Assessment of microbial degradation in factory mixed juice and filtrate

C Shi, DW Rackemann, CH Bakir and WOS Doherty

Sugar Research and Innovation, Queensland University of Technology, Brisbane, Qld 4000;
c.shi@qut.edu.au

Abstract Undetermined sucrose loss is a serious problem in raw-sugar manufacturing. Laboratory deterioration experiments were conducted at ambient temperature using factory mixed juice (MJ) and filtrate (FIL). Well-known metabolic products, including mannitol, lactic acid, polysaccharides, oligosaccharides and organic acids, were detected. Unexpectedly, methanol was found in both the untreated and deteriorated juices and is suspected to be caused by the action of micro-organisms on the pectin present in sugarcane juice. The deterioration rate of filtrate was generally slower than that of mixed juice, but the formation of exocellular polysaccharides was significantly higher. The mannitol concentration was a good indicator to predict sucrose loss in MJ, but not in FIL. It is suggested that an evaluation of the use of biocides, particularly in the filter station to reduce the negative impact of polysaccharides due to filtrate recycling to mixed juice, should be undertaken.

Key words Mixed juice, filtrate, microbial deterioration, sucrose

INTRODUCTION

Sucrose loss has long been a common problem for the sugar industry, and occurs between harvesting and milling, and during the sugar-manufacturing process. The three main reaction pathways that result in sucrose loss are microbial, enzymatic and chemical processes. Results of the effects of these processes on mixed juice (MJ) have indicated that 93% of sucrose deterioration is caused by microbial action, while 5.7% and 1.3% is by enzymatic and chemical processes, respectively, within the first 14 h of the deterioration process (Eggleston 2002).

Previous juice-deterioration studies have indicated that even under Australian harvesting and processing conditions, where the lag time between harvesting and milling (i.e. cut-to-crush) is short, and the maintenance of good hygiene practices through sanitisation of the cane milling train, mixed-juice screen, filters and drains, juice deterioration by micro-organisms still occurs (Tilbury *et al.* 1997). Despite this, little work has been conducted by Australian sugar mills to accurately determine the extent of sucrose loss caused by microorganisms, and the use of biocides approved by the Food and Drug Administration (USA) to mitigate sucrose loss. As a consequence of this, the contribution of microbial deterioration to undetermined sucrose loss is unknown.

In addition, microbial deterioration of sucrose also produces many metabolic products that detrimentally affect the evaporation and crystallisation rates, and molasses exhaustion (sugar recovery). These products include glucose-derived polysaccharides (e.g. dextran), fructose-derived polysaccharides (e.g. levan), mannitol, oligosaccharides (e.g. 1-kestose), organic acids (e.g. lactic acid), and ethanol. Rackemann *et al.* (2015) established that clarifying the filtrate (FIL) and directing it to evaporator supply juice (ESJ) improved performance and throughput of the clarification station due to the removal of insoluble solids, polysaccharides and scale-forming calcium-organic acid complex.

Despite the successful outcome of this study, there has been no adoption of filtrate-clarification technology in Australia.

Assessing the impact of microbial degradation on MJ and FIL will establish whether it is beneficial to use a biocide not only to reduce sucrose loss, but also to reduce the formation of metabolic products such as polysaccharides. This control strategy would be a more cost-effective way to reduce the impact of impurities recycled with the FIL in the sugar-manufacturing process than using filtrate-clarification technology.

Here, we examine and compare microbial deterioration of MJ and FIL at ambient temperature (23°C) over a period of up to 129 h to clearly show differences in microbial deterioration between the two types of juices. The evaluations include analysing the initial MJ and FIL compositions and the deterioration products formed at various times, including reducing sugars, organic acids, alcohols, mannitol, 1-kestose and polysaccharides.

