WHOLE STALK GREEN CANE DETERIORATION INDICATORS TO ASSESS MIXED JUICE QUALITY

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Abstract

A deterioration trial was conducted in Reunion Island on two main sugarcane CERF varieties (R570 and R579). Whole stalk green canes were harvested, left in the field and sampled every two days. This trial was carried out to find new deterioration indicators using chromatography analyses (HPIC, HPLC). In addition to standard parameters (i.e. purity, cane weight, pH, reducing sugars), oligosaccharides, polyols, organic acids, and amino acids were measured. Results highlighted that 1-kestose, proline and *cis/trans* aconitic acid ratio were closely linked to cut to crush delay (respectively r^2 = 0.98, 0.82 and 0.76). To a lesser extent citrate and amino acids (alanine, cysteine, isoleucine) were also correlated to cut to crush delay. These results were compared to mixed juice analysis to assess freshness of sugarcane delivered to the mills. According to Le Gol sugar mill mixed juice analysis, estimated freshness of delivered cane was variable. Purity, reducing sugars and Pol/sucrose highlighted a delay below four days after harvest. Aconitic ratio, proline and 1-kestose indicated a cut to crush delay between four and seven days.

Keywords: sugarcane, green cane, deterioration, sugar losses, kestose, proline, mixed juice

Introduction

In Reunion Island, most of the cane is grown by small-scale farmers, with the average area of cane plantation being 6 ha. Moreover, because of geographical and environmental constraints, 75% of the cane is harvested as hand-cut whole stalk green cane. Thus, cut to crush delay is estimated to be up to seven days or more in some areas of the Island.

Quality of juice after delayed extraction has been studied according to harvest method (Wood, 1976) as a means of determining good storage conditions (Yusof *et al.*, 2000) and consequences in the factory (Morel du Boil, 1995; Ravelo *et al.*, 1991). In this study, common analyses (*i.e.* pol and brix, pH, sucrose and reducing sugars) were conducted and compared with Reunion sugar industry data and reports in the literature.

Oligosaccharides, polysaccharides and dextrans in juice are well-known indicators for determining the degree of deterioration of mechanically cut and burned cane (Ravelo *et al.*, 1991) but there are few reports on indicators of the freshness of cane juice from green cane delivered to the mill. Bacci and Guichard (1994) showed that ethanol correlated well with deterioration in Reunion Island conditions, although the increase in ethanol was not as fast in green cane as in burned cane.

A previous study conducted at CERF (Centre d'Etudes de Recherche et de Formation - Sugar Cane Research and Training Institute) showed that acid aconitic ratio could be used as an indicator of cut to crush delay in the mill for green cane (Corcodel *et al.*, 2006). This paper

goes further, studying cane juice composition during deterioration with novel analytical methods.

Metabolites involved in the physiological response of sugarcane to water deficient stress, were the focus of the investigation because after the cane has been cut it receives no more water. Sugars, polyols, oligosaccharides or amino acids (mainly proline) were found to be related to water-deficient stress (Folkert *et al.*, 2001; Errabi *et al.*, 2006). They were then monitored in deteriorated canes. Quantification of these metabolites in sugarcane tissue requires sophisticated analytical techniques and instrumentation, including HPAE-PAD (High Performance Anionic Exchange Chromatography - Pulsed Amperometric Detector), HPAE-CD (High Performance Anionic Exchange Chromatography - Conductimetric Detector) and HPLC (High Performance Liquid Chromatography) assessment (Gzik, 1996; Farine *et al.*, 2001).

This study had two goals: (i) finding new deterioration indicators by using new analytical methods, and (ii) assessing freshness of cane delivered to the mill by analysing mixed juice. The trial was planted to Reunion varieties R570 and R579.

Canes were sampled every two days after harvest. Analyses were performed in the CERF laboratory. This paper discusses changes in commonly measured parameters (*e.g.* pol, brix, starch), as well as in less well-known parameters such as organic acids, oligosaccharides and amino acids. The results are also compared to mixed juice composition of Le Gol mill, a sugar factory located in the south of Reunion Island.

Materials and Methods

Trial design and cane sampling

The trial was conducted on two main R-varieties: R570 and R579. Table 1 shows the repartition of those two varieties in Bois Rouge mill and Le Gol mill area, the two sugar mills of Reunion Island.

Table 1. Changes in cane varieties grown in the Bois Rouge and Le Gol areas (CTICS, 2005).

Year	Bois Rouge			Le Gol		
	R570	R579	Other	R570	R579	Other
1996	85%	11%	4%	82%	4%	14%
1999	69%	28%	3%	72%	8%	14%
2005	49%	50%	1%	74%	12%	14%

Two 300 kg bundles of each of the two varieties were harvested on November 2007. The bundles were left in the field under ambient conditions. A sub-set of 10 canes per bundle was weighed at harvest (day 0) and again at 2, 4, 7, 9 and 11 days after harvest. On the same days, 2x10 canes were randomly selected from each bunch, and cut into halves. The 10 cane sample was sub-sampled and only five bottoms and five tops were kept.

