlations of ASI-II and Haze dextran and mannitol with the two filterability methods used, although the pol-filterability method was easier to undertake and more precise.

However, as pH ($r^2 = 0.594$) and ethanol ($r^2 = -0.521$) were moderately fitted with percentage pol-filterability (see Fig. 9) 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 at 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, Chap. 9), and most likely interfere with the filterability of deteriorated juices as well.

3.5. Mannitol degradation reactions

As mannitol showed great potential as an indicator of sugarcane deterioration which predicts processing problems, degradation reactions were undertaken to ensure that mannitol does not degrade across the factory, although large quantities of mannitol are often present in clarified juices, evaporator syrups, and massecuites (Eggleston, personal observation). If mannitol

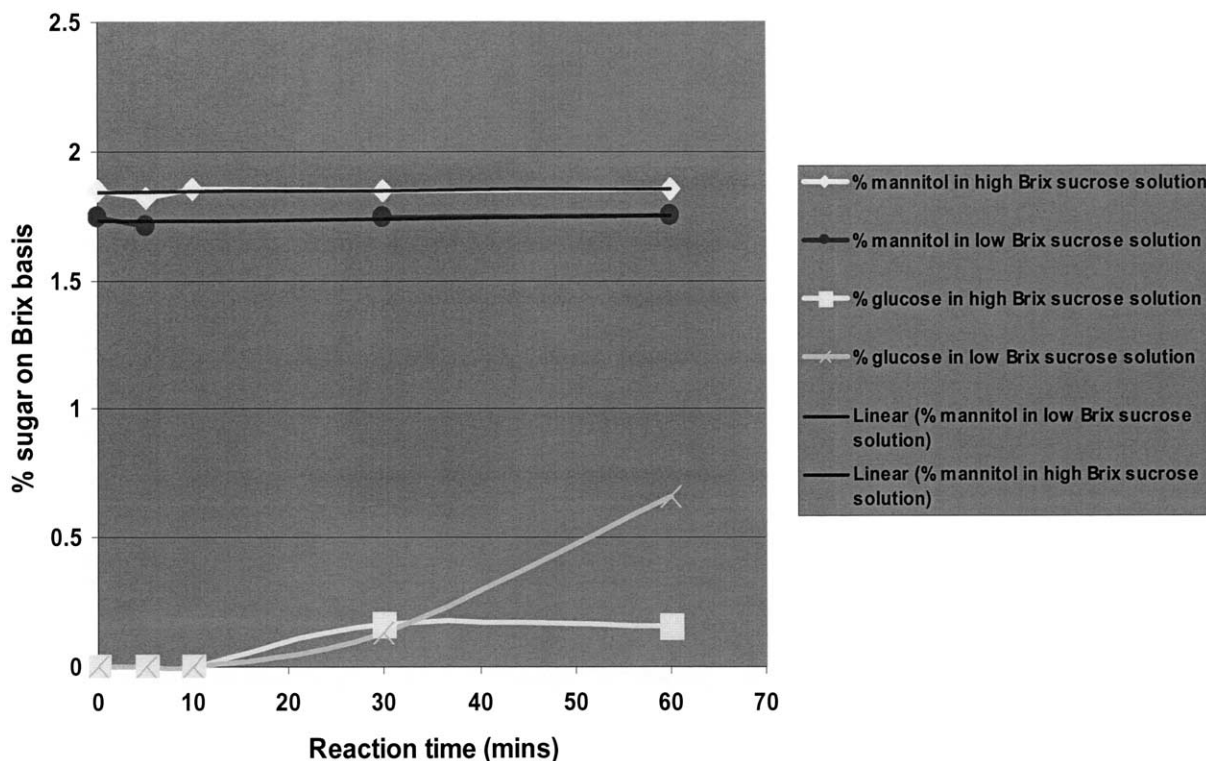


Fig. 10. Model reactions of mannitol under simulated industrial conditions (99 °C; pH 5.4). See Experimental section for full conditions.

