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Deterioration of cane juice—sources and indicators

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Abstract

Laboratory tests, comparing microbial, enzymic, and chemical deterioration of sucrose in factory cane juice, were undertaken. Heat (boiling temperature) and biocide treated juice, as well as untreated juice, were deteriorated at 27 °C (to simulate factory ambient temperature) in an incubator over 71 h. The biocide-treated juice retained its dark brown colour, fresh odour, initial pH and °Brix levels over 71 h. In strong contrast, after 71 h the untreated juice was light brown, had a strong alcohol odour and markedly lower pH and °Brix levels. The colour of the heated juice decreased only after 23 h, and the juice was viscous after 71 h and had neither a fresh nor alcoholic odour. Sucrose, glucose, and fructose were analyzed using gas chromatography. Over the first 14 h of deterioration, 93.0% of sucrose losses were microbial, 5.7% enzymic and 1.3% were chemical (acid degradation). Ion chromatography with integrated pulsed amperometric detection (IC–IPAD) was used to simultaneously determine ethanol, mannitol, and oligosaccharides in deteriorated cane juice. The rate of formation of mannitol, produced from the reduction of fructose by mannitol dehydrogenase in dextran forming *Leuconostoc* bacteria, was much higher than associated oligosaccharides or ethanol formation. A further investigation of the use of mannitol as a sensitive indicator of future dextran-related processing problems at the cane factory is warranted. Ethanol was shown not to be very useful as an indicator of *Leuconostoc* bacterial cane deterioration.

1. Introduction

Cane deterioration in the field, factory storage pile, or during factory milling processes, has become a topic of major concern in recent years, particularly in the US where mechanical harvesting of billeted sugar cane has increased dramatically. Sucrose destruction reactions in cane deterioration include chemical (acid) and enzymic inversion reactions, and those from microbial activity, and can be influenced by cane health and environmental conditions.

Sugar technologists (Eggleston, Legendre, & Richard, 2001a,b, in press; Lionnet, 1996; Morel du Boil, 1995) have reported a variety of cane deterioration products to confirm cane deterioration and delay (cut-to-crush time), which have been used to predict and control processing problems at the factory. Such deterioration products have included high invert concentrations, microbial (yeast, bacteria, and fungi) contamination (e.g. ethanol and lactic acid concentrations) and polysaccharides, but not all deterioration products impact future factory processes. Lionnet (1996) stated that a cane deterioration product "will be useful only if it can be related to some aspect of the operations of the factory". Dextran polysaccharide (formed mainly by *Leuconostoc* bacteria) has often been reported as a cane deterioration indicator, and is responsible for many of the numerous negative impacts that cane deterioration has on factory processing, mostly associated with the rise in viscosity from this polysaccharide. Oligosaccharides are also products of cane deterioration (Eggleston et al., 2001; Morel du Boil, 1995; Ravelo, Ramos, & Torres, 1995) and are responsible for crystal deformation problems (Morel du Boil, 1991). Ravelo, Ramos, and Mejiras (1991) reported that the formation of total oligosaccharides was greater than the formation of dextran and ethanol in cane subjected to delays and is, therefore, a more sensitive indicator of cane deterioration.

Little work has been accomplished on accurately elucidating the relative contributions of microbial, enzymic, and chemical reactions to sucrose losses on cane deterioration at factory ambient temperatures. This is

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partly because inaccurate purity measurements using optical rotation (referred to as "pol" in the sugar industry) have been too frequently used to evaluate sucrose losses (Kulkarni, 2001). The present investigation was undertaken to determine the relative sources of sucrose degradation in cane juice by using laboratory juice deterioration experiments. By using biocide and heat treatments on factory cane juice, the elucidation of sources was made more clear. Investigation was also undertaken to identify deterioration products which can be used as sensitive indicators of deterioration of cane in the field and at the factory, and which could predict future processing problems. Carbohydrates serve as carbon sources for many microbes, while sugar alcohols (alditols), alcohols, and organic acids are metabolic products. Ion chromatography with integrated pulsed amperometric detection (IC–IPAD) was used to simultaneously determine sugars, alditols, and alcohols in cane juice.

2. Materials and methods

2.1. Factory cane juice deterioration experiments

Factory mixed juice (MJ) of pH 5.16, with no preservatives added, was collected from a Louisiana raw sugar factory before screening, and stored in a laboratory −40 °C freezer until required. MJ (300 ml) was placed in three separate beakers. In the first beaker, 0.08% sodium azide (0.24 g/300 ml juice) was added; in the second beaker, the MJ was brought to a boil and boiled for 1 min, then cooled immediately on ice; in the third beaker, juice was left untreated. The pH values of the three samples were adjusted after treatment to the initial pH of 5.16. Adding azide increased the pH to 5.46 so dilute HCl was added to adjust the pH to 5.16. Boiling of the juice reduced the pH to 5.07, and this was raised to pH 5.16 with dilute NaOH. Subsamples (30 ml) from each treatment were then transferred to smaller beakers representing each time of deterioration, and were lightly covered with parafilm and aluminium foil to prevent evaporation, and to simulate juice in closed pipes and tanks in the factory. All beakers were placed in an non-sterile incubator (VWR General Purpose Incubator, 1500 Series) at 27.1 °C (approx. ambient temperature in the factory). A beaker representing each of the three treatments was removed after 0, 7, 14, 23, 31, 38, 47, 55, and 71 h and immediately placed on ice. °Brix and pH were measured before storage at −40 °C, until further analyses.