Cane analysis

Cane analysis followed the press method used in Reunion for sugarcane payment (CTICS, 2007). Cane samples were cut into ±10 cm billets, crushed in a FAPMO cutter-grinder, and approximately 1 kg of pulp was pressed at 200 bars for 1 min 30 sec with a Pinette-Emidecau

hydraulic press. Press cake was weighed and juice collected. Juices of the two sub-samples (700 mL each) of one bundle were mixed and frozen at -18ºC for further analysis.

Fibre was determined according to an equation established for the Pinette-Emidecau press under Reunion Island conditions:

$$\text{Fibre (\%)} = 0.55 \times b \times 100$$

where $b = \frac{\text{cake weight}}{\text{pulp weight}}$

For pol and brix, all the samples were analysed following the non-lead method (ICUMSA Draft Method No. 9, 2007) developed by CERF. Juices were filtered with a paper filter (Fiorini 1105 and a Clarcel CBL filter aid). The filtration unit was a Schmidt and Haensch Autofilt equipped with a compressor (Jun-Air at 5 bars pressure). The method consisted of preparing 2x200 mL solution and 2x15 g Clarcel CBL. The filter aid was added to the juice and then the solution was vigorously mixed. The first 200 mL sample was used to clean the filtration apparatus, the other sample was used for reading. Before each measurement, filtrates were cooled at 20°C in a bath. The same filtrate was used for pol and brix reading.

Pol and brix were used to measure apparent purity (App Pu):

$$\text{App Pu} = \frac{\text{Pol}}{\text{Brix}} \times 100$$

Pol in cane was calculated with the following equation:

$$\text{Pol in Cane} = \frac{\text{Pol J} \times (1 - b)}{1.012 - 0.41 \times b}$$

where Pol J = Pol of extracted juice in g%g

Conductivity and pH were performed directly in raw juice with a CONSORT C532.

Starch

Starch assessment was based on the ICUMSA method (GS1-17) modified with a centrifugation step for a faster analysis. This method was validated by comparing a few samples with both methods. Starch content of a 12.5 g juice sample was precipitated in 50 mL absolute ethanol. After centrifugation (4000 rpm), the supernatant was discarded. Starch was then solubilised in a boiling $CaCl_2$ solution (25% m/m) for 15 min and, after cooling, the pellet was eliminated by centrifugation (4000 rpm). Finally starch content was measured by reaction with potassium iodate and potassium iodide under acidic conditions (acetic acid). A five point standard curve enabled quantification of starch concentration.

Chromatography analyses

Sucrose, glucose and fructose

Analyses of pressed juices were performed on the filtrate used for pol and brix assessments. Following appropriate dilution (around 1/100 000), sucrose, glucose and fructose were quantitatively analysed using Dionex High Performance Anionic Exchange Chromatography

with Pulsed Amperometric Detector (HPAEC-PAD) including CarboPac PA1 column and electrochemical detector with Au working electrode and Ag/AgCl reference electrode. Column eluent conditions were: 0-10 min 150 mM NaOH isocratic, flow rate 1 mL/min, temperature 30ºC (ICUMSA method GS7/8/4-24, 1998). Before each analysis, the syringe and injection loop were both automatically flushed by 250 µL deionised water. Quantification was based on one standard with lactose as internal standard. Sucrose was used to measure true purity and sugar weight of cane:

$$\text{True Purity} = \frac{\text{Sucrose}}{\text{Brix}} \times 100$$

$$\text{Sugar Weight} = \text{Cane Weight x Sucrose}$$

Polyol and oligosaccharides

Analysis of polyol and oligosaccharides were performed as described for sucrose and invert sugars. Dilutions (around 1/1000) were adapted to enable detection of polyol (mannitol) and oligosaccharides (palatinose, isomaltotriose and 1-kestose). Column eluent conditions were: 0-30 min 150 mM NaOH isocratic, flow rate 1 mL/min at a temperature of 30°C. Before each analysis, the syringe and injection loop were both automatically flushed by 250 µL deionised water. A three point standard curve for each compound was used without internal standard.

Organic acids

Organic acids (lactic, citric, isocitric, *cis*-aconitic and *trans*-aconitic acids) were quantitatively analysed by Dionex HPAE with Conductivity Detector (HPAE-CD) including IonPac AS11 HC column and ASR 4 mm suppressor to improve conductivity signal. Column eluent conditions were: NaOH gradient (2 mM from 0-1 min, 2-4 mM from 1-10 min, 4-30 mM from 10-25 min, 30-60 mM from 25-35 min and 2 mM from 35-38 min). The flow rate was 1.5 mL/min at a temperature of 30°C. Before each analysis, the syringe and injection loop were both automatically flushed with 250 µL deionised water. A three point standard curve for each compound was used.

Amino acids

Amino acid assessments were performed with a commercial Waters AccQ.Tag Chemistry Package. The AccQ.Fluor reagent converts, in a borate buffer, primary and secondary amino acids to stable fluorescent derivatives which were separated by reversed-phase Waters HPLC (Nova-Pak^{TM} C_{18}). Column eluent conditions were commercial buffer: acetonitrile: deionised water gradient, and flow rate was 1.0 mL/min at a temperature of 37°C. Derivatised amino-acids were detected by UV (254 nm) and quantified with a one point standard curve passing through zero using a commercial amino-acid standard solution.

