

CANE DETERIORATION – OLIGOSACCHARIDE FORMATION AND SOME PROCESSING IMPLICATIONS

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Abstract

Several cane deterioration trials were carried out in the period 1992 to 1993. The accumulation of individual oligosaccharides during this deterioration is reported. Comparisons between the deterioration of burnt and trashed cane are drawn. Pilot plant studies have been used to indicate potential effects on cane processing and sugar quality.

Introduction

Sucrose is lost whilst numerous compounds are formed in the time between harvesting and processing cane. Both the type and quantity of these deterioration products will be dependent on several factors including cane variety, cane maturity and cane health. Harvesting practices such as burning, topping or billeting, as well as environmental conditions such as post-harvest temperature and rainfall will also play a part. Earlier investigations associated with cane deterioration have been reported, the emphasis usually being on monitoring sucrose losses using parameters such as purity (Anon., 1964; Irvine and Legendre, 1977; Davis and Dasrat, 1985), commercial cane sugar (CCS) (Egan, 1968; Ivin and Bevan, 1973), recoverable sugar (Bacic *et al.*, 1977) and estimated recoverable sugar (Wood *et al.*, 1972). Wood and co-workers have carried out extensive trials to assess the effects of several seasonal and harvesting factors on post-harvest cane deterioration under South African conditions (Wood *et al.*, 1972; Wood, 1973a, 1973b, 1976; Clowes and Wood, 1978).

In some cases attempts have been made to measure deterioration products to infer the cane delay and so control the expected processing problems. Dextran has received the most attention (Fulcher and Inkerman, 1974; Wells and James, 1976). Egan (1967) reported that increased gum content was often the first indication of deterioration and also monitored reducing sugars (1966). Irvine and Legendre (1977) also found increased gums a particularly sensitive indication of deterioration in chopped cane. Bruijn (1966) monitored a variety of impurities, including gums (Anon., 1964). Blake and McNeil (1978) quantified the ethanol produced, as did Lionnet (1986). More recently the development and extensive use of ethanol for assessing post-harvest delays under South African conditions (*i.e.* whole stalk cane generally burnt) has been found to be a useful indication that cane delays have occurred. The analysis is easy to carry out and the information can be readily used to highlight problem areas (Lionnet and Pillay, 1987, 1988; de Robillard *et al.*, 1990; Cox *et al.*, 1992; Koster *et al.*, 1992).

However, relatively few investigators have examined the formation of post-harvest deterioration products which might impact directly on processing efficiency. The polysaccharides and oligosaccharides are two groups of compounds that may have such an effect. This is due to their specific physical or chemical properties which can influence sucrose crystallisation kinetics. These compounds include dextran (Foster *et al.*, 1977) which is predominantly a problem when chopped cane deteriorates (Ravelo *et al.*, 1991a) or polysaccharides or oligosaccharides (Ravelo *et al.*, 1992). Ivin (1986) used

high performance liquid chromatography (hplc) (C₁₈ or amine modified silica) to measure oligosaccharides on stored cane. Recent work in Cuba compared the merits of using ethanol, polysaccharides or oligosaccharides as indicators of cane deterioration on mechanically harvested or manually cut billeted cane (Ravelo *et al.*, 1991b).

The present study focuses on only one aspect of cane deterioration, *viz.* the accumulation of specific oligosaccharides during the delays between harvesting and crushing whole stalk cane, and attempts to highlight how the presence of these sugars may impact on aspects of cane processing and sugar quality.

Experimental

Unless stated otherwise, analyses followed the procedures described in the SASTA manual (Anon., 1985). Affination was carried out according to the ICUMSA cane sugar method (Schneider, 1979).

Oligosaccharides were measured on direct analysis of cane (DAC) extracts, pilot plant products and selected factory or refinery samples using isocratic high performance anion exchange chromatography with a sodium hydroxide-sodium acetate mobile phase and pulsed amperometric detection. Raffinose peak height was used for quantitation. A factor was used to compensate for the change in area to height ratio at longer retention times. The procedure is described in Appendix 1.

Theandrose (in selected syrup and sugar samples) was measured using gas chromatography. Samples were concentrated by freeze drying and derivatised with the usual procedure (Schäffler and Morel du Boil, 1984). Splitless injection was used and separations were obtained on an HP-5 column (25 m × 0.32 mm × 0.52 μm) at 290°C with H₂ as carrier, detector 300°C, injector 300°C.

DAC extracts and pilot plant products were obtained from the cane deterioration trials carried out in the NB cane supply area during spring 1992 (where only burnt cane was used). The effect of deterioration on burnt or green cane was studied in later trials in conjunction with the SA Sugar Association Experiment Station (SASEX) during 1993. The cut cane was laid in windrows. Conditions are summarised in Table 1. Trials were generally sampled every second day for DAC analysis and once a week when pilot plant processing was included. The operation of the pilot plant has been described (Lionnet and Reid, 1993).