did degrade it would not be as useful as an indirect measure of dextran, which degrades very little if at all in the factory unless dextranase is applied. Model thermal reactions of mannitol in a low Brix sucrose solution (15.7) at pH 5.4 to simulate a juice, and in a high Brix sucrose solution (66.7 Brix) at pH 5.4, to simulate a syrup, were undertaken. The concentration of mannitol was 1.9% on a Brix basis in the low and high Brix solutions, respectively. This represents a worse case scenario of extensive sugarcane deterioration and results are illustrated in Fig. 10. No mannitol degradation was detected in either the low or high Brix sucrose solutions over 1 h at 99 °C and pH 5.4, even though some sucrose degradation had occurred after 30 and 60 min, as indicated by the increase in glucose concentrations (Fig. 10). After 60 min, 1.25% and 0.30% sucrose solutions were degraded by inversion in the low and high Brix sucrose solutions, respectively. The non-detection of mannitol degradation under simulated industrial conditions, gives further support to the use of mannitol to indirectly measure dextran and/or sugarcane deterioration in factory core or press juices.

4. 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 percentage pol-filterability, increased and decreased, respectively, on freeze-deterioration. Strong polynomial trends or quadratic fits existed between ASI-II dextran and titratable acidity ($r^2 = 0.916$) and pH ($r^2 = -0.883$). 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 at ~ 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 at ~ 1000 ppm/Brix in mixed juice, it is 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-lactic acid, may be, worthwhile considering the strong overall correlations observed with dextran. Mannitol, produced by mannitol dehydrogenase, an extracellular enzyme of *Leuconostoc*, predicted viscosity better ($r^2 = 0.838$) than both ASI-II ($r^2 = 0.802$) and haze ($r^2 = 0.814$) dextran, because it can indicate all *Leuconostoc* polysaccharides, including dextran, levan, and alternan. In comparison, ethanol ($r^2 = 0.676$), leucrose ($r^2 = 0.711$), and pH ($r^2 = -0.711$) were only moderately correlated with viscosity. Mannitol was also better than dextran at predicting percentage 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. Model mannitol degradation reactions, simulating industrial conditions, also showed that no mannitol degradation occurred, even after 1 h at 99 °C, pH 5.4 and high or low Brix, which gives further support to the use of mannitol to indirectly measure dextran and/or sugarcane deterioration in the factory, in press or core juices. The ultimate goal of this research is to develop a reliable, fast, and simple method to indicate to factory personnel whether a load of sugarcane can be processed economically or if at all.

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 ($P < 0.05$) 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. This was because it was selected in Argentina where freezes seldom occur.