Results and Discussion

Change in concentration of common parameters with cut to crush delays

The method to follow changes of one parameter with cut to crush delay consisted in drawing a regression line between the parameter and post-harvest days. For example, sugar weight loss is shown in Figure 1A for R570 an R579 (mean of the two bundles). Sugar weight decreased very regularly with post-harvest days with a slope of 0.03 kg of sugar per day (R570 r^2=0.94

and R579 r²=0.87). As indicated in Figure 1A, sucrose and cane weight decreased. This resulted in a global decrease of sugar weight. Regular loss of weight has already been described in deterioration trials and is attributed to dehydration (Lionnet, 1996; Corcodel and Mullet, 2007).

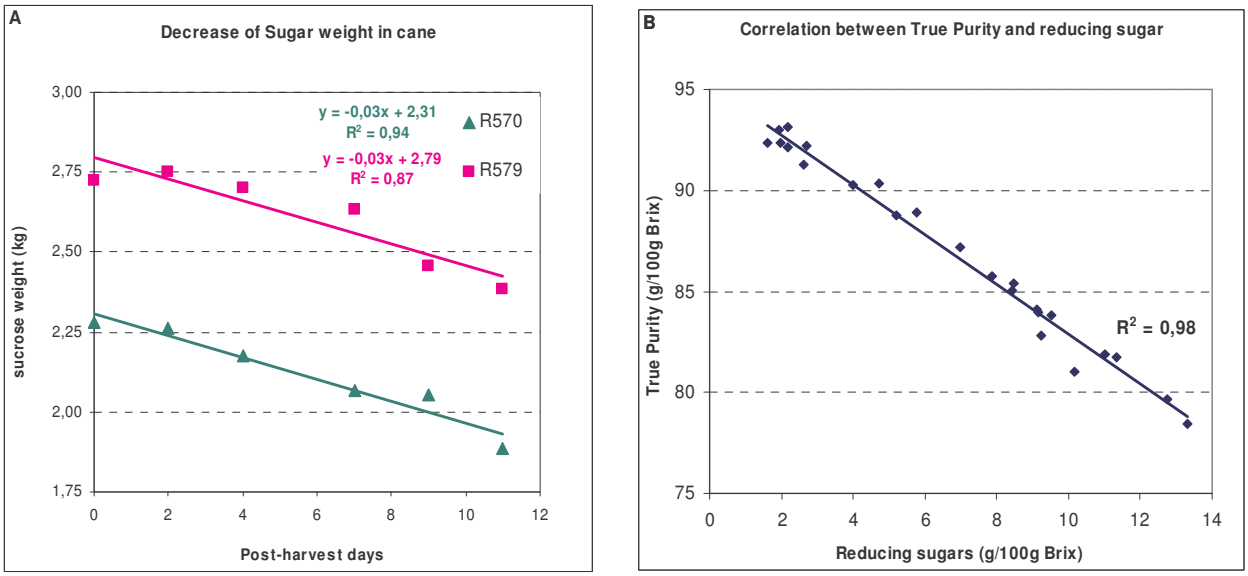

Figure 1. Decrease of sucrose weight (cane weight x sucrose in g/100 g of cane) with post-harvest days (A), and correlation line between purity and reducing sugars (B).

True Purity decreased at a rate of 1.11 points per day (Table 2) and apparent purity decreased at 1.2 points per day. Under similar conditions, Bacci and Guichard (1994) had found a drop of apparent purity of 0.87 and 1.24 points per day in September and December, respectively. The decrease in purity was due to both brix increase and to pol and sucrose decrease. An increase of reducing sugars (0.92/100 g brix per day) was strongly correlated with the observed drop of True Purity (figure 1B). These results are in accordance with other deterioration trials and highlight sucrose inversion (Irvine, 1971; Yusof *et al.*, 2000).

Sucrose destruction has also already been associated to microbial activity (Lionnet, 1996; Eggleston, 2002), particularly lactic acid Leuconostoc bacteria. For billeted or burnt canes, a fast drop of pH was attributed to microbial activity (Wood, 1976), whereas in whole stalk (Wood, 1976; Sens *et al.*, 2005) a lower acidification was noted but no microbial activity was highlighted. But in this trial, the acidification observed is slight and mannitol, isomaltotriose and lactic acid concentrations do not show any evident microbial deterioration of whole stalk green sugar cane (data not shown).

Table 2 shows regression coefficients and slopes obtained in this trial for the most commonly analysed parameters in deteriorated cane. Regression lines have been calculated between each parameter and post-harvest days for the four trials (2 bundles x 2 varieties). R² and slopes correspond to the mean of the four regression lines. Parameters with a r² greater than 0.75 shows a high linear correlation with cut to crush delays and are shown in bold type.

Table 2. Correlation of classical parameters with post-harvest days (mean of four curves).