Results and discussion

In this study only those oligosaccharides readily detected using isocratic anion exchange chromatography and pulsed amperometric detection (PAD) have been considered since this technique readily lends itself to monitoring large numbers of samples with minimal sample preparation. Furthermore, the study has been limited to those oligosaccharides observed in the sugar crystal. It can be seen from Figure 1 that juice from deteriorating cane [chromatogram (a)] shows a wide spectrum of PAD responsive components (typically

Table 1

Summary of conditions for deterioration trials

Trial	Date	Variety	Age	Purity	Temp (°C)	Treatment
1	Sept/Oct 92	N 12	24 m	94,0		Burnt
2	Oct/Nov 92	Mixed	22 m	92,6		Burnt/standing vs Burnt
3	Apr/May 93	N 14	14 m	90,1	17-22	Burnt vs Trash
4	May/June 93	N 14	15 m	89,1	15-20	Burnt vs Trash
5	Aug 93	NCo 376	13 m	90,7		Burnt vs Trash vs Burnt/standing
6	Nov 93	NCo 376	17 m	87,6	17-29	Burnt vs Trash vs Burnt/standing

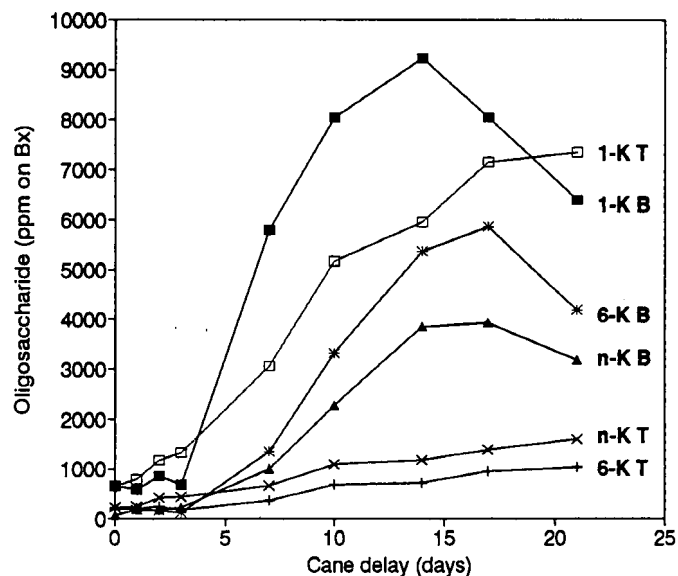


FIGURE 2 Kestoses in DAC extract during deterioration of burnt or trashed windrowed cane (1-K = 1-kestose, 6-K = 6-kestose, n-K = neo-kestose, B = burnt, T = trashed)

cedure used did not allow the measurement of the androse in the presence of the large amounts of 1-kestose formed). The predominant oligosaccharide in both burnt and trashed cane was 1-kestose. All three kestoses formed more rapidly in deteriorating burnt cane than in green cane subjected to similar post-harvest conditions. Eventually with very stale cane 1-kestose levels declined. Typical trends with delay time are shown in Figure 2.

The increases in kestose levels as cane purity decreased are presented in Table 2. The accumulation of 6-kestose and neo-kestose relative to the sucrose lost was considerably less when trashed whole stalk cane deteriorated. However, burning or trashing had little effect on the relative extent of 1-kestose formation.

The levels of 1-kestose in freshly harvested cane can vary considerably. During these trials the level in similar purity juice from fresh cane ranged from about 600 to almost 4 000 ppm on brix (Table 3). Higher concentrations have

Table 2

Formation of kestoses (mg/g sucrose destroyed) as cane deteriorates

	Treatment	
	Burnt	Trashed
1-kestose	26	26
6-Kestose	12	3
neo-Kestose	19	6

Table 3

Range of 1-kestose concentrations in DAC extracts from freshly harvested cane

Date	Variety	Age	Purity	1-kestose (ppm on Bx)
Sept 92	N 12	24 m	94,0	620
Nov 93	NCo 376	17 m	87,4	650
Aug 93	NCo 376	13 m	90,6	850
Oct 92	N 12/NCo 293/NCo 376	22 m	92,6	1115
Apr 93	N 14	13 m	91,1	3920
May 93	N 14	14 m	88,9	3930

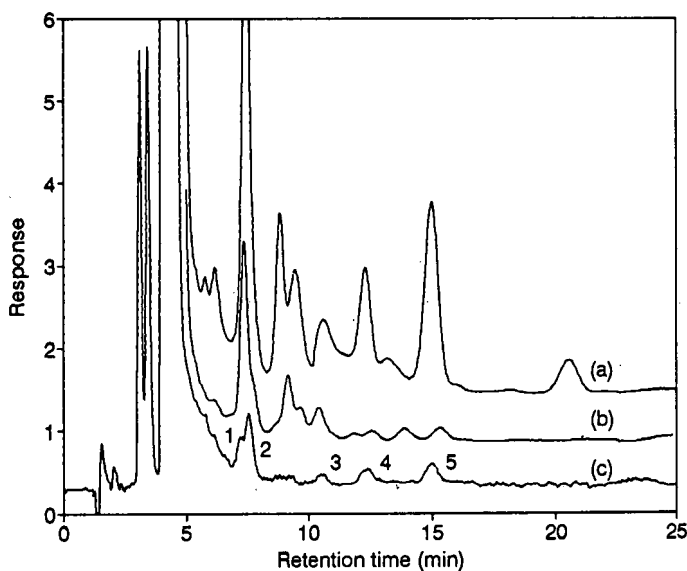


FIGURE 1 Anion exchange chromatograms showing oligosaccharides in (a) DAC extract from deteriorated cane (b) DAC extract from fresh cane (c) affinated A-sugar (1 = 1-kestose, 2 = theandrose, 3 = unknown, 4 = 6-kestose, 5 = neo-kestose) (Note: Peaks eluting before 5 minutes have been excluded from chromatograms (a) and (b) to avoid confusion from overlapping off-scale peaks).

a consistent pattern of about 15 to 20 compounds), whilst relatively few of these are detected in the sugar crystal [chromatogram (c)]. These have been tentatively identified as 1-kestose, theandrose, 6-kestose, neo-kestose and an unknown component which is not always present.