LABORATORY MIXED JUICE AND FILTRATE DETERIORATION EXPERIMENTS

Composite samples of MJ and FIL were collected from a sugar mill. The samples were immediately frozen and stored in a freezer at -40°C until required, with no preservatives added. Prior to the experiments, MJ and FIL were placed in separate containers and covered with Parafilm® and aluminium foil to prevent evaporation (to simulate juice in closed pipes and tanks in a factory) and thawed to ambient temperature (23°C). The initial brix and pH of the samples were measured, and 50 mL aliquots were used in the deterioration experiments that were conducted at 0, 4, 6, 8, 24, 48, 72, 82, 96, 120 and 129 h. The MJ used in this study did not contain FIL.

ICP-OES analysis of metal ions in mill juices

Concentrations of inorganic ions were analysed using an inductively coupled argon plasma optical-emission spectrometer (ICP-OES). To reduce the interference of the organic sugar matrix, samples were diluted to a sucrose concentration of 2 wt% with 1% nitric acid.

Sucrose, glucose and fructose by HPLC

Concentrations of sucrose and reducing sugars in the juice samples were determined by high-performance liquid chromatography (HPLC) analysis based on ICUMSA Method GS7/8/7–24. Samples were first diluted to adjust the sucrose concentration to ~1% and then filtered through a 0.45 µm nylon filter prior to analysis. We used an anion-exchange column Dionex Carbopac PA1 (250 mm X 4.0 mm ID) with a guard column. Sugars were separated using this column equilibrated at 35 ± 2°C and detected by a Waters 2465 electrochemical detector. The eluent was 150 mM sodium hydroxide at a flow rate of 1 mL/min.

1-kestose and mannitol analysis

Concentrations of 1-kestose and mannitol in the samples were determined using HPLC. The samples were diluted 50 times to achieve a sucrose concentration within the appropriate range and filtered with a 0.45 µm nylon filter prior to analysis. We used an Aminex HPX-87P Column with a guard column to separate 1-kestose, sugars and mannitol equilibrated at 85 ± 2°C and detected by a Waters 2414 refractor index detector. The eluent was MilliQ water at a flow rate of 0.5 mL/min.

Organic acid and alcohol analysis

Organic acids and alcohols (methanol and ethanol) in the samples were extracted by solid-phase extraction with Waters QMA Sep-pak cartridges. Their concentrations (measured in duplicate) were determined using Waters 2487 dual λ absorbance and Waters 410 refractive index (RI) detectors after they were eluted with 5 mM sulphuric acid at a flow rate 0.5 mL/min from two BioRad Aminex HPX-87Hx columns (300 mm × 7.8 mm ID) connected in series and equilibrated to 35°C and 85°C, respectively (Blake *et al.* 1987).

Polysaccharide analysis

Total polysaccharides were determined from triplicate measurements using the Sugar Processing Research Institute (SPRI) procedure (Roberts 1981). Polysaccharides in the samples were precipitated with absolute ethanol. The precipitate was treated with 1% (w/v) phenol and concentrated sulphuric acid prior to analysis using a Cintra 40 double-beam UV–visible absorption spectrometer at 485 nm.

Brix

The brix of the samples was measured at ambient temperature (~23°C) using a Bellingham and Stanley RFM 342 digital refractometer (Tunbridge Wells, Kent, UK) accurate to ± 0.01 Bx. Triplicate measurements were taken.

pH

The pH of each of the samples was measured at ambient temperature (~23°C) using the Mettler pH meter calibrated at room temperature using three different pH buffers (pH 4, 7 and 10).

RESULTS AND DISCUSSION

Mixed juice and filtrate compositions

It is generally accepted that undetermined sucrose loss does occur during the milling stage. Sucrose losses of the order of 0.1% are difficult to measure directly as the traditional pol method is unable to differentiate the contributions of metabolic products and sucrose. The presence of metabolic products such as mannitol, lactic acid and ethanol are indicators that can be used to predict sucrose loss (Jones *et al.* 1997; Lionnet and & Pillay 1987) and are used as tools to compare the rate and extent of juice deterioration.