Parameter	r^2 (mean)	Slope mean	General tendency
Relative weight (%/day)	**0.99**	**-1.07**	**Linear loss of weight of 1%/day**
Fibre (g/100 g cane)	0.34	0.12	Tends to increase
Brix (g/100 g juice)	**0.93**	**0.26**	**Linear increase**
Pol (g/100 g juice)	0.40	-0.08	Irregular loss
Pol in cane (g/100 g cane)	0.48	-0.08	Bell curve
Apparent purity	**0.95**	**-1.20**	**Linear loss of 1.2%/day**
True purity %	**0.94**	**-1.11**	**Linear loss of 1.1%/day**
Glucose (g/100 g brix)	**0.96**	**0.46**	**Linear increase for reducing sugars**
Fructose (g/100 g brix)	**0.96**	**0.46**	
pH	0.63	-0.03	Slight acidification
Conductivity (mS)	0.42	0.06	No tendency
Starch (mg/kg brix)	**0.77**	**-77.04**	**Linear loss**

Change of novel parameters with cut to crush delays

Pol/Sucrose

Figure 2A shows a decay of pol/sucrose ratio during deterioration. Both pol and sucrose decreased with deterioration. The trend of the ratio indicates that the loss in pol was greater than the loss in sucrose. The drop of pol/sucrose ratio is strongly correlated with reducing sugars uptake (r^2>0.8). Schoonees (2003) described the influence of glucose and fructose on pol final value (called pol derived) with the formula:

$$\text{Pol derived} = S + [(52.50 + 0.005902 * G + 1.4872E\text{-}5 * G^2) * G + (-91.33 - 0.04264 * F + 5.8136E\text{-}5 * F^2) * F] / 66.59$$

where S = sucrose (g/100 g juice), G = glucose (g/100 g juice), F = fructose (g/100 g juice)

The comparison between measured pol and derived pol did not show that optically active components other than sucrose, glucose and fructose have an effect on pol results (Figure 2B).

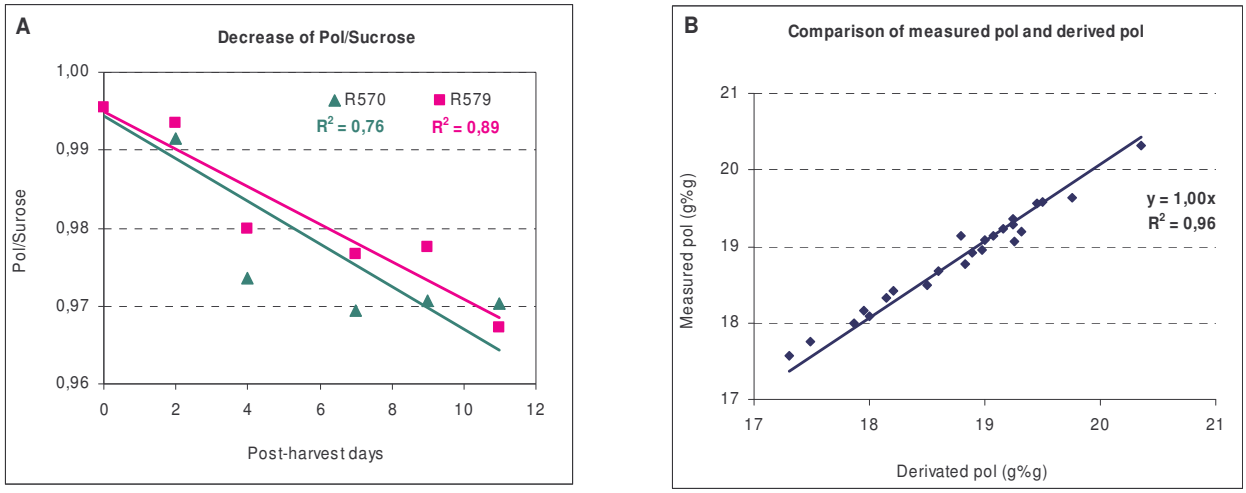

Figure 2. Pol/sucrose decrease with post-harvest days (A) and correlation between measured pol and derivated pol (B).

Organic acids

Aconitic acid

Aconitic acid is the major non nitrogen organic acid in sugarcane, with two isomer forms: trans-aconitic and cis-aconitic acid. The concentrations of the two isomers seem linked and two opposing hypotheses have been proposed. Brauer (1981) demonstrated that trans-aconitic accumulation in maize was due to citrate dehydrase activity, whereas other studies showed aconitate isomerase activity (Altekar *et al.*, 1965; Thomson *et al.*, 1997). Results of this study did not permit a conclusion to be made on this link, although an increase of cis-aconitic and a decrease of trans-aconitic was noticed. Change in the concentration of total aconitic acid was more irregular. Corcodel and Mullet (2007) reported that the cis aconitic/trans-aconitic ratio was a good cut to crush delay indicator and established the formulae shown in the equation below.

$$\text{Days post-harvest} = \left(\frac{\text{Cis Aconitic (g\%g)}}{\text{Trans Aconitic (g\%g)}} \times 100 - 0.0117\right) / 0.0026 \quad (r^2 = 0.88)$$

Figure 3 compares results of 2007 formulae with data from this trial. Results obtained for varieties R579 and R570 were in accordance with those of 2007 except at harvest (day 0) and 11 days after harvest for R570. Given the fact that the 2007 formulae was established from a 12 trial mean, and the 2008 graph from a single trial with two varieties and two replicates, this difference was probably be due to sample variation. Aconitic ratio is confirmed as an interesting cut to crush indicator.