2.2. Chemicals and reagents

HPLC grade sodium hydroxide and sodium acetate trihydrate were obtained from Fisher Scientific. Millipore water (18 MΩ) was used to prepare eluents and samples. Standard sugars and sugar alcohols were analytical grade. Sodium azide was from Sigma. 1-Kestose, 1,1 nystose and 1,1,1 fructofuranosylnystose were generously donated by Dr. Takahisa Tokunage of Meiji Seika Kaisha, Ltd. Theanderose (6-α-D-glucosylsucrose) was purchased from Wako chemicals and kindly purified by Dr. Greg Cote of USDA-ARS. Absolute ethanol was from Aaper.

2.3. IC–IPAD analysis of carbohydrates and sugar alcohols

Carbohydrates (mainly oligosaccharides from 2 to 12 degrees of polymerization) and alcohols were determined on duplicate juice samples, diluted 1 g/25 ml, then filtered through a 0.45-μm filter. Carbohydrate IC–IPAD chromatograms were obtained on a Dionex BioLC instrument. The carbohydrates and alcohols were separated on Dionex CarboPac PA-1 guard and analytical anion-exchange columns (250×4 mm), at ambient temperature (~25 °C). Flow rate = 1.0 ml/min. Eluent conditions were: 100 mM NaOH isocratic (0.0–1.1 min; inject 1.0 min), a gradient of 0–300 mM NaOAc in 100 mM NaOH (1.1–40.0 min), and return to 100 mM NaOH (40.1–45.0 min) to re-equilibrate the column. Oligosaccharides and alcohols (from 100 μl injections) were detected with a PED-2 detector; detector conditions are listed in Eggleston and Clarke (1997). Using a Spectra-Physics SP8880 autoinjector and Dionex Peaknet chromatography software, runs were accumulated of multiple samples and check standards. Oligosaccharides, mannitol, and ethanol were identified by comparing retention times with standards and by spiking with standards. Peak heights were measured to reduce the effect of interfering adjacent peaks. Oligosaccharides, palatinose mannitol, and ethanol were quantified in reference to glucose raffinose, myo-inositol, and ethanol standards, respectively.

2.4. Sucrose, glucose and fructose by gas chromatography (GC)

The determination of sucrose, fructose and glucose in cane juice by GC was based on ICUMSA method GS7/4-22 (1998) with modifications. Duplicate samples were derivatized following the oximation–silylation procedure in ICUMSA GS7/4-22 (1998), and heating blocks were used to heat the derivatized samples at 80 °C. Gas chromatograph conditions varied considerably from ICUMSA GS7/4-22 (1998) because further separation of sugars was required for increased accuracy. Separation of sugars occurred on a DB-5 capillary (5%-phenyl)-methyl polysiloxane column (30 m × 0.25 mm i.d., column film thickness was 0.1 mm) on a Hewlett Packard 5890A gas chromatograph, equipped with a

flame ionization detector. Operation conditions: injection port 300 °C; detector 310 °C; column started at 100 °C for 3 min; then it was programmed at 5°/min until 150 °C, then 10°/min until 300 °C, remaining at 300 °C for 10 min. Head pressure was 21 psi with a 25:1 split ratio; sample volume was 1 μl. Trehalose dihydrate (Alltech) was the internal standard for sucrose, and methyl-α-D-glucopyranoside (Sigma) the internal standard for glucose and fructose.

2.5. °Brix

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

2.6. pH

Measurements of pH were at room temperature (~25 °C), using an Ingold™ combination pH electrode calibrated at room temperature using two different pH buffers (pH 7 and 10). The electrode was connected to a Metrohm 716 DMS pH meter.

2.7. Dextran

Duplicate samples were analyzed for dextran using the ASI (Audubon Sugar Insitute) II method (Sarker & Day, 1986) which uses dextranase enzymes.

3. Results and discussion

3.1. Deterioration of cane juices

In order to determine the relative contributions of microbial, enzymic, and chemical deterioration of sucrose in cane juice, laboratory manipulations of cane juice were undertaken. These included the addition of an effective biocide (sodium azide) or heat (at juice boiling temperature). The biocide, sodium azide, was chosen because of its effectiveness to destroy all microbes, which was a requirement of this laboratory study if the relative sources of deterioration were to be quantified. However, because of its toxic character, it would not be appropriate for use as a biocide in the factory. The pH of the treated juices was adjusted to the initial untreated value, in order to eliminate initial differences caused by acid sucrose inversion.