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References

- Bliss, L. Q. (1975). Los sistemas de cosecha y su influencia en la cristalización de los azúcares. *Miscellaneous publication, No. 53 EEAT, Hoy EEAOC, Tucuman, Argentina*.
- Chen, J. C. P. (1993). Raw sugar quality criteria. In J. C. P. Chen & C. C. Chou (Eds.), *Cane sugar handbook* (12th ed., p. 359).
- Côté, G. L., & Robyt, J. F. (1982). Isolation and partial characterization of an extracellular glucanase from *Leuconostoc mesenteroides* NRRL B-1355 that synthesizes an alternating (1→6), (1→3)- α -D-glucan. *Carbohydrate Research*, *101*, 57–74.
- Eggleston, G. (2002). Deterioration of cane juice – Sources and indicators. *Food Chemistry*, *78*, 99–107.
- Eggleston, G., & Legendre, B. L. (2003). Mannitol and oligosaccharides: Potential new criteria for determining cold tolerance in sugarcane varieties. *Food Chemistry*, *80*, 451–461.
- Eggleston, G., Legendre, B. L., & Richard, C. (2001). Effect of harvest method and storage time on cane deterioration I: Cane quality changes. *International Sugar Journal*, *103*, 331–338.
- Eggleston, G., Monge, A., & Ogier, B. (2003). Sugarcane factory performance of cold intermediate and hot lime clarification systems. *Journal of Food Processing and Preservation*, *26*(6), 433–454.
- Erten, H. (1998). Metabolism of fructose as an electron acceptor by *Leuconostoc mesenteroides*. *Process Biochemistry*, *33*(7), 735–739.
- Godshall, M. A., Legendre, B. L., Richard, C., & Triche, R. (2000). Effect of harvest system on cane juice quality. In *Proceedings of the 2000 Sugar Proc. Research Conf.* (pp. 222–236), Porto, Portugal.
- Grobben, G. J., Peters, S. W. P. G., Wisselink, H. W., Weusthus, R. A., Hoefnagel, M. H. N., Hugenholz, J., & Eggink, G. (2001). Spontaneous formation of a mannitol producing variant of *Leuconostoc pseudomesenteroides* grown in the presence of fructose. *Applied Environmental Microbiology*, *67*, 2867–2870.
- Imrie, F. K. E., & Tilbury, R. H. (1972). Polysaccharides in sugar cane and its products. *Sugar Technology Reviews*, *1*, 291–361.
- Irvine, J. E., & Legendre, B. L. (1985). Resistance of sugarcane varieties to deterioration following freezing. *Sugar Cane*, *2*(March/April), 1–4.
- James, G. P. (1972). The depressing effect of deteriorated cane on raw sugar filterability. In *Proceedings of the 39th Session of the Queensland Soc. Sugar Cane Technol.* (pp. 275–277).
- Knipling, E., Brown, W. H., & Gray, J. (2003). Notice of release of sugarcane variety HoCP 96-540. *Sugar Bulletin*, *81*(9), 14–15.
- Legendre, B. L. (2001). Sugarcane production handbook. *Louisiana State University Agricultural Center Pub.* 2859.
- Legendre, B. L., Tsang, W. S. C., & Clarke, M. A. (1985). Changes in juice composition of sugarcane as affected by post-freeze deterioration in Louisiana. In *Proceedings of the 1984 Sugar Proc. Res. Conf.* (pp. 92–107) New Orleans, LA.
- Lillehoj, E. B., Clarke, M. A., & Tsang, W. S. C. (1984). *Leuconostoc* SPP in sugarcane processing samples. In *Proceedings of the 1984 Sugar Proc. Res. Conf.* (pp. 141–151) New Orleans, LA.
- Lionnet, G. R. E., & Pillay, J. V. (1987). Ethanol as an indicator of burn to crush delay. In *Proceedings of the 1987 S. Afr. Sugar Technol. Ass. Mtg.* (pp. 5–7).
- Mariott, J. A., Levi, C. A., Duncelman, P. H., & Legendre, B. L. (1991). Registration of TucCP 77-42 sugarcane. *Crop Science*, *31*(2), 492.
- Ness, J. N. (1984). Viscometry in cane sugar processing. In *Proceedings of the Aust. Soc. Sugar Cane Technol.* (Vol. 6, pp. 271–277).
- Payne, G. W. (1953). The physical and technical conditions in sugar manufacture (temperature, density and pH). In P. Honig (Ed.), *Principles of Sugar Technology* (pp. 431–481).
- Robyt, J. F., & Walseth, T. F. (1979). Production, purification, and properties of dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F. *Carbohydrate Research*, *68*, 95–111.
- Sarkar, D., & Day, D. F. (1986). Dextran analysis: A modified method. *Journal of ASSCT*, *6*, 102–107.
- Shafiq, M., & Samaniego, R. (1974). Filterability of some Philippine commercial sugars. *Sugar News*, *50*(11), 412–426, 422–428.
- Steinmetz, K., Buczy, R., & Bucholz, K. (1998). The quality of frost-damaged sugar beet. *Zuckerindustrie*, *123*(12), 933–942.
- Thielecke, K. (2002). Oligosaccharides and polysaccharides. Referee's Report Subject 2. ICUMSA Reports, pp. 107–122.
- Vandamme, E. J., Raemaekers, M., Vekemans, N., & Soetart, W. (1996). Polysaccharides, oligosaccharides, special sugars and enzymes via *Leuconostoc mesenteroides* sp. fermentations. In T. Faruk Bozoglu & B. Ray (Eds.), *NATO ASI Series: Vol. H 98. Lactic acid bacteria: Current advances in metabolism, genetics and applications* (p. 205). Berlin: Springer.