Table 1 shows the compositions of MJ and FIL in our samples. As expected, the brix of the FIL (10.75 Bx) is lower than that of MJ (13.2 Bx), whereas the pH and calcium content are higher because of the addition of lime saccharate by the factory to the mingler to achieve a pH of 7.5. The % sucrose on brix, % glucose on brix and % fructose on brix of MJ are higher than those of FIL. The higher glucose/fructose ratio in MJ may be because of the formation of a higher proportion of fructose-polysaccharides in the FIL. This assumption may be partly true because of the higher total polysaccharide concentration in the FIL. However, as the polysaccharide concentration is more than 2 times in FIL than in MJ (Table 1), it is more likely due to the contributions of polysaccharides leached out of the mud in the filter station and the formation of polysaccharides by microbes using the sugars as a feed source.

Table 1. Composition of mixed juice and filtrate.

Parameter	pH	Brix (Bx)	Mannitol (mg/mL)	% Sucrose on brix	% Glucose on brix	% Fructose on brix	G/F ratio	Polysaccharide (mg/kg·Bx)	
MJ	5.38	13.20	0	86.28	1.50	1.67	0.94	62.057	
FIL	7.50	10.75	0	84.27	1.13	1.24	0.91	146.887	
Organic acid (mg/kg·Bx)	Oxalic acid	Cis-aconitic acid	Citric acid	D-gluconic acid	L-malic acid	Trans-aconitic acid	Succinic acid	Lactic acid	Acetic acid
MJ	0.245	0.940	1.700	0.887	1.830	11.538	0.515	0.041	0.916
FIL	0.163	0.551	2.121	0.885	1.094	8.605	0.626	0.556	4.593

Metal ions (mg/kg·Bx)	Ca	K	Mg	Na	P	Al	Fe	Si
MJ	8.14	112.48	8.17	1.71	5.38	0.11	0.017	1.83
FIL	25.09	75.52	13.10	3.89	2.80	0.05	0.012	1.44

Mannitol was not detected in both samples, indicating that dextran (a glucose polysaccharide) produced by the bacterium *Leuconostoc mesenteroides* is absent, and the lactic acid *Leuconostoc* that produces it from fructose is dormant (Ravelo *et al.* 1991). No ethanol was detected in these samples, an indication of inactivity of the yeast *Saccharomyces* reported to be present in sugarcane juices (Chen and Chou 1993).

There are no significant differences between the total organic acid concentration in MJ (18.612 mg/kg·Bx) and FIL (19.194 mg/kg Bx), although the concentration of individual acids differed. This is the case for lactic and acetic acids. There is a 13-fold increase in the lactic acid concentration, from MJ to FIL, indicating microbial/enzymatic deterioration. Lactic acid is one of the indicators of sucrose loss by microbial deterioration (Rein 2018). The 5-fold increase in acetic acid concentration in FIL is mainly attributed to hydrolysis of bagacillo by high temperature and high pH during the clarification stage, though acetic acid is also formed by micro-organisms (Eggleston 2002).

Analysis of deteriorated juices

The juice deterioration studies were carried out at ambient temperature, and no heat treatment of the juices was carried out and neither was a biocide added. However, sucrose loss in the milling stage is mainly caused by micro-organisms, with minor contributions by enzymatic and chemical processes (Tilbury *et al.* 1997; Eggleston 2002).

Figure 1(a) shows that the brix of MJ decreased slowly up to 8 h and thereafter decreased sharply up to 72 h, before gradually decreasing with time. Figure 1(a) also shows that the brix of the FIL decreased slightly in the first 48 h, before decreasing sharply between 48-72 h. The little or no change in the brix at the initial stage of the deterioration process is because the soluble solids were utilised as the carbohydrate source for the micro-organisms. The subsequent rapid decreasing brix with time meant that the sugars (including sucrose) were quickly utilised by the microbes with increasing formation of the metabolic products. These results indicate that the activities of these micro-organisms were lower in FIL than MJ, and that the colony and proportions of these micro-organisms are likely to be different. There is also the destruction of enzymes during the clarification of MJ from where FIL originates, which will have some impact on the deterioration process.