Formation of oligosaccharides in burnt or trashed whole stalk cane during cane delays

Four of the deterioration trials allowed the behaviour of burnt and trashed cane to be compared. Trials were generally carried out over extended periods in order to enhance the effects measured. The results for the DAC extracts from these trials are included in Appendix 2. Figure 1 gives a comparison of the relatively simple chromatogram obtained with fresh cane [chromatogram (b)] and the fairly complex profile given by badly deteriorated cane [chromatogram (a)].

The three kestoses (1-, 6- and neo-kestose) were the main deterioration products. (The simple chromatographic pro-

also been recorded. Hence high levels of oligosaccharides do not necessarily indicate cane deterioration. However, cane delays will inevitably lead to increased oligosaccharide loads to the factory.

Pilot plant processing

Cane from several trials was processed in the SMRI pilot plant (Lionnet and Reid, 1993). This enabled an evaluation of the effects of different stages of processing on oligosaccharide levels to be made. The results are given in Appendix 3. The following points can be made.

Extraction. The levels of 1-kestose in mixed juice (MJ) and DAC are compared in Figure 3. During Trial 1 the levels in mixed juice were considerably higher than those in DAC extracts when badly deteriorated cane was processed (6-kestose showed similar effects). This was attributed to enzymic activity as a result of the ideal incubation conditions present during preparation and extraction of infected cane. During subsequent trials precautions were taken to minimise this effect by dosing the imbibition water with a bactericide (Busan 881). It can be seen from Figure 3 that such treatment was generally successful in preventing further oligosaccharide formation during extraction.

Although oligosaccharides are generated during cane delays, extraction conditions may also influence the type and level of oligosaccharides introduced into process. Mill sanitation would be particularly important when crushing deteriorated cane.

It is tempting, but highly speculative, to draw parallels between milling and diffusion. The higher temperatures maintained in a diffuser probably inhibit enzymic activity and so limit oligosaccharide formation during extraction. By contrast, conditions in a milling tandem are probably conducive to microbial and enzymic activity and thus enhance the accumulation of oligosaccharides in mixed juice from deteriorated cane.

Clarification and evaporation. The effect of processing on 1-kestose levels for the trials incorporating biocide is summarised in Figure 4. In general, processing (other than extraction as discussed in the previous section) had little effect

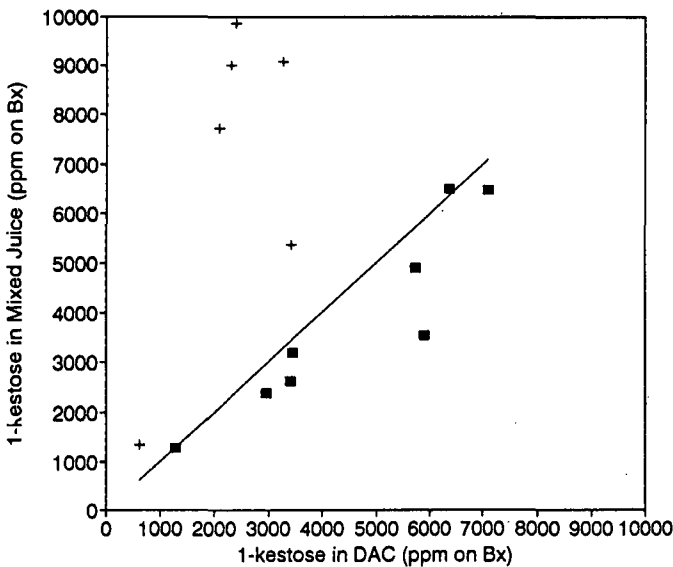


FIGURE 3 Comparison of 1-kestose concentrations in DAC extracts and mixed juice from deteriorated cane (a) in the absence of bactericide (+) (b) with bactericide addition to imbibition water (■). (c) DAC and MJ concentrations equivalent (-)

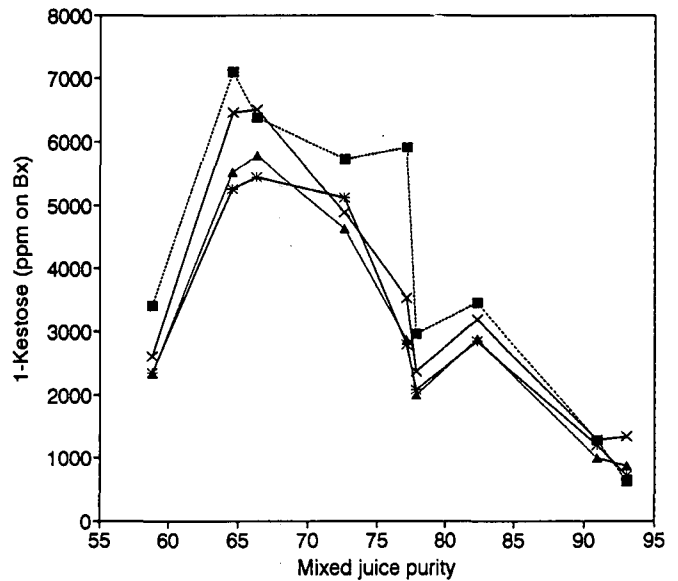


FIGURE 4 Effect of processing on 1-kestose concentrations (DAC = ■, MJ = x, clear juice (CJ) = ▲, Evaporator syrup = *).

on oligosaccharide levels. In broad terms, as these incoming levels increased this was reflected throughout the process. There were indications that slight decreases occurred during clarification. However, this might be attributed to product storage difficulties associated with the pilot plant as noted by Lionnet and Reid (1993). In any case such effects were relatively minor when compared to the large increases associated with the initial cane delay.