Initially all samples were dark brown and had the characteristic fresh odour of factory cane juice. The untreated juice began to change to a paler colour after 7 h, and by 71 h was a very pale brown and had a "wine" odour. In comparison, the addition of a biocide had a remarkable effect: after 71 h the fresh odour was still apparent and the colour unchanged from dark brown. Sodium azide is an effective biocide, which does not allow any microbial growth to occur; therefore, this was the first indication that much of the deterioration was microbial. In further comparison, the heated juice, changed to a pale colour only after 23 h, and after 71 h had neither a fresh nor "wine" odour. Moreover, the sample was viscous and gummy in appearance, indicating that polysaccharides had formed.

3.2. Analysis of deteriorated cane juices

The pH changes with deterioration time are shown in Fig. 1. Cane deterioration, as indicated by a reduction in pH, started immediately in the untreated cane juice, with the largest changes occurring in the first 14 h, the rate of pH change decelerating thereafter. The pH also decreased in the heated juice but only after a 14 h delay, indicating that either enzymes or microbes (the heat would have denatured the enzymes and destroyed or vastly reduced the numbers of microbes) are responsible for the initial change in pH. In the biocide control sample, there were no marked changes in pH over 71 h, further evidence that microbial growth is mostly responsible for cane deterioration.

°Brix (% dissolved solids) also changed dramatically in the juices on deterioration, as illustrated in Fig. 2. °Brix of the untreated juice decreased slightly over the first 23 h, but the rate of °Brix reduction then accelerated, indicating solids were being utilized as a biomass source and converted into a liquid and/or gas state. In

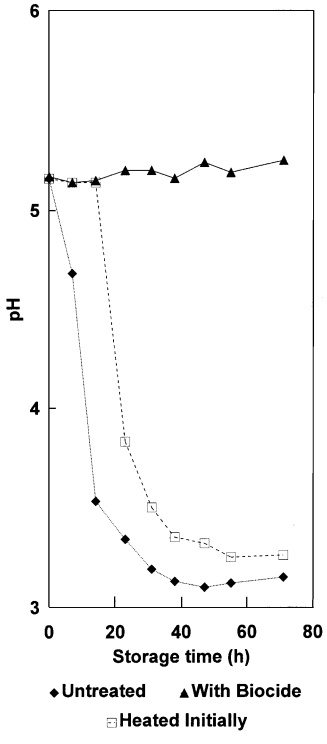

Fig. 1. Changes in pH on deterioration.

comparison, the °Brix of the heated juice decreased only slightly on deterioration, suggesting that deterioration, especially after 14 h, was because of the metabolic change of biomass into another solid source. In the biocide control juice, there was no significant change in °Brix across 71 h.

High and low molecular weight dextran polysaccharide formation in the three juice treatments is shown in Fig. 3. In the untreated juice, dextran formation was slow in the first 7 h, then accelerated between 7 and 14 h. A slowdown then occurred up to 31 h, and then a second acceleration phase (this may just be characteristic of the microbial load). In contrast, in the pre-heated juice, no dextran was formed in the first 14 h, because the heat would have destroyed or vastly reduced the numbers of most of the *Leuconostoc* bacteria initially present in the juice. The large formation of dextran between 14 and 23 h could be because of re-innoculation in the incubator from the non-sterile experimental conditions. A further explanation is that the heat treatment just reduced the number of viable *Leuconostoc* bacteria to a level where lag phase growth occurred, and it took 14 h for the bacteria to recuperate and produce dextran, especially in exponential phase growth. After 23 h no significant dextran was formed which was likely because the very low pH stopped *Leuconostoc* growth and/or the activity of dextransucrase. As expected, in the biocide control juice, there was no formation of dextran over 71 h. Ravelo et al. (1995) applied the disinfectant IFOPOL™ to stored billeted cane and observed that the formation of polysaccharides, as well as total oligosaccharides, was greatly reduced.

Changes in sucrose, glucose, and fructose concentrations, on a °Brix basis, are illustrated in Fig. 4. Degradation of sucrose in the factory can occur via a variety of mechanisms. It can be hydrolysed into glucose and fructose by either acid (acid inversion of sucrose) or by naturally-occurring cane invertase enzymes (sucrose inversion). Another mechanism of sucrose loss is by its utilization by microbes. High infections and stagnant zones occur often in the cane factory, particularly in the milling station, and these act as 'open fermentors'. *Leuconostoc* bacteria are able to utilize the glucose in the sucrose molecule to form dextran (a glucose polysaccharide). Yeast, particularly *Saccharomyces,* often found at factories (Chen & Chou, 1993), can convert sucrose into ethanol and carbon dioxide, especially under anaerobic conditions, often found in cane storage piles and at the factory. Yeasts, and other microbes, are also known to secrete periplasmic invertase enzymes (Hanko & Rohrer, 2000).