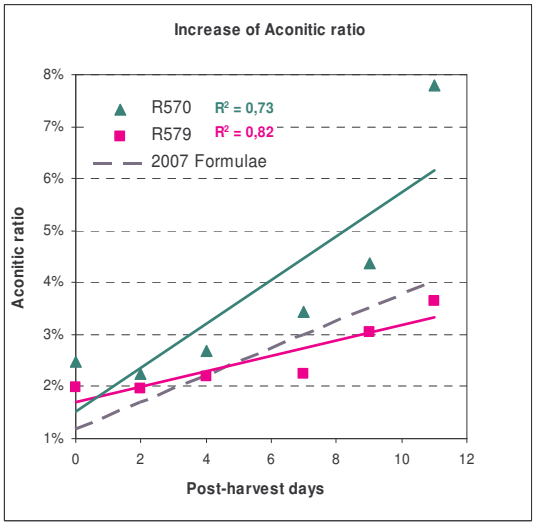

Figure 3. Increase of aconitic acid ratio with post-harvest days and comparison with 2007 trial.

Citrate and isocitrate

In this trial, no trends emanated from isocitrate analysis, although citrate concentration showed linear relationship with cut to crush delay (Figure 4). To explain this data, enzymatic flows have to be investigated. As respiration is disturbed, reduced cofactors accumulation will block acetyl-CoA synthesis, which is the main way to citrate and the Krebs cycle. A small accumulation of pyruvate is visible on a HPAE-CD chromatograph (data not shown), which

supports the idea of decreased pyruvate dehydrogenase activity. As the Krebs cycle is still working to produce precursors, citrate cellular concentration decreases.

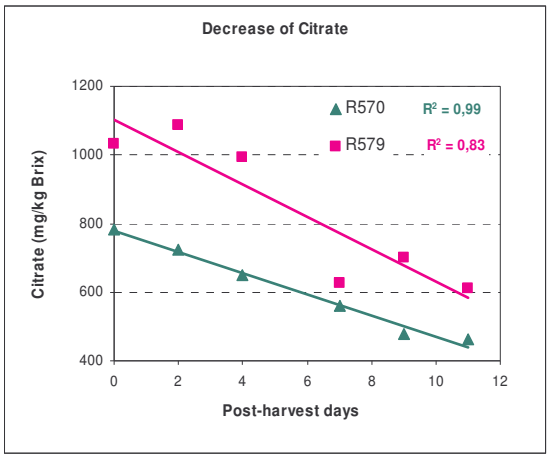

Figure 4. Decrease of citrate with post-harvest days.

Amino acids

The concentration of 15 amino acids, quantified by HPLC, was investigated during deterioration. Amino acids are involved in many biochemical reactions and proline, in particular, has been studied for many years (Errabii *et al.*, 2006).

Under water deficient stress, proline synthesis plays two physiological roles. The first is in maintaining the balance of cellular redox potential. Indeed plants are frequently exposed to excessive light intensities compared to energy necessary for carbon assimilation. Thus proline synthesis and degradation maintain a $NADP^+$/NADPH ratio at values compatible with metabolism (Hare *et al.*, 1998).

Proline synthesis also enables regulation and biophysical protection. In response to cellular dehydration, many plants accumulate compatible solutes which prevent ionic interactions amongst proteins. Many compounds have been identified as osmoprotectants, e.g. proline, glutamate, glycine betaine, oligosaccharides and fructans. Although these metabolites are chemically different, they share the property of being excluded from the surface of proteins which are still hydrated. The accumulation of proline under various stress conditions (salt, drought, metal) has been documented in many plants (Errabii *et al.*, 2006; Folkert *et al.*, 2001).

Other free amino acids also appear to be linked to drought stress and osmotic adjustment. Gzik (1996) concluded that under stress conditions the entire pool of amino-acids has to be considered.

The results of amino acid monitoring are shown in Table 3 for R570 and Table 4 for R579. The results confirm that proline can be considered as a good potential cut to crush delay indicator for the two predominant R varieties. Indeed, the fours trials showed a linear increase of proline (r^2=0.82) with post-harvest days and a very low concentration at harvest (day 0).

However, concentrations of proline in fresh canes that have been under water deficient stress during growth have not been measured in this trial. This will be done in other studies carried out at CERF. Alanine, cysteine, isoleucine and leucine showed a linear increase (r^2>0.70) in

concentration over the cut to crush delay. Variety R570 doubled its free amino acid content only two days after harvesting, whereas variety R579 took nine days. Measurements were done on two bundles per variety. In these conditions, it was not relevant to confirm them by statistical analysis.

Table 3. Free amino acid contents in sugarcane variety R570 depending on the cut to crush delay.