The reduction of pH is a qualitative indicator of juice deterioration (Figure 1(b)). While the pH of the MJ decreased sharply at the start of the deterioration process, that of FIL initially decreased slowly before dropping significantly. The difference in the extent of the pH drop in the initial stage of the deterioration process between the two juices may be due to the differences in the initial juice pH - the pH of MJ was 5.38 and that of FIL was 7.50. In addition, as the juices have different proportions of the individual organic acids, they are likely to have different buffering capacity. The sharp drop in pH in the second stage of the process is associated with increasing formation of organic acids by the micro-organisms. We suggest that the differences in the pH profile observed between MJ and FIL is also related to the number and types of micro-organisms and enzymes.

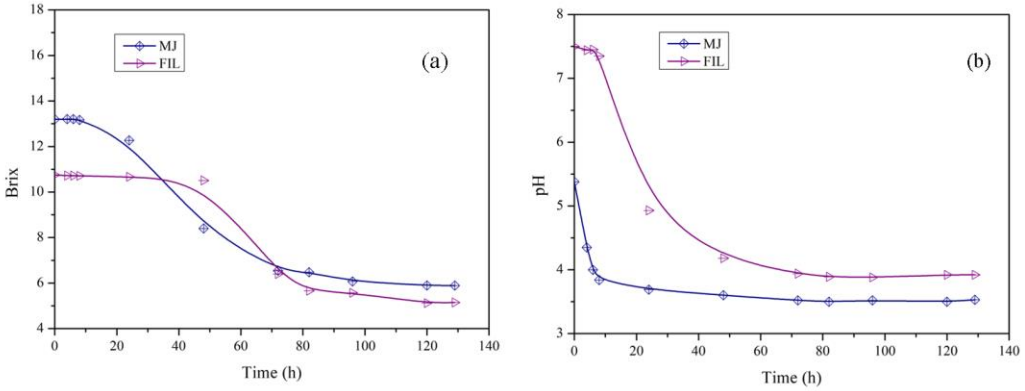

Figure 1. Juice brix and pH during the deterioration process of factory mixed juice and filtrate: (a) brix; (b) pH.

Figure 2(a) shows that the sucrose concentration of MJ appears stable during the first 8 h, and then decreases sharply at a rate of 2.70 mg/mL·h up to 48 h. The glucose concentration increased to a maximum value of 19.20 mg/mL during the first 24 h, before decreasing to ~0.41 mg/mL afterwards. The fructose concentration increased up to a maximum of 46.16 mg/mL during the initial 48 h and then decreased to ~27.90 mg/mL at 129 h. The decrease of sucrose concentration was not proportional to the increments of glucose and fructose concentrations. This is because reducing sugars are continuously utilised by the micro-organisms relative to sucrose. The fructose concentrations are higher than the

glucose concentrations during the whole process, which may be due to the polymerisation of glucosyl moieties from sucrose by the glucosyltransferases secreted by the *Leuconostoc*, resulting in the liberation of the fructose (Eggleston 2002).

In addition to the loss of sucrose and gaining of glucose and fructose, a number of microorganisms secrete enzymes which utilise sucrose as substrate for synthesis of oligo- and polysaccharides. Oligosaccharides can be formed from both enzymatic activity and acidic degradation of sucrose (Richards 1988). Some of these oligosaccharides (e.g. 1-kestose) have been reported to affect the morphology of sucrose, resulting in the formation of needle-like sucrose crystals (Ramos and Ravelo 2009). In our study, the kestose family was the major oligosaccharides identified during the deterioration process. The changing pattern of 1-kestose during the process was similar to that of glucose, as it increased in the first 24 h to a maximum concentration of 2.925 mg/mL and then decreased to zero (Figure 2 (a)). Mannitol was formed and accumulated throughout the deterioration process reaching a final concentration of 9.166 mg/mL.