In the untreated juice, sucrose degraded rapidly (Fig. 4a), particularly over the first 14 h (29.0% sucrose loss), which is further shown by the concomitant, sharp increase in glucose and fructose concentrations (Fig. 4b and c). Although, after 39 h, sucrose loss decelerated, by 71 h very little sucrose, glucose, and fructose remained, because the solids had been transformed by microbes (see °Brix results). In comparison, the sucrose in the biocide-treated juice was only slightly degraded in the juice (1.7% after 14 h). This slight degree may be because the biocide is unable to stop the enzymic and

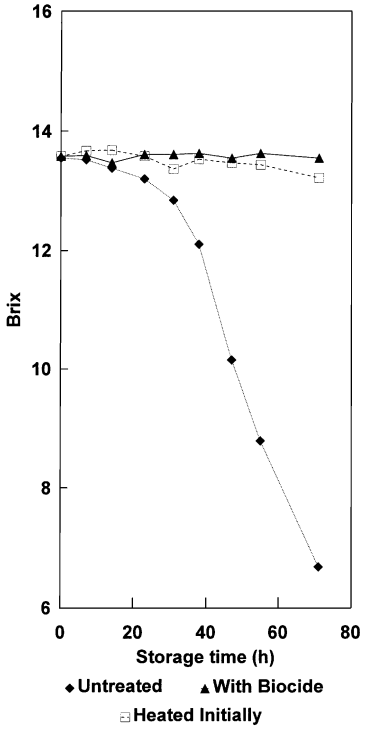

Fig. 2. Changes in Brix on deterioration.

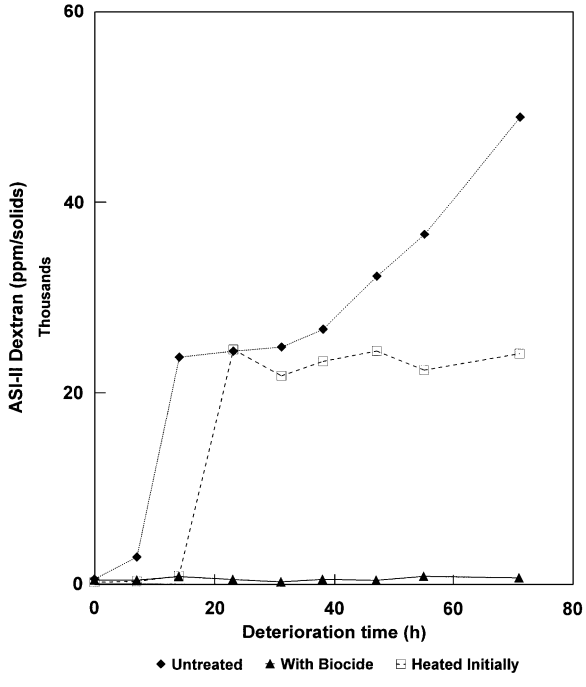

Fig. 3. Dextran formation on deterioration.