Crystallisation and oligosaccharide transfer. It is during crystallisation that certain oligosaccharides exert a profound influence. Some oligosaccharides poison specific faces of the sucrose crystal causing habit modification leading to reduced crystal growth rates (Smythe, 1967). It has been demonstrated that crystal growth rates in South African refineries are adversely affected by oligosaccharides (Morel du Boil, 1985, 1991). The increased levels of oligosaccharides re-

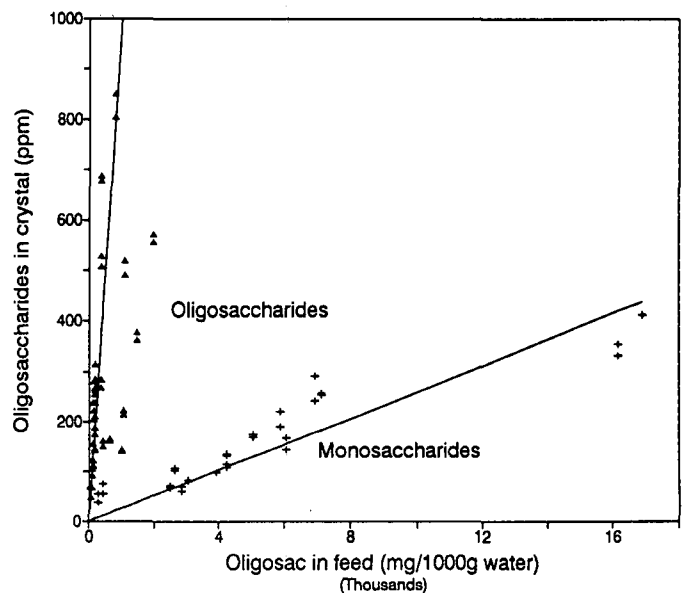


FIGURE 5 Transfer of monosaccharides (+) and oligosaccharides (▲) from mother liquor to the crystal during crystallisation

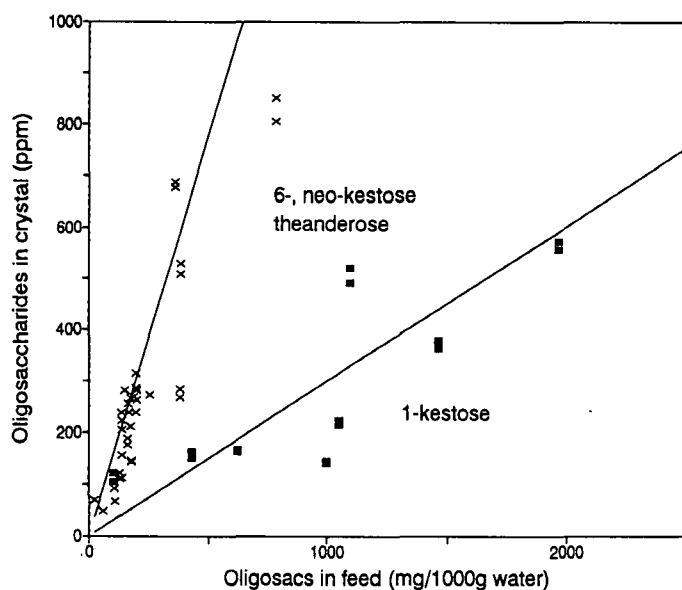


FIGURE 6 Comparison of transfers of 1-kestose (■) and other oligosaccharides (x) from mother liquor to the crystal during crystallisation

sulting from cane deterioration are reflected in higher oligosaccharide levels in evaporator syrup with concomitant higher concentrations in the crystal. During this investigation there were no clear indications that theandrose increased as cane deteriorated (Table A3.1 in Appendix 3). The transfer of sugars from the feed to the crystal under pilot plant conditions is presented graphically in Figures 5 and 6.

The slopes of these plots represent the amount of oligosaccharides in the crystal as a fraction of that in the mother liquor during boiling. The values are not absolute, but will change with conditions. However, with the controlled conditions maintained in the pilot pan, these transfer factors indicate the relative adsorption of the different oligosaccharides (Table 4).

Table 4

Relative transfer of oligosaccharides to the sucrose crystal

Sugar	n	r	Slope
Glucose	16	0,91	0,024
Fructose	16	0,92	0,028
1-Kestose	24	0,66	0,300
6-Kestose	24	0,77	0,870
neo-Kestose	24	0,87	1,300
Theandrose	15		1,530

Figure 5 illustrates that, although large amounts of monosaccharides are present in syrup from stale cane, their transfer to the crystal is clearly much less than that of the oligosaccharides. It can be seen from Figure 6 that 1-kestose (the major deterioration product) is adsorbed less strongly than theandrose, 6- or neo-kestose (about 25 to 30% as much). These last two oligosaccharides are formed rapidly in deteriorating burnt cane. Reduced crystal growth rates will be encountered when processing such cane.