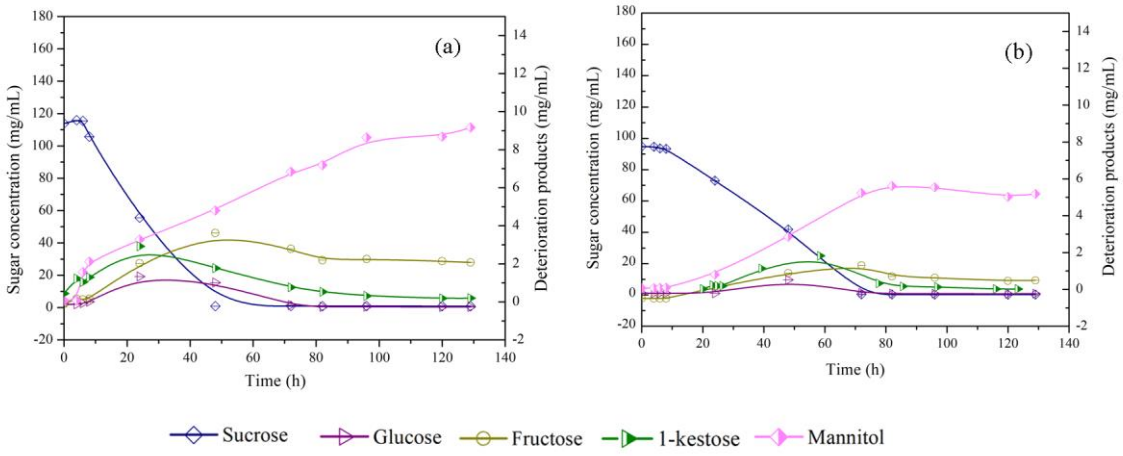

Figure 2. Concentrations of sugars and deterioration products during the deterioration process of factory (a) mixed juice and (b) filtrate.

The concentrations of sugars and deterioration products of FIL are shown in Figure 2(b). The sucrose concentration appears stable during the first 8 h, but it then decreases gradually at a rate of 1.44 mg/mL·h to 0.37 mg/mL at 72 h. The rate of decrease in sucrose concentration in FIL is much lower than that of MJ, providing further evidence that sucrose deterioration rate in FIL is slower than that of MJ. The glucose and fructose concentration profiles follow similar trends as that for MJ, but the recorded values at any time were lower than that of MJ. The 1-kestose concentration increased during the first 24 h period to a maximum value of 1.826 mg/mL at 24 h and then decreased. The mannitol concentration increased during the first 90 h, while the rate of formation was much slower than that of MJ, reaching a maximum concentration of 5.618 mg/mL.

Figure 3 shows that both the total polysaccharides in MJ and FIL increase with time, but the rate of increase in FIL was significantly higher than that in MJ. The total polysaccharides concentration at the end of the experiment (i.e. 129 h) was ~11.3 times higher in FIL than in MJ (i.e. 469.970 mg/kg Bx for MJ and 5,312.680 mg/kg Bx for FIL).

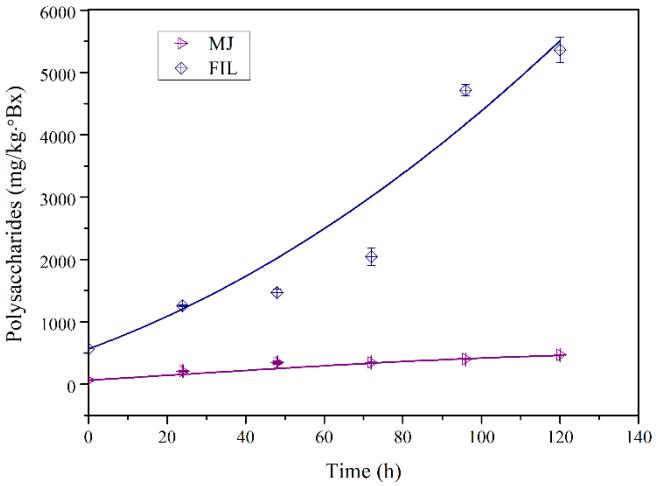

Figure 3. Concentrations of polysaccharides during the deterioration process of factory mixed juice (MJ) and filtrate (FIL).