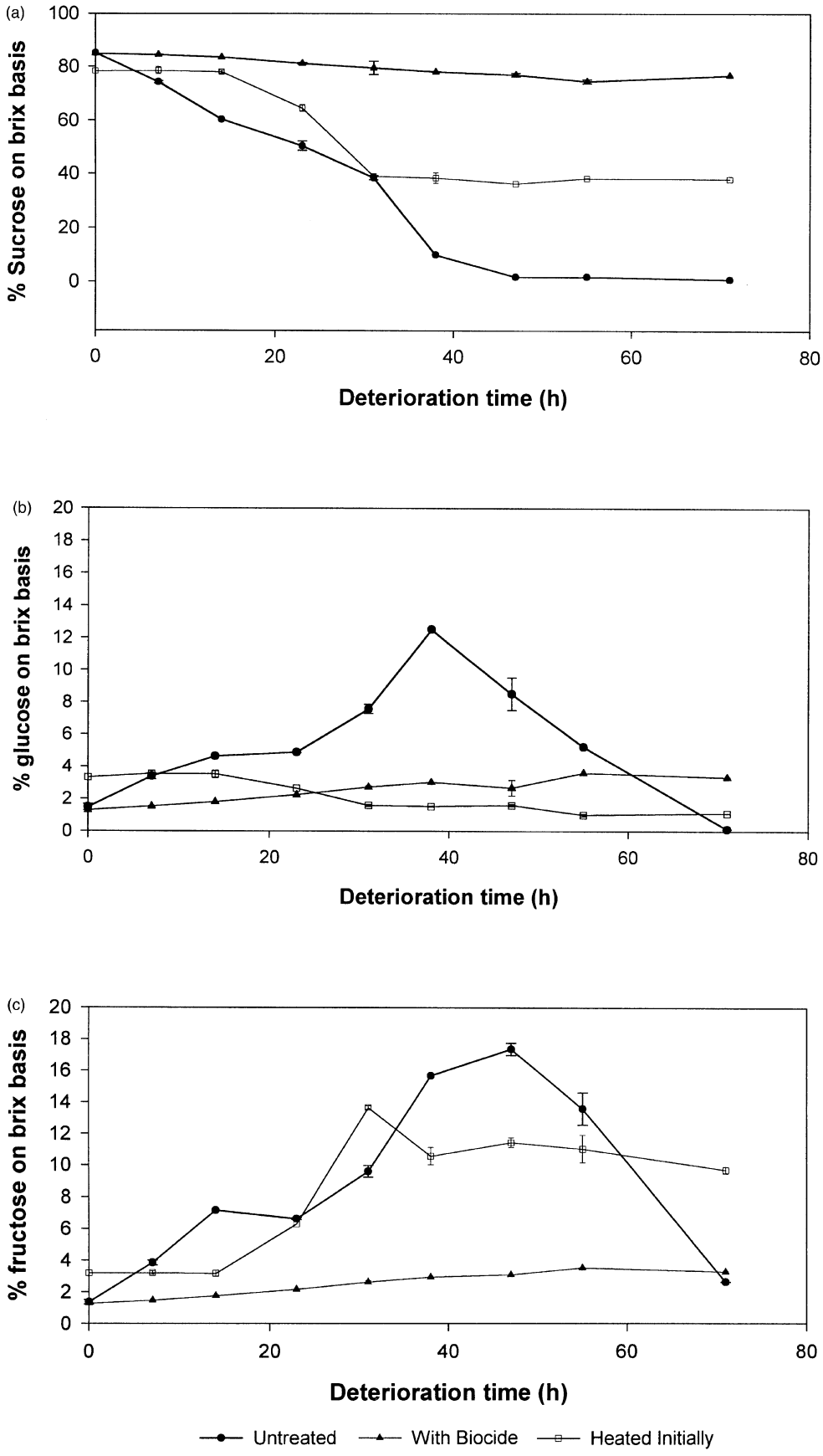

Fig. 4. (a) Sucrose losses on deterioration. (b) Glucose contents on deterioration. (c) Fructose contents on deterioration.

acid inversion of sucrose. In the juice pre-heated before deterioration, only 0.4% sucrose was measurably lost during the first 14 h. This strongly suggests that the heating treatment denatured the invertase enzymes, as well as markedly reducing the levels of microbes (including thermophilic bacteria), and that at ambient temperatures, acid sucrose inversion contributes very little to sucrose loss in the factory. Glucose and fructose similarly increased slightly on sucrose inversion and G/F ratios stayed constant (Table 1).

Using the combination of untreated, biocide-treated, and heat-treated juices after 14 h deterioration, it was possible to calculate the contributions of the different sucrose loss mechanisms. The untreated juice was taken as equivalent to total deterioration, biocide-treated juice as equivalent to enzymic and chemical deterioration, and the pre-heated juice as chemical (acid) deterioration only. It was calculated that 93.0% of deterioration was microbial, 5.7% enzymic, and 1.3% was chemical. As microbiological deterioration is such a major source of loss, the need to use biocide agents, or other aseptic conditions at the factory is highlighted.

Dextran formation on deterioration of the untreated juice was also indicated by the change in G/F ratios (Table 1). Low G/F ratios indicate a relative increase in fructose to glucose, which occurs when dextran is formed because *Leuconostoc* bacteria utilize glucose to form dextran, leaving fructose from the sucrose molecule as a by-product.

3.3. Sensitive indicators of cane deterioration

The author previously developed and used an ion chromatography with pulsed amperometric detection (IC–IPAD) method (Eggleston et al., in press) to study deterioration products of cane, with an emphasis on oligosaccharides. Oligosaccharides are known to form in deteriorated cane during delays between cutting and crushing, but their formation has usually been attributed to microbial (mainly bacterial) activity (Ravelo et al., 1991, 1995) and enzymic activity (Morel du Boil, 1995). However, kestose oligosaccharides, which can form from enzymic activity in the cane, are also degradation products from the acid degradation (inversion) of sucrose (Richards, 1988) and are, therefore, formed from chemical reaction activity as well. The major oligosaccharides formed on cane deterioration (Eggleston et al., 2001b; Morel du Boil, 1995) are from the kestose family: 1-kestose (GF_2), 6-kestose (GF_2), neo-kestose (GF_2), nystose (GF_3), and kestopentaose (GF_4) and kestohexaose (GF_5) isomers, as well as oligosaccharides formed as acceptor (secondary) products (Demuth, Jordening, & Buchnolz, 1999; Robyt & Eklund, 1982) from the action of dextransucrase in *Leuconostoc* strains. Examples of dextran-associated oligosaccharides are isomaltotriose, isomaltotetraose, leucrose, and palatinose.