Factory examples

Although the main source of oligosaccharides in mixed juice comes about as a result of cane delays and deteriora-

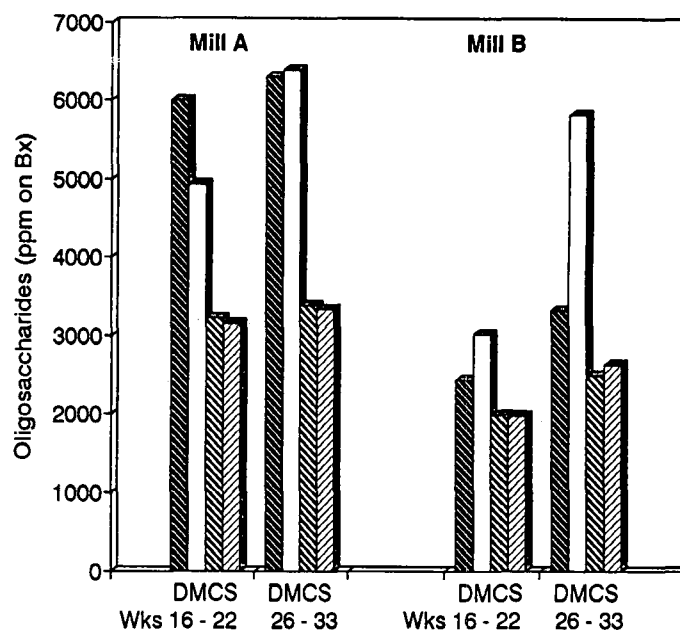


FIGURE 7 Trends in oligosaccharide levels during factory processing (D = DAC, M = MJ, C = CJ, S = Syrup) (based on data from Day-Lewis, 1995)

tion, some seasonal physiological contribution is also to be expected. However, whatever the origin, the effects can be expected to be detrimental. Pilot plant experiments have indicated that the oligosaccharide concentration in processing streams

- can be enhanced by the extraction technique
- may be influenced during clarification
- will affect crystal growth rates.

(a) A survey carried out by Day-Lewis (1995) at two factories showed similar extraction and clarification trends for total oligosaccharide contents to those noticed during pilot plant trials. This is summarised in Figure 7.

Table 5

Oligosaccharide concentration in affinated export VHP sugar during 1991

Mill	Month	Oligosaccharide (ppm)			
		theandrose	1-kestose	6-kestose	neo-kestose
NB	May	260	70		100
	Jun	300	80	60	120
	Jul	270	70	55	100
	Aug	320	60	70	145
	Oct	280	165	70	180
UC	Nov	275	150	120	190
	Jun	200	60	5	50
	Aug	210	65	5	65
	Sept	240	70		180
	Oct	225	100	50	130
AK	Nov	250	100	65	135
	Jul	180	80	60	110
	Aug	190	60		110
	Sept	240	80		125
	Oct	250	100	65	175
SZ	Dec	290	90	55	150
	May	245	25		125
	Jun	220			100
	Jul	200			115
	Aug	175	35		115
	Sept	230	90		175
	Oct	210	35	95	155
	Nov	200		85	140
	Dec	240	65	75	140

(b) The oligosaccharide levels in affinated export very high pol (VHP) crystals from four factories were monitored during 1991. The concentrations of the oligosaccharides are shown in Table 5.

Although not necessarily as a direct result of cane deterioration, all sugars showed increased oligosaccharide concentrations towards the end of the season which would have impeded crystallisation rates.

Refining examples

Certain oligosaccharides tend to transfer preferentially to the crystal so that the VHP sugar entering the refinery is enriched with compounds that will prevent efficient crystallisation. The levels vary as can be seen from Figure 8 where the oligosaccharides in incoming affinated raw sugar to a refinery increased at the end of the season.

The evidence presented here illustrates that the effects of oligosaccharide accumulation during cane deterioration (and, to a lesser extent variety and maturity differences) will carry through to the sugar and ultimately to the refinery where their influence on crystallisation rates will be particularly marked. Impurities associated with cane processing cannot be avoided, but the effects of oligosaccharides can be reduced by minimising cane delays and maintaining good mill sanitation.

Conclusions

Although there are undoubtedly some seasonal and varietal differences in oligosaccharide concentrations in cane juice, the main source is as a result of deterioration during cane delays. The kestoses were the main oligosaccharides formed. The rate of formation of the kestoses was faster for burnt than green cane. Similar amounts of 1-kestose were formed per unit purity drop with either treatment. However, two to three times as much 6- or neo-kestose was formed per unit purity lost when burnt, compared with green, cane deteriorated. Theanderose did not appear to increase as cane deteriorated. The method of juice extraction and mill sanitation played some part in increasing juice oligosaccharide levels, particularly when stale cane was processed. There were indications that small decreases in oligosaccharides occurred during clarification. However, this effect is difficult

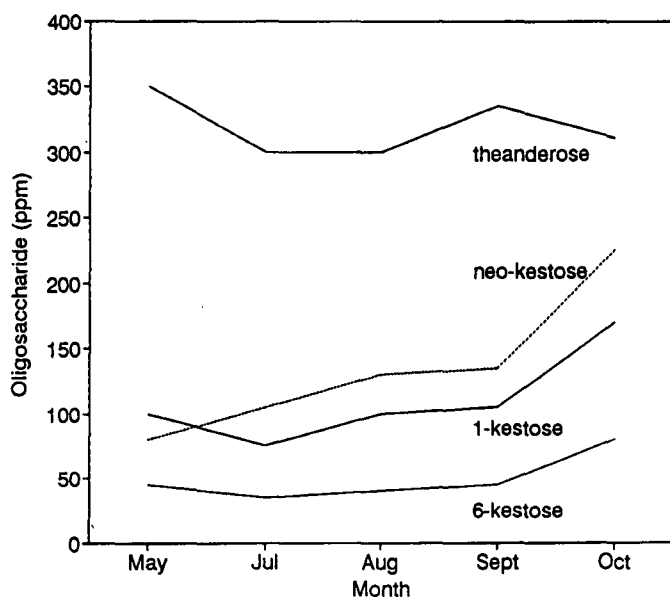


FIGURE 8 Seasonal variation of oligosaccharides in affinated sugar entering a refinery

to explain and might be partly attributed to problems with sampling. In general, the concentrations throughout the process were related to the amounts included with the cane.