Mannitol has been adopted as an indicator for juice deterioration as it has a good correlation with dextran (Eggleston 2002; Eggleston *et al.* 2004; Eggleston and Harper 2006). The two juice samples in our study both showed increasing concentrations of mannitol and polysaccharide with increasing deterioration time to at least 90 h. However, when mannitol concentrations of the juices are compared, the values were higher with MJ (even when brix is taken into account), while much lower polysaccharides concentrations were obtained. This is likely due to other extracellular polysaccharides, apart from dextran, present in juice that are in higher proportions in FIL than in MJ. Hector *et al.* (2015) have shown that, besides *L. mesenteroides* that predominantly produce dextran, microorganisms such as *Penicillium* sp., *Streptococcus* spp., *Lactobacillus* spp., *Xanthomonas albilineans* and *Acetobacter diazotrophicus* present in sugarcane juice are involved in the production of other polysaccharides. The proportion of these micro-organisms are, therefore, likely to be higher proportion in FIL. The reason for this is not known.

Bacterial metabolism also leads to the formation of organic acids. Lactic acid in raw juice has been a determinant for microbial activity and has been cited as a useful indicator for the process (Rein 2018). We detected 11 organic acids, oxalic acid, cis-aconitic acid, citric acid, D-gluconic acid, L-malic acid, trans-aconitic acid, succinic acid, glycolic acid, lactic acid, formic acid and acetic acid ,throughout the deterioration process.

Figure 4 shows the concentrations of the six major organic acids present in the juices. For MJ, the total organic acid concentration increased with time, and the maximum rate occurred between 24-72 h (2.49 mg/kg·Bx·h). The maximum acid concentration occurred at 72 h (165.248 mg/kg·Bx), and then remained relatively constant (Figure 4(a)). L-malic acid present in the fresh juices disappeared instantly when the deterioration process started, while oxalic acid and cis-aconitic acid concentrations fluctuated during the process (data not shown), although the variation was smaller than that of the six major acids. In MJ, citric acid and trans-aconitic acid concentrations decreased from initial values of 2.41 mg/kg·Bx and 11.53 mg/kg·Bx to 0.16 mg/kg·Bx and 3.26 mg/kg·Bx, respectively, while lactic acid, gluconic acid, acetic acid and succinic acid concentrations progressively increased during the process. Lactic acid showed the most significant increase from 0.57 to 65.99 mg/kg·Bx at 72 h, followed by succinic acid, D-gluconic acid and acetic acid in that order. Lactic acid and acetic acid are produced with mannitol as the deterioration products of *Leuconostoc* bacteria (Eggleston 2002). Succinic acid is the intermediate product of tricarboxylic acid cycle (or the Krebs' cycle) process of aerobic organisms. The significant increases in succinic acid concentrations are due to the presence of the succinic acid

producing microorganisms. D-gluconic acid can be derived from micro-organisms and through the transformation of D- glucose by chemical oxidation, which is unlikely under these conditions.

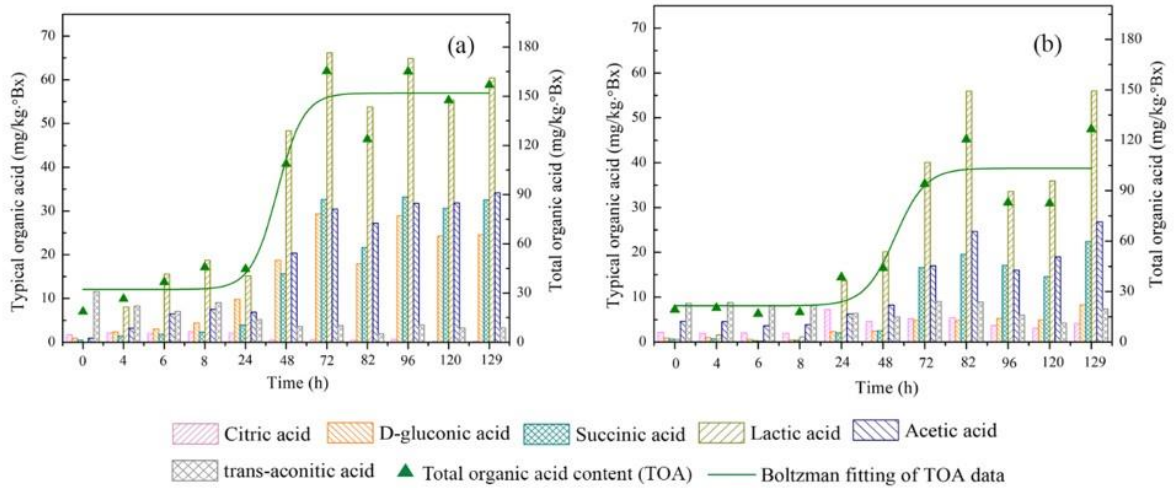

Figure 4. Concentrations of organic acids during the deterioration process of factory (a) mixed juice and (b) filtrate.