Amino acid (mg/kg DM)	Days after harvesting variety R570					
	0	**2**	**4**	**7**	**9**	**11**
Alanine	26.5	61.0	42.8	140.1	123.4	155.9
Proline	3.0	14.8	23.3	80.8	77.9	88.8
Cysteine	7.5	6.6	14.0	42.7	34.0	70.3
Isoleucine	1.4	3.5	1.0	8.2	6.9	13.2
Leucine	6.8	13.5	10.8	40.1	35.8	55.5
Other	138.9	267.2	189.9	423.8	380.0	450.8
Total (% day 0)	100.0	197.1	159.1	393.5	356.5	449.5

Table 4. Free amino acid contents in sugarcane variety R579 depending on the cut to crush delay.

Amino acid (mg/kg DM)	Day after harvesting variety R579					
	0	**2**	**4**	**7**	**9**	**11**
Alanine	71,4	72,5	60,3	114,8	135,1	119,4
Proline	5.1	13.7	43.0	109.8	120.7	95.2
Cysteine	8.4	17.0	9.8	27.9	34.3	49.9
Isoleucine	1.3	2.2	1.6	3.0	4.4	4.9
Leucine	15.4	17.7	14.4	22.4	28.5	28.7
Other	280,1	360,4	261,4	287,5	409,3	294,4
Total (%Day 0)	100.0	132.8	108.2	147.7	196.5	156.3

1-kestose : a promising cut to crush indicator

Polyols and oligosaccharides have been measured since 2007 at the CERF laboratory. Thus far, mannitol, isomaltotriose and 1-kestose have been identified. Mannitol and isomaltotriose did not show any regular accumulation whereas kestose (Figure 5) increased in a very linear way. 1-kestose seems to be a good cut to crush delay indicator for green cane. Indeed, for fresh cane 1-kestose concentrations are practically nil (less than 0.1% Brix) and the increase according to days after harvest is regular (0.05 g/100 g Brix per post-harvest day).

The oligosaccharides 1-kestose could be associated with two enzymatic activities: sucrose:sucrose 1-fructosyl transferase (EC 2.4.1.99) and invertase (EC 3.2.1.26) which also catalyses fructotransferase reaction. Indeed previous studies on yeast invertase activity enabled an understanding of enzymatic hydrolysis of sucrose and production of significant amounts of fructans. Legendre and Eggleston (2002) described the formation of oligosaccharides for concentrated sucrose solutions (>10 g%g) and Farine *et al.* (2001)

identified 1-kestose as one of the most predominant. Presence of kestose in sugarcane mills was widely discussed by Eggleston (2002) and Morel du Boil (1995).
1-kestose could be measured in cane delivered to assess freshness of cane with basis.

For R570, Post Harvest Days = 17.87 × kestose%Brix – 1.26
For R579, Post Harvest Days = 18.94 × kestose%Brix – 0.40

A global equation taking into account both of the varieties has been established:

Post Harvest Days = 18.51 x kestose%Brix – 0.88

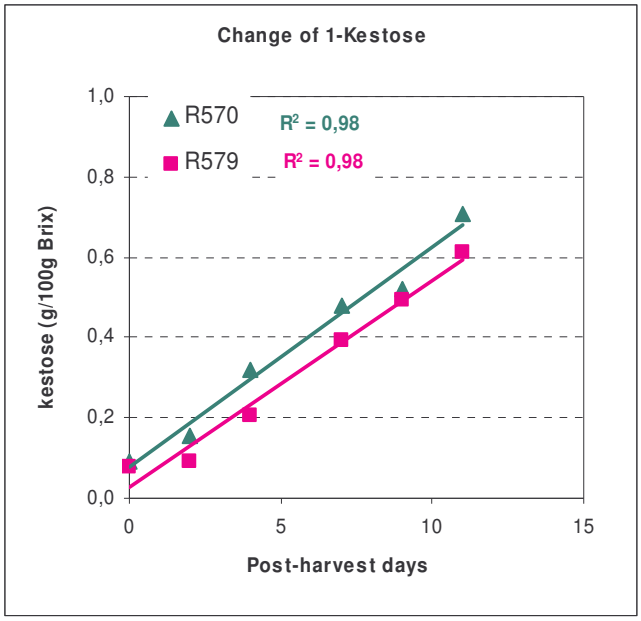

Figure 5. Changes in 1-kestose with post-harvest days.

Mixed Juice Survey
Comparing levels in mixed juice of various compounds presented previously can give an idea of freshness of cane delivered to Le Gol mill.

Cane freshness assessment using Mixed Juice common analysis
Every week, a mixed juice sample from Le Gol mill, representative of the week, is analysed at the CERF laboratory. As R570 represents 70% of the cane delivered to Le Gol mill, juice composition of R570 fresh cane and of cane delivered 4 and 7 days after harvest are compared to Le Gol mixed juice analysis. Unfortunately, this reasoning could not be applied to Bois-Rouge mill, because of the mixed juice sampling method. Figure 6A shows that according to reducing sugars, mixed juice analysed corresponded to a 0-4 day cut to crush delay.

True purity (Figure 6B) and pol/sucrose (Figure 6C) show a slightly higher delay reaching more than seven days in weeks 46 and 47 of the harvest season. Composition of mixed juice of true purity and pol/sucrose is more variable, probably because of other environmental and agronomical parameters influencing them.