Increased oligosaccharide loads to the factory adversely affect crystallisation rates by selective adsorption on different crystal faces. Some oligosaccharides (e.g. 6-kestose, neo-kestose and theanderose) were transferred more readily than others such as 1-kestose, leading to crystal elongation and retarded crystal growth rates. The extent of this transfer was related to the concentration in the feed liquor. By contrast, despite high fructose and glucose levels in syrup from deteriorated cane, very little invert was transferred to the crystal. Factory produced export VHP sugars showed increased oligosaccharide levels towards the end of the crushing season.

Oligosaccharides in the crystal are subsequently transported to the refinery where they are rapidly concentrated leading to exhaustion difficulties. The variability in oligosaccharide concentrations in sugars is reflected in refinery melt. Oligosaccharides continue to be concentrated throughout the refining process, exacerbating slow crystallisation rates.

Hence it can be seen that increased juice oligosaccharide concentrations, which occur mainly as a result of cane delays, will influence crystallisation rates right through factory processing and ultimately into refining.

Acknowledgements

An investigation of this scope could not have been carried out without the co-operation and assistance of many people. The contributions of all those involved – including cane growers, SASEX extension officers and other SASEX staff, mill personnel, as well as the staff of the Processing, Analytical Services and Chemical Divisions at the SMRI – were invaluable.

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- Panose was used as a retention time marker and peaks were identified by expressing their retention times relative to that of panose injected before and after batches of samples.
- There were some limitations imposed by the essential sucrose overload, although the CarboPac columns can tolerate a fair degree of overload from sugars not being quantified. Solvent strength was adjusted so that the main peaks eluted in less than 30 minutes since some peak clusters could not be resolved with isocratic elution regardless of solvent composition. Oligosaccharides eluting before or with sucrose were ignored.
- Because of the considerable peak overlap, peak heights were used for quantitation. A factor was used to compensate for the change in area to height ratio at longer retention times so that heights could be converted to the corresponding areas. Typically

$$(\text{Area})_{\text{olig}} = H_{\text{olig}} \times [K_1 + K_2 \times (\text{Rel } R_{i,\text{olig}})]$$

where K_1 and K_2 are constants obtained from the raffinose and panose retention times and responses.

Experimental conditions are listed below.

Equipment:

Pump	Waters M-45 hplc pump
Column	CarboPac PA I (Dionex) column (250 mm × 4 mm) PA Guard column (Dionex) (25 mm × 3 mm)
Detector	Dionex Model PAD - 2
Cell	: Standard (with thin (0,005") gasket)
Electrode	: Gold
Reference	: Silver
Settings	: Pulse range (2)
	: Display (+)
	: Response (3 sec)
	E_1 0,05V $t_1 = 4 = 420$ mS
	E_2 0,75V $t_2 = 3 = 180$ mS
	E_3 -0,20V $t_3 = 6 = 360$ mS
PAD range	: 300 nA
Detector temperature (°C)	: 22 - 25

Integrator : HP 3396 Series II (height mode)

Conditions:

Solvent	: 15 mM sodium acetate/100 mM sodium hydroxide (de-gassed with helium)
Flowrate	: 1,0 ml/min
Column temperature	: 27°C
Sample volume	: 20 µl

Sample Preparation:

(a) Reference standard:

Raffinose	: 3,5 ppm raffinose pentahydrate
Panose	: 3 ppm

(b) Samples:

Samples were diluted with water (reverse osmosis) and filtered (0,45 µm) before injection. Typical dilutions are indicated below.

(i) DAC extracts	- 1 g in 50 ml
(ii) Mixed/clear juice	- 1 g in 100 ml
(iii) Syrup	- 0,5 g in 250 ml
(iv) A-Molasses	- 1 g in 100 ml and 10 ml in 100 ml
(v) B-Molasses	- 0,75 g in 100 ml and 10 ml in 100 ml
(vi) C-Molasses	- 0,5 g in 100 ml and 10 ml in 100 ml
(vii) VHP sugar	- 0,5 g in 250 ml
(viii) Melt	- 5 g in 100 ml and 15 in 100 ml
(ix) Jet 4	- 1 g in 100 ml and 15 ml in 100 ml

APPENDIX 1

The measurement of oligosaccharides using anion-exchange high performance liquid chromatography (hplc)

Anion exchange hplc was used to separate the oligosaccharides and pulsed amperometric detection (PAD) to quantify the peaks. Several assumptions were necessary.

- Raffinose was selected for calibration because of its commercial availability. It was assumed that the PAD response would be similar for all oligosaccharides. External calibration was used and the linearity of response was confirmed in the range 0,5 to 10 ppm ($r = 0,9997$ for 7 calibration pairs).