The changing patterns of these organic acids in FIL during the deterioration process are similar to those inf MJ. The initial concentration of organic acids was 19.194 mg/kg·Bx, which then increased, reaching a maximum concentration of 126.60 mg/kg·Bx at 82 h and then remained relatively stable. The maximum rate of increase occurred between 48 and 72 h (2.08 mg/kg·Bx·h), which is lower than that in MJ.

We detected no ethanol in MJ or FIL, which is in strong contrast to Eggleston (2002) or our previous work (unpublished) that showed that a significant amount of ethanol was formed in the untreated juice samples during the deterioration process. However, reasonable amounts of methanol were produced in both MJ and FIL (Table 2), although its concentration fluctuated throughout the process. This may simply be due to the volatility of methanol, as it boils at 65°C.

Table 2. Methanol concentration during the deterioration process.

Time (h)		0	4	6	8	24	48	72	82	96	120	129
Methanol	MJ	0.06	0.12	0.05	0.25	0	0.20	0.05	0.16	0.27	0.02	0.21
(mg/mL)	FIL	0.18	0.24	0.22	0.26	0.11	0.16	0.004	0.13	0.13	0.01	0

In traditional beverage fermentation processes, methanol production is linked to the activities of pectinase-producing yeast, fungi and bacteria (Ohimain 2016). Pectinolytic enzymes are classified into esterases and depolymerase (lyases and hydrolases). Hydrolysis of pectin by lyases produces oligo-or mono-galacturonate, while hydrolysis of pectin by esterase produces pectic acid and methanol (Sieiro *et al.* 2012). Our work on monosaccharide analysis of sugarcane juice showed the presence of pectin in sugarcane juices (Shi *et al.* 2019). The formation of methanol indicated the presence of the esterase-producing microbes.

DISCUSSION AND CONCLUSIONS

We evaluated the deterioration of MJ and FIL at ambient temperature in the laboratory. Sucrose loss and contents of reducing sugars and metabolic products were determined. The significantly higher proportions of the metabolic products, lactic acid, acetic acid and polysaccharides in FIL compared to MJ, prior to the deterioration experiments, implied microbial activities in the milling stage. The deterioration results indicated that the rate of formation of the metabolic products (except polysaccharides) in MJ was faster than FIL. This could be due to the lower initial juice pH value and less harsh environmental conditions (lower temperature) that allowed micro-organisms to thrive. The significantly higher polysaccharide content in FIL is because of the activity of micro-organisms other than *L. mesenteroides*, which is associated with dextran. A reasonable amount of methanol was detected in all the samples and is a product of yeast fermentation of pectin.

There were linear relationships (R^2=0.987-0.999 for MJ; R^2=0.899-0.976 for FIL) between mannitol concentration and time, total polysaccharide concentration and time, and between lactic acid and time for both juices. There were also linear correlations between sucrose loss and mannitol concentration for both juices. Therefore, using mannitol as the indicator, the sucrose loss in MJ with a residence time of 1 h (typical in Australian sugar mills) is 0.062%. A negative sucrose loss was obtained with FIL, perhaps suggesting that mannitol is not a suitable indicator to predict FIL sucrose loss. The relationships between sucrose loss and total polysaccharides/lactic acid concentration were non-linear. Sucrose losses of 0.296% and 0.898% were obtained for FIL and MJ, respectively, when the total polysaccharide content was used as the predictor. Lactic acid was a poor predictor for sucrose loss.