In most cases common parameters highlight a delay below four days after harvest.

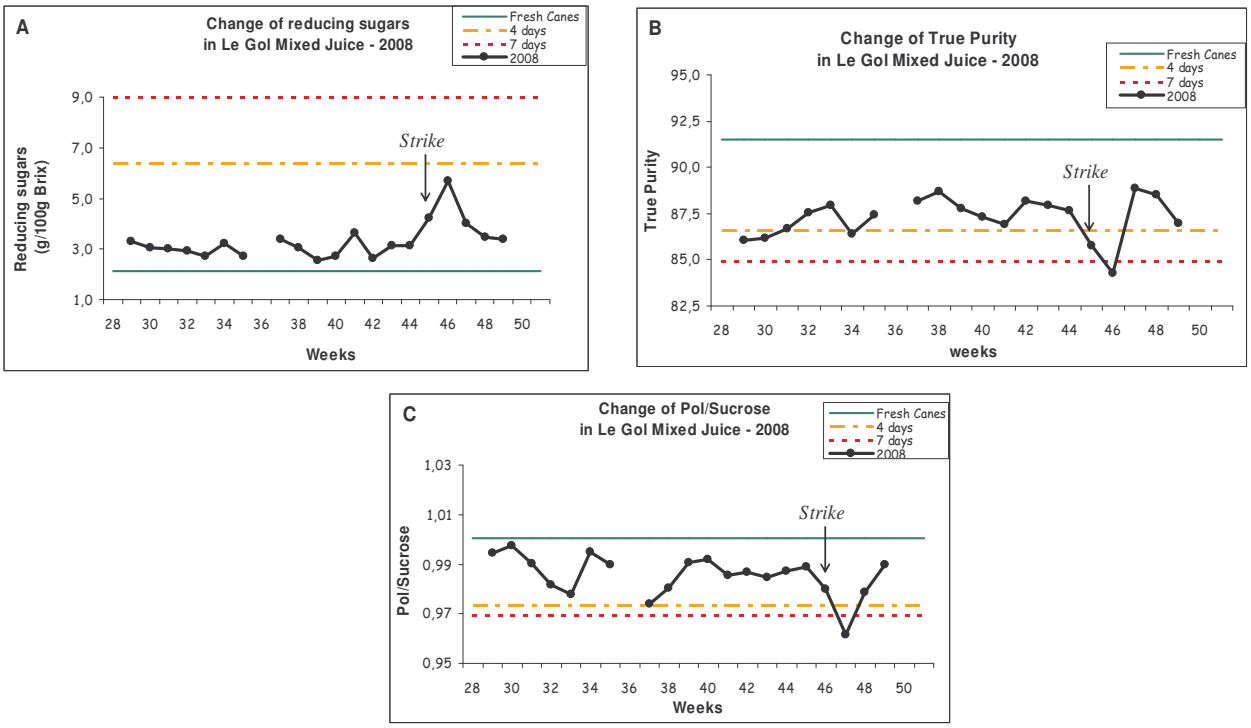

Figure 6. Mixed juice analysis from Le Gol mill during 2008 crushing season and post-harvest delay threshold of variety R570 for reducing sugars (A), true purity (B) and pol/sucrose ratio (C).

Cane freshness assessment using novel traditional analysis

Influence of extraction method (press and mills) on analytical results

It has been observed that amino acids and aconitic acid were three times more concentrated in mixed juice than in press juice extracted from deterioration trial cane samples. Differences between mixed juice and pressed juice composition indicated a better extraction of amino acids with a 5-roller mill tandem than with the press method (Table 5). In this case, it is not possible to match deteriorated levels and mixed juice composition with a cut to crush delay. Proline is under-estimated with the press method and the values on Figure 7B do not correspond to the real cut to crush delay. However, proline concentration can be used in relative terms, when comparing one week with another. Extraction of proline according to the press method or mill extraction will be further studied at CERF.

The same extraction problem was noted for *trans* and *cis* aconitic acid composition but the use of the aconitic ratio enabled the comparison and indicated an average delay of 4-7 days (Figure 7A).

Despite citrate being a promising measured parameter in pressed juice, no particular trends were noted in mixed juice (data not shown). It was not a suitable parameter to assess quality in mixed juice.

Table 5. Amino acid and aconitic acid concentrations in mixed juice and extracted juice from deterioration trial.

	Deterioration trial	Mixed juice (Le Gol 2008)
Amino acids (mg/kg Brix)	534.01	1873.62
Aconitic acid (mg/kg Brix)	2904.97	9297.92

Measurements of cut to crush delay from a mixed juice survey

Aconitic acid ratio measured in mixed juice indicated a cut to crush delay of 4-7 days until week 40, and 7-11 days until the end of the season (Figure 7A). For proline (Figure 7B), the comparison revealed a cut to crush delay of 4-7 days for most of the weeks, and more than 11 days in weeks 37 and 45 to 48. 1-kestose concentration in mixed juice indicated also a 4-7 day cut to crush level except in weeks 45 to 47 (Figure 7C).