Table 1
Effect of deterioration time on glucose/fructose ratios

Deterioration time (h)	Unadulterated juice ratio	Juice with biocide ratio	Heated juice ratio
0	1.08	0.99	1.10
7	0.88	1.022	1.11
14	0.65	1.02	1.12
23	0.74	1.04	0.42
31	0.79	1.04	0.12
38	0.79	1.03	0.15
47	0.49	1.03	0.14
55	0.39	1.03	0.09
71	0.05	1.03	0.12

Mannitol is also formed from fructose by the lactic acid *Leuconostoc* bacteria (Soetart, 1991). Fructose, as an alternative electron acceptor, is reduced to mannitol by the enzyme mannitol dehydrogenase (D-mannitol: NAD-2-oxidoreductase, EC 1.1.1.67). During this process, the reducing equivalents are generated by the coupled conversion of glucose into D(−)-lactic acid and acetic acid. The following theoretical fermentation equation was derived by Vandamme, Raemaekers, Vekemans, and Soetart, 1996:

$$2\ \text{Fructose} + \text{Glucose} \rightarrow 2\text{Mannitol} + \text{D(}-\text{)-Lactic Acid} + \text{Acetic Acid} + CO_2$$

Mannitol has recently been identified (Steinmetz, Buczys, & Bucholz, 1998) as a sensitive quality criterion for frost-damaged sugarbeets and, consequently, was analyzed in this study to ascertain if it could be used to indicate cane deterioration.

Ethanol has also been advocated as a cane deterioration indicator (Lionnet, 1996; Lionnet & Pillay, 1988), particularly in burnt whole-stalk cane (Lionnet & Pillay, 1987). It is a metabolic by-product of many microbial reactions, and the amount formed depends on the type of microbe, as well as microbial growth parameters, including temperature and humidity. Ethanol is a major by-product of yeast fermentation reactions, with yeast converting sucrose into ethanol and carbon dioxide, especially under dry and anaerobic conditions. It was asserted by Mackrory, Cazalet, and Smith (1984) that *Leuconostoc* bacteria, besides forming dextran, can also be heterofermentative and produce lactic acid, ethanol and carbon dioxide, although Lillehoj, Clarke, and Tsang (1984) and Erten (1998) reported that this is only the case if glucose, not sucrose, is the carbohydrate carbon source.

Recently, Hanko and Rohrer (2000) used an IC–IPAD method to simultaneously determine sugars, sugar alcohols (alditols), and alcohols produced by growing yeast (*Saccharomyces cerevisae*) cultures and,

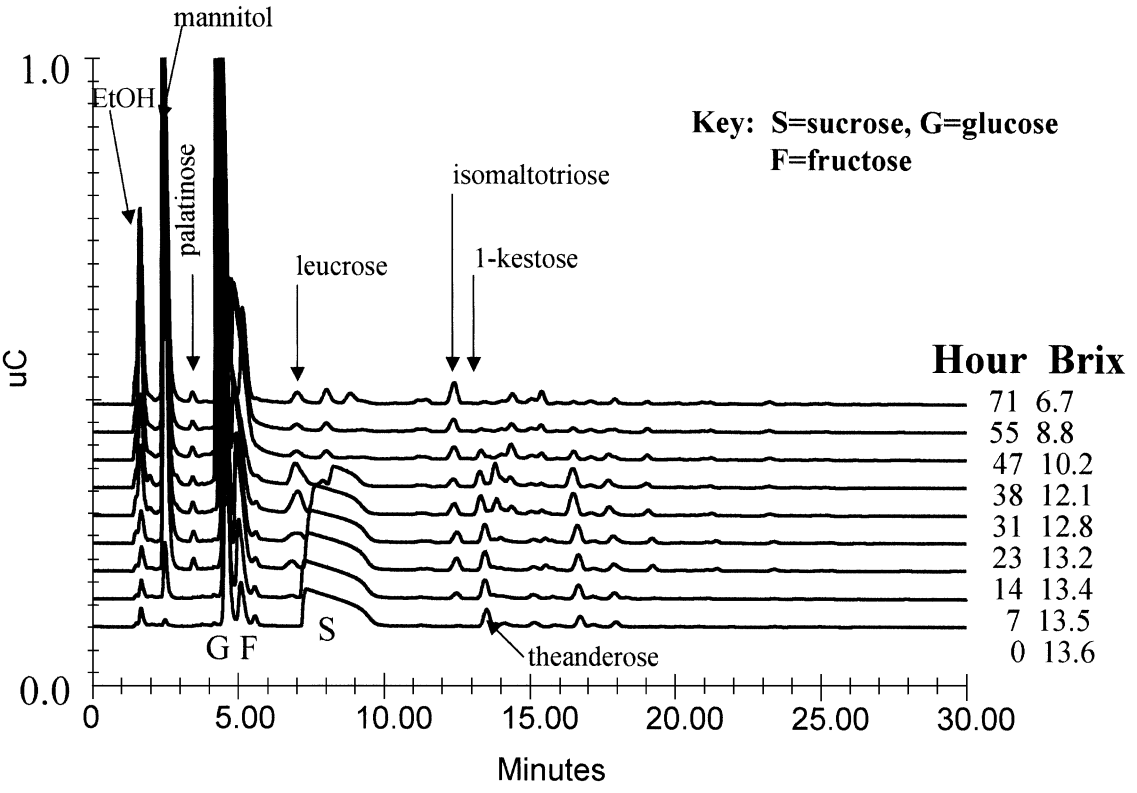

Fig. 5. IC–IPAD chromatograms of the untreated juice on deterioration.

in this study the author was able to use an IC–IPAD method to simultaneously detect oligosaccharides, mannitol (alditol), and ethanol cane deterioration products. The IPAD method is relatively insensitive to ethanol, compared with mannitol, oligosaccharides, and other sugars. However, this may be an advantage when large concentrations of ethanol are forming on microbial deterioration.