In summary, our results indicate that:

- sucrose loss occurs in MJ and FIL by the activity of micro-organisms;
- recycling of impurities in the FIL to MJ contributes not only to sucrose loss, but will impact negatively on the overall sugar-manufacturing process because of significant contribution of metabolic polysaccharides; and
- both laboratory and factory trials should be conducted with the use of biocides in the milling stage (including the filter station) of Australian sugar mills.

ACKNOWLEDGEMENTS

We acknowledge the support of Sugar Research Australia Limited, Sugar Research Institute and Queensland University of Technology for project funding, and the assistance of Mr Tal Cooper from the Centre for Analytical Research Facility (CARF) for help in analysing the metal-ion concentrations.

REFERENCES

Blake JD, Clarke ML, Richards GN (1987) Determination of organic acids in sugar cane process juice by high-performance liquid chromatography: improved resolution using dual Aminex HPX-87H cation-exchange columns equilibrated to different temperatures. *Journal of Chromatography A* 398: 265–277.

Chen JC, Chou CC (1993) *Cane sugar handbook: a manual for cane sugar manufacturers and their chemists*. Wiley, New York.

Eggleston G (2002) Deterioration of cane juice—sources and indicators. *Food Chemistry* 78: 95–103.

Eggleston G, Harper W (2006) Determination of sugarcane deterioration at the factory: Development of a rapid, easy and inexpensive enzymatic method to measure mannitol. *Food Chemistry* 98: 366–372.

Eggleston G, Legendre B, Tew T (2004) Indicators of freeze-damaged sugarcane varieties which can predict processing problems. *Food Chemistry* 87: 119–133.

Hector S, Willard K, Bauer R, Mulako I, Slabbert E, Kossmann J, George GM (2015) Diverse exopolysaccharide producing bacteria isolated from milled sugarcane: implications for cane spoilage and sucrose yield. *PloS One* 10(12): e0145487.

Jones B, Joyce J, Shipton W, Rodman D (1997) ATP as an indicator of microbial contamination in crushing juices. *Proceedings of Australian Society of Sugar Cane Technologists* 19: 396–401.

Lionnet GRE, Pillay JV (1987) Ethanol as an indicator of burn to crush delay. *Proceedings of the South African Sugar Technologists' Association* 1987: 5–7.

Ohimain E I (2016) Methanol contamination in traditionally fermented alcoholic beverages: the microbial dimension. *SpringerPlus* 5(1): 1607.

Rackemann D W, Bakir H, Edwards J, Cripps L, Doherty W O (2015) Evaluation of mud filtrate clarification to improve factory performance. *Proceedings the Australian Society of Sugar Cane Technologists* 37: 416–424.

Ramos EL, Ravelo S (2009) Sugarcane oligosaccharides and their impact on sucrose quality and factory efficiency. *Sugar Tech* 11: 185-188.

Ravelo B, Ramos S, Mejias R (1991) Sugar cane deterioration and its implications in the factory. *International Sugar Journal* 93(1108):82-6.

Rein P (2018) *Comparison of Sugar Losses in Milling and Diffusion*. 2018 ISSCT Factory Workshop.

Richards GN (1988) Effects of impurities on degradation of sucrose under processing conditions. Sugar Series 9: 253–264.

Roberts EJ (1981) Estimation of the soluble polysaccharides in sugar–A rapid test for total polysaccharides. *Proceedings of the 1980 Technical Session on Cane Refining Research* 130–133.

Shi C, Rackemann DW, Moghaddam L, Wei B, Li K, Lu H, Doherty WO (2019) Ceramic membrane filtration of factory sugarcane juice: Effect of pretreatment on permeate flux, juice quality and fouling. *Journal of Food Engineering* 243: 101–113.

Sieiro C, García-Fraga B, López-Seijas J, da Silva AF, Villa TG (2012) Microbial pectic enzymes in the food and wine industry. In *Food Industrial Processes - Methods and Equipment*. 2:1-8.

Tilbury RH, Hollingsworth BS, Graham SD, Pottage P (1977) Mill sanitation: a fresh approach to biocide evaluation. *Proceedings of the International Society of Sugar Cane Technologists* 16: 2749–2768.