In most cases those parameters highlight a delay of 4-7 days after harvest.

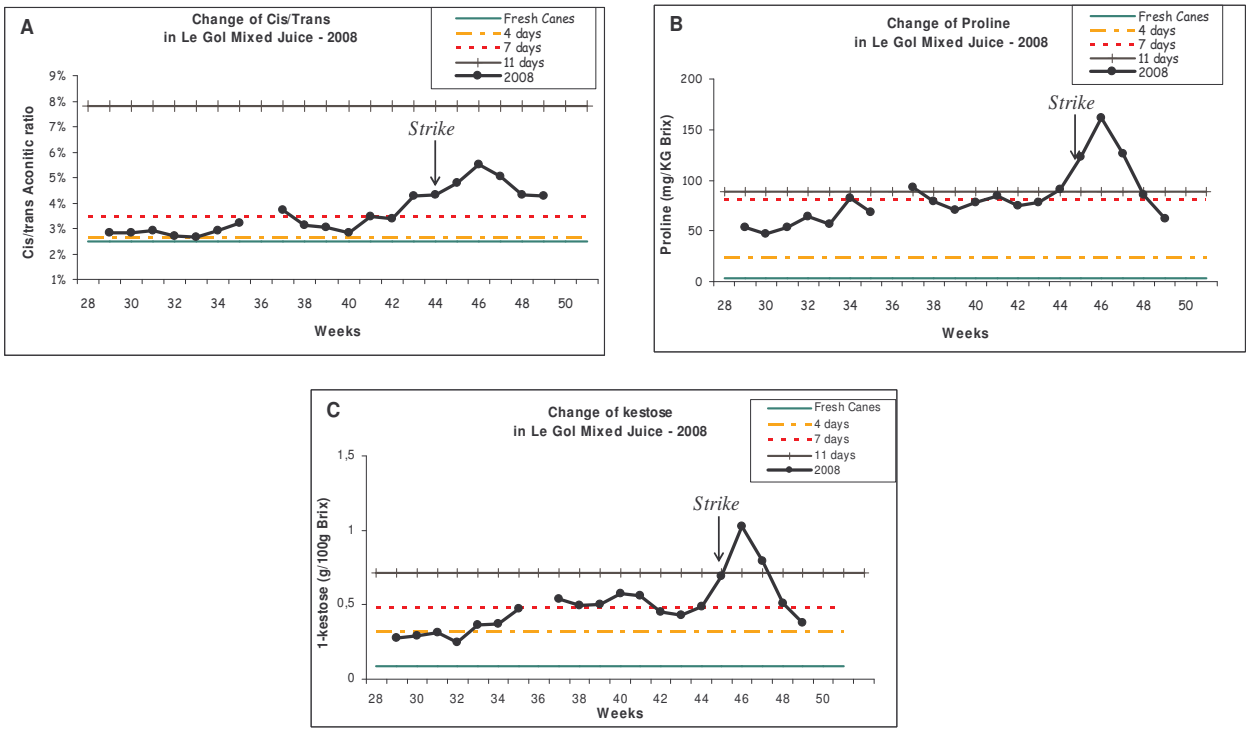

Figure 7. Mixed juice analysis from Le Gol Mill during 2008 crushing season and post-harvest delay threshold of R570 for cis/trans ratio (A), proline (B) and 1-kestose (C).

Although these parameters are in discordance between a 0-4 day delay or a 4-7 day delay between harvest and crush, all are concordant on the quality of cane delivered. For example, they all indicate a poor cane quality in week 46. This can be explained because during week 45, mills had to stop crushing because of a labour strike. Then, when the mills started again, cane crushed had been cut the previous week.

Further studies are needed to evaluate which parameter is the more accurate to assess global freshness of cane delivered to the mill.

Conclusions

Loss of weight, purity and true purity, and an increase in of reducing sugars have been observed in whole stalk green cane after harvest.

Aconitic ratio is confirmed as a good cut to crush delay indicator. This study highlights new and promising research leads for assessment of cane quality. Among amino-acids, proline gathers low concentration in fresh cane and a highly linear increase with deterioration. Other amino acids such as alanine, cysteine, leucine and isoleucine also showed interesting trends. Studies on variability of proline and other amino-acids in fresh cane grown under water-deficient stress have been planned.

Among oligosaccharides, 1-kestose showed a linear correlation with cut to crush delay. With a low concentration in fresh cane and a linear increase, it behaves in hand-cut green cane in the same manner as ethanol in burned cane (Lionnet and Pillay, 1987). The method used in this paper is time consuming, but if this parameter is relevant enough to assess freshness of cane, a simple colorimetric method can be developed.

The drop in juice purity associated with cut to crush delay reduces the factory performances (Ravelo *et al.*, 1991). Quality of cane delivered to the mill can be assessed according to mixed juice analysis. Further studies would enable a more precise deterioration delay using relevant indicators.

Acknowledgments

The authors would like to thank CERF breeding department staff for providing the canes and CTICS workers for their help with the press method. The many analyses done by colleagues in the CERF laboratory are appreciated.

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