There were dramatic changes in the IC–IPAD chromatograms of the untreated juice over 71 h, which are illustrated in Fig. 5. Even after the first 7 h, mannitol and isomaltotriose had increased, and leucrose was visible, confirming that mannitol dehydrogenase and dextransucrase activities were present. However, ethanol had also formed slightly and the °Brix had begun to decrease (Fig. 2) which strongly suggests that *Leuconostoc* bacterial deterioration and other microbial (most likely yeast reactions are involved because of the reduction in °Brix) deterioration reactions were simultaneously occurring in the juice. As expected, for the biocide-treated juice, *Leuconostoc* metabolites including mannitol and isomaltotriose, within experimental error, did not form over the 71 h deterioration time, and leucrose could not be measured (Fig. 6); furthermore, no additional ethanol was formed, and even the initial concentration decreased, which may be because of evaporation. However, the trisaccharide kestoses, neo-, 6-, and 1-kestose, increased over the 71 h, which further confirms that the enzyme, and acid, inversion of sucrose makes a small, but significant, contribution to cane deterioration.

In the pre-heated juice, none of the deterioration products studied increased over the first 14 h (Fig. 7),

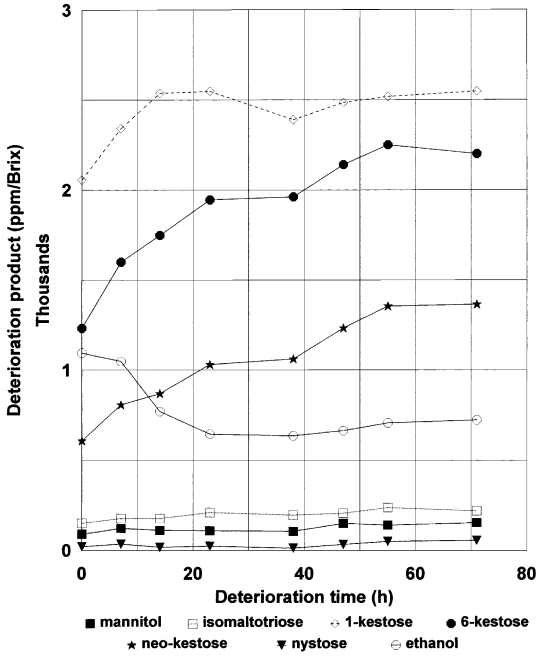

Fig. 6. Deterioration products in biocide treated juice.

confirming previous results that the initial heat treatment delayed deterioration. After 14 h, however, there was a marked increase in dextransucrase metabolites, including isomaltotriose, isomaltotetraose, leucrose, and palatinose. Moreover, as can be seen in Fig. 7, mannitol, also produced by *Leuconostoc* bacteria, but via a different mechanism from dextran and dextransucrase acceptor products, increased even more dramatically. As the rate of formation of mannitol was much higher than the other metabolites, including ethanol (Fig. 7), it would make a sensitive indicator for cane deterioration that could be used by factory staff to check if the load of cane delivered to the factory is going to cause dextran-associated problems in subsequent processing. Moreover, there were very high correlations between mannitol and dextran in the pre-heated ($R^2 = 0.952$, $P < 0.005$) and untreated ($R^2 = 0.98$, $P < 0.015$) juices. Results here warrant an investigation into the use of mannitol as a cane deterioration indicator under industrial conditions.

In marked contrast to the formation of dextran in the pre-heated juice after a 14 h delay, there were no increases in the concentrations of sucrose inversion products, the trisaccharide kestoses. This confirms that inversion of sucrose at ambient temperatures is more attributable to enzymic rather than acid inversion (the pH was very acidic at this stage of deterioration). After 14 h deterioration of the pre-heated juice, results have indicated that the sample was either re-inoculated in the non-sterile incubator or *Leuconostoc* bacteria growth recuperated. A surprising observation was that, even though after re-inoculation dextran, dextran-associated oligosaccharides, and mannitol formed, only a relatively small amount of ethanol formed (Figs. 7 and 8). This strongly suggests that ethanol is only slightly associated with dextran formation in cane juice by *Leuconostoc* bacteria, which may be because it is formed only when glucose is the carbohydrate carbon source (Lillehoj et al., 1984). Furthermore, in strong contrast to the heat- and biocide-treated juices, marked ethanol formation occurred in the untreated juice (Figs. 5 and 8). As the untreated juice clearly had associated yeast fermentation, dextran forming, and other microbial deterioration reactions, these results may explain, to some extent, why ethanol has proven not always to be a good indicator of processing problems associated with deteriorated cane in South Africa (C. Richard, personal communication). Most processing problems, particularly in the US, including increased viscosity reducing flow throughput and slowing of filtration and crystallization rates, are because of the dextran polymer. Ethanol, as suggested from results in this study, is more an indicator of yeast and possibly other, non *Leuconostoc,* bacterial deterioration in cane and, therefore, cannot always predict processing problems associated with dextran.