APPENDIX 2

Cane deterioration trials

Table A2.1

Accumulation of kestoses in DAC extracts during cane delays

Trial	1																	
Treatment	Burnt																	
Day	Bx	S	Pty	Oligosaccharide (ppm on Bx)														
				1-K	6-K	n-K												
0	7.12	6.69	94.0	620	325	420												
2	6.96	6.32	90.8	1090	260	360												
4	6.99	6.42	91.9	1700	380	825												
7	7.02	6.39	91.0	3420	430	1900												
9	6.82	6.22	91.2	3515	545	2065												
11	7.18	6.41	89.3	3010	500	1470												
14	7.10	6.41	90.3	2080	540	1125												
16	7.41	6.70	90.4	2010	775	1600												
18	6.95	6.04	86.9	2665	860	1720												
21	6.65	5.80	87.2	3265	1050	2225												
23	6.80	6.09	89.6	2360	1020	2160												
28	6.64	5.18	78.0	2400	1390	2850												
30	7.03	5.52	78.5	2000	1190	2190												
32	7.35	5.73	78.0	2065	1080	2175												
35	6.89	4.80	69.7	2310	1310	2700												
37	6.86	4.58	66.8	2090	1500	2860												
Trial	2																	
Treatment	Burnt						Burnt/standing											
Day	Bx	S	Pty	Oligosaccharide (ppm on Bx)									Bx	S	Pty	Oligosaccharide (ppm on Bx)		
				1-K	6-K	n-K										1-K	6-K	n-K
0	6.65	6.16	92.6	1115	390	510						6.65	6.16	92.6	1115	390	510	
2	6.08	5.14	84.5	1700		610						6.80	6.29	92.5	710		490	
4	6.60	6.17	93.5	890		465						6.64	6.22	93.7	725		350	
7	6.77	6.07	89.7	1300	380	620						6.72	6.20	92.3	1280	390	700	
9	6.54	5.88	89.9	1720	400	760						6.21	5.57	89.7	1985	500	795	
11	6.85	6.16	89.9	1435	455	690						6.56	5.99	91.3	1090	390	575	
14	7.17	6.10	85.1	2165	700	1400						6.85	5.87	85.7	1495	390	860	
16	7.07	6.10	86.3	2180	700	1370						6.61	5.62	85.0	2380	760	1375	
18	7.00	5.89	84.1	2600	890	1640						6.63	5.63	84.9	1875	495	1365	
23	6.75	4.93	73.0	2040	620	1610						6.30	5.01	79.5	2910	340	775	
25	6.75	5.29	78.4	2165	820	1765						6.59	5.46	82.9	1900	525	1330	
28	6.74	4.53	67.2	3530	2085	4010						6.25	4.28	68.5	2970	1155	2790	
30	7.24	5.16	71.3	1920	985	2065						6.64	4.78	72.0	1890	775	2165	
35	9.29	5.89	63.4	1700	1690	3235						9.34	4.61	49.4	1290	1320	2360	
Trial	3																	
Treatment	Burnt						Trashed											
Day	Bx	S	Pty	Oligosaccharide (ppm on Bx)			Bx	S	Pty	Oligosaccharide (ppm on Bx)								
				1-K	6-K	n-K				1-K	6-K	n-K						
0	6.04	5.50	91.1	3920	295	520	5.51	4.91	89.1	3185	565	745						
2	5.69	5.02	88.2	3180	420	460	5.96	5.33	89.4	2930	395	720						
5	5.43	4.54	83.6	4295	690	665	6.02	5.08	84.4	4345	385	850						
7	5.69	4.88	85.8	5855	790	1080	5.31	4.37	82.3	5495	655	925						
9	5.95	4.97	83.5	6670	800	1690	5.48	4.15	75.7	7025	865	1285						
12	6.16	5.12	83.1	6735	775	1770	5.75	4.45	77.4	5570	555	1045						
14	6.42	5.17	80.5	7280	1845	3185	5.60	4.27	76.3	5770	760	1575						
16	6.16	4.85	78.7	7790	1365	2575	5.50	3.85	70.0	7450	765	1755						
19	5.56	3.86	69.4	9995	2310	4680	5.87	4.30	73.3	7510	1045	1700						
21	6.50	4.40	67.7	8460	3475	6710	6.07	4.19	69.0	8415	1295	2505						
23	5.84	3.46	59.3	9415	3460	7080	5.34	3.67	68.7	9515	1095	2025						
26	5.22	2.59	49.6	10590	3685	6070	5.69	3.60	63.3	9050	1775	2745						
28	5.35	2.95	55.1	6720	1790	3185	5.82	3.69	63.4	4900	735	1335						

Appendix 2 cont.