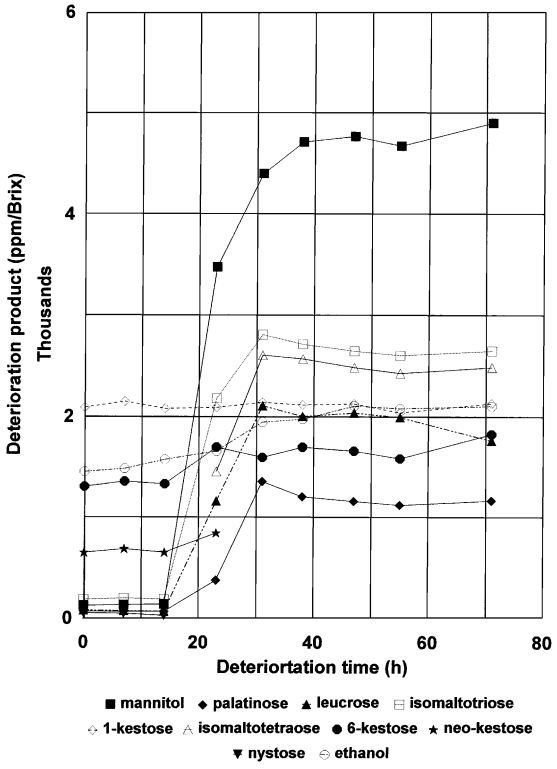

Fig. 7. Deterioration products in heat-treated juice.

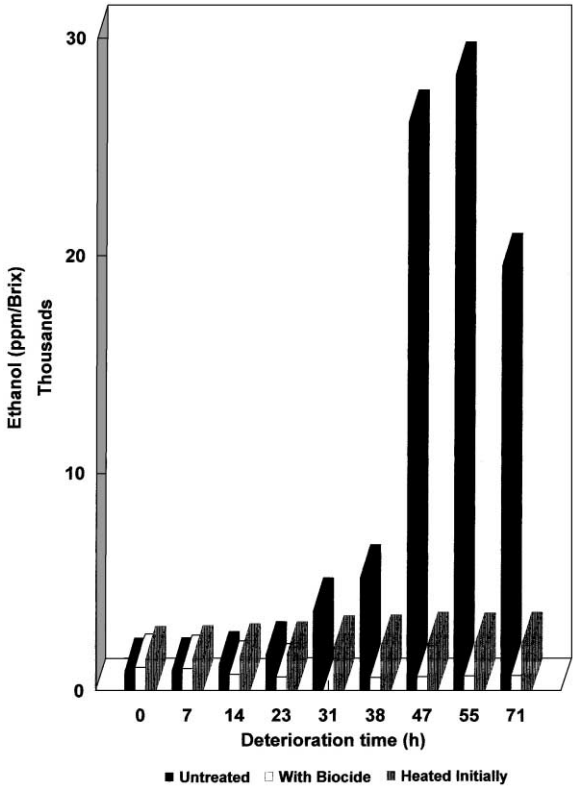

Fig. 8. Ethanol changes on deterioration.

4. Conclusions

By comparing the deterioration (at 27 °C) of heat- and biocide-treated cane juice with untreated juice, it was possible to quantitatively compare the relative contributions of microbial, enzymic, and chemical deterioration on sucrose losses. Over the first 14 h of deterioration, 93.0% of sucrose deterioration was microbial, 5.7% enzymic and 1.3% was chemical (acid degradation). Ion chromatography with integrated pulsed amperometric detection (IC–IPAD) was used to simultaneously analyse for ethanol, mannitol, and oligosaccharides in deteriorated cane juice. The rate of formation of mannitol, produced from the reduction of fructose by mannitol dehydrogenase in dextran-forming *Leuconostoc* bacteria, was much higher than associated oligosaccharides or ethanol formation. A further investigation of the use of mannitol as a sensitive indicator of future dextran-related processing problems at the factory is warranted. Ethanol was shown not to be very useful as an indicator of cane *Leuconostoc* bacterial deterioration. There is now a need to undertake further deterioration studies, with different sources of cane juice and specific inoculations, with yeast and *Leuconostoc* bacteria, which are frequently found in cane juice.

Acknowledgements

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