Trial	4																	
Treatment	Burnt						Trashed											
Day	Bx	S	Pty	Oligosaccharide (ppm on Bx)			Bx	S	Pty	Oligosaccharide (ppm on Bx)								
				1-K	6-K	n-K				1-K	6-K	n-K						
0	5,29	4,72	89,2	2750	355	350	5,42	4,82	88,9	3930	510	630						
2	5,59	5,00	89,5	2660	400	330	4,79	4,05	84,6	5535	1245	745						
4	5,17	4,47	86,5	3700	590	475	5,18	4,34	83,8	5720	1190	840						
8	5,43	4,57	84,2	3725	495	535	6,02	5,15	85,6	4520	460	775						
10	5,80	4,97	85,7	4675	570	940	5,60	4,76	85,0	4055	355	720						
14	4,70	3,58	76,2	6845	1025	1455	6,02	4,90	81,4	6875	885	1175						
16	5,20	4,01	77,1	6505	1175	1655	6,10	5,14	84,3	6395	770	1180						
18	5,49	4,43	80,7	5450	895	1780	5,24	4,01	76,5	8090	1335	1215						
21	5,71	3,99	69,9	10485	3485	5020	5,24	3,56	67,9	9580	1875	2000						
25	6,09	3,94	64,7	8325	3560	6900	5,75	4,08	71,0	7435	1325	2030						
28	5,07	3,22	63,5	7740	2065	3325	5,86	4,03	68,8	8710	1430	2665						
Trial	5																	
Treatment	Burnt						Trashed						Burnt/standing					
Day	Bx	S	Pty	Oligosaccharide (ppm on Bx)			Bx	S	Pty	Oligosaccharide (ppm on Bx)			Bx	S	Pty	Oligosaccharide (ppm on Bx)		
				1-K	6-K	n-K				1-K	6-K	n-K				1-K	6-K	n-K
0	4,64	4,21	90,7	835	260	200	4,51	4,08	90,5	895	285	185	4,66	4,23	90,8	960	190	
1	4,60	4,19	91,1	1005	220	165	4,54	4,12	90,8	750	145	170	4,59	4,12	89,8	1095	215	220
2	4,59	4,21	91,7	945	160	175	4,54	4,09	90,1	1235	415	230	4,47	3,99	89,3	765	210	190
3	4,60	4,19	91,1	1230	175	245	4,77	4,32	90,6	1000	180	240	4,45	3,99	89,7	705	150	225
7	4,34	3,91	90,1	4485	390	725	4,67	4,10	87,8	2505	375	500	4,56	4,10	89,9	1970	290	350
10	4,82	4,11	85,3	9065	540	1055	4,89	4,02	82,2	3045	380	650	4,19	3,80	90,7	1425	155	240
14	4,80	3,87	80,6	7685	620	1660	5,08	3,90	76,8	4365	470	855	4,26	3,73	87,6	4245	375	510
17	4,86	3,60	74,1	7025	645	1600	4,95	3,68	74,3	4740	465	935	4,13	3,70	89,6	4075	430	525
21	4,92	3,38	68,7	13665	1645	3925	5,14	3,69	71,8	8395	700	1390	4,21	3,45	82,0	5685	500	630
28	4,86	3,42	70,4	17400	1790	3645	5,17	3,59	69,4	8450	1000	1520	4,20	3,45	82,1	4720	600	740
Trial	6																	
Treatment	Burnt						Trashed						Burnt/standing					
Day	Bx	S	Pty	Oligosaccharide (ppm on Bx)			Bx	S	Pty	Oligosaccharide (ppm on Bx)			Bx	S	Pty	Oligosaccharide (ppm on Bx)		
				1-K	6-K	n-K				1-K	6-K	n-K				1-K	6-K	n-K
0	5,62	4,91	87,4	650	210	60	6,28	4,69	74,7	650	190	225	5,49	4,87	88,7	620	155	150
1	5,93	5,19	87,5	565	165	170	5,68	4,97	87,5	770	195	245	5,38	4,64	86,3	630	145	170
2	5,80	5,01	86,4	845	150	170	5,85	5,04	86,2	1160	230	415	5,09	4,45	87,4	795	200	175
3	5,69	4,98	87,5	670	110	215	5,63	4,88	86,7	1315	160	425	5,09	4,48	88,0	660	155	180
7	5,94	5,00	84,2	5780	1345	1000	5,92	4,85	81,9	3040	370	655	4,93	4,25	86,2	5170	1065	475
10	6,17	4,73	76,7	8035	3300	2260	6,28	4,69	74,7	5165	675	1085	5,05	3,83	75,8	6565	1590	715
14	6,45	4,52	70,1	9225	5365	3835	6,43	4,68	72,8	5935	715	1170	4,52	3,31	73,2	6290	2090	1135
17	6,33	4,25	67,1	8020	5850	3930	6,43	4,49	69,8	7125	945	1375	4,52	3,27	72,4	6375	2275	1190
21	7,15	4,58	64,1	6380	4185	3175	6,60	4,35	65,9	7325	1020	1596	5,23	2,92	55,8	3400	1810	1180

APPENDIX 3
Pilot plant trials

Table A3.1

Oligosaccharides in process products

Trial	Treatment	Day	1-kestose (ppm on Bx)					6-kestose (ppm on Bx)					neo-kestose (ppm on Bx)					theandrose (ppm on Bx)	
			DAC	MJ	CJ	Evap Syr	Sugar	DAC	MJ	CJ	Evap Syr	Sugar	DAC	MJ	CJ	Evap Syr	Sugar	Evap Syr	Sugar
1	Burnt	0	620	1340	865	710	115	325	465	400	290		420	580	565	400	70	1060	240
		7	3420	5350	3000	2645	140	435	440	500	410		1895	1630	905	885	145	845	240
		14	2080	7710	4000	4240	220	540	620	510	660	50	1125	2060	1185	1415	300	1000	260
		21	3265	9075	4785	4500	370	1045	1165	920	855	135	2225	2925	1955	1955	520	1055	270
		28	2400	9850	4970	5210	500	1390	1665	1285	1300	180	2850	3600	2500	2535	680	1185	280
		35	2300	9005	5110	4405	565	1310	2065	1790	1630	275	2695	4000	3215	3015	830	1165	275
2	Burnt/standing	7	1280	1275	995	1200	250	390				155	700	595	555	595	200	830	205
		21	3460	3190	2890	2860	165	985	765	865	850	80	1880	1495	1340	1440	240	890	240
		28	2970	2375	1995	2075	160	1155	870	805	625	120	2790	1800	1565	1525	270	960	225
5	Burnt	28			5310	5115	335	1745	1400	1120	1100	205	3430	3235	2480	2375	685	700	200
	Burnt/standing	30	5900	3525	2870	2800	175	1560	540	475	490	105	1520	1225	1065	1060	190	595	145
6	Trashed	35	6370	6500	5780	5445	460	895	790	750	680	145	1590	1735	1580	1585	485	430	150
	Trashed	22	5730	4890	4620	5120	260	705	600	580	595	105	1495	1260	1155	1285	290	825	225
	Burnt	27	7100	6465	5515	5260		4145	2830	2930	2730		4665	4490	4610	3860			
	Burnt/standing	30	3415	2620	2345	2355		2410	1340	1125	1115		2165	1635	1485	1465			