

AN ANALYTICAL APPROACH FOR THE DETERMINATION OF COLOUR AND OTHER IMPURITIES IN CANE

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Abstract

An analytical technique for the determination of the soluble constituents of sugarcane has been developed and found to be satisfactory. The technique allows the cane to be analysed for colour bodies and colour precursors, under specified conditions of pH and temperature. It has been used to investigate the changes in impurity content due to the presence of tops and trash, due to burning as opposed to trashing and due to the various parts, such as rind and node, of the clean cane stalk. The effects of water stress have also been investigated. The results show that on a comparable basis, tops introduce more impurities than trash. The rind of a stalk of cane contributes significant quantities of colour impurities. Finally, drought causes large increases in the concentrations of colour impurities and colour precursors, particularly amino nitrogen.

Introduction

The direct analysis of sugarcane was investigated by Buchanan^{1,2,3} over the period 1966–1968 and has since been adopted at all the South African mills. The method is used very satisfactorily for the determination of pol, brix, fibre and moisture in cane but has not been extended to the determination of specific impurities, particularly those that are present in cane at low concentrations.

From 1981, Spiro⁵⁻⁸ has been investigating the mechanisms through which soluble constituents of tea and coffee are extracted when these materials are mixed with water. He has proposed a number of simple and elegant models to describe these extraction processes and has shown that his models fit the experimental results very satisfactorily.

The extraction of brix and of a number of impurities from prepared cane has been extensively investigated by Rein.⁹ His results show that the mechanisms involved in the case of the cane/water system are not fundamentally different from those of the tea/water or coffee/water systems of Spiro.

These two approaches were combined to give a method for the determination of a number of the constituents of cane.

Theory

A mass W^* of cane pulp, containing a fraction X_o of a given constituent, is immersed at time $t = 0$, in a volume V of water, the system being kept at a constant temperature. Immersion has two main consequences:-

- the cane pulp absorbs water
- various soluble constituents are extracted into the liquid.

At equilibrium, the net volume of water taken up by unit mass of pulp is V_n and Y is the mass of soluble constituents extracted per unit mass of cane.

Then, the mass of the swollen cane pulp, W_s , is $W_s = W \cdot (1 + V \cdot \rho_w) - Y \cdot W$ (1)

The mass of the given constituent in the extract, at equilibrium, W_a , is

$$W_a = C_\infty \cdot (V - W \cdot V_n)$$

* Nomenclature in Appendix 1.

But the total mass of the constituent is $W \cdot X_o$.

Thus, the mass of the constituent in the cane pulp, W_p , is

$$W_p = W \cdot X_o - C_\infty \cdot (V - W \cdot V_n)$$

and the concentration of that constituent in the pulp is then $C_s = [W \cdot X_o - C_\infty \cdot (V - W \cdot V_n)] / [W + W \cdot V_n \cdot \rho_w - Y \cdot W]$

The concentration of the constituent in the extract is C_∞ / ρ_s .

A partition coefficient, K , may be defined by

$$K = \frac{\text{Concentration of constituent in extract}}{\text{Concentration of constituent in pulp}} \quad \dots (2)$$

Then

$$K = \frac{C_\infty / \rho_s}{W \cdot X_o - C_\infty \cdot (V - W \cdot V_n) / (W + W \cdot V_n \cdot \rho_w - Y \cdot W)}$$

which may be rearranged to give

$$\frac{1}{C_\infty} = \frac{V}{X_o \cdot W} + \frac{1}{X_o} \left(\frac{1 + V_n \cdot \rho_w - Y}{K \cdot \rho_s} - V_n \right) \quad \dots (3)$$

Equation 3 shows that a plot of $1/C_\infty$ versus $1/W$ should yield a straight line with slope equal to V/X_o and intercept equal to $\left(\frac{1 + V_n \cdot \rho_w - Y}{K \cdot \rho_s} - V_n \right)$

X_o , the mass fraction of the constituent in the cane, can be obtained from the slope of the line since V is known.

K , the partition coefficient, can also be obtained from the intercept if the quantities V_n , ρ_w , Y and ρ_s are known. Of these only V_n is not available but may be obtained through the following procedure.

A mass of cane W_c is mixed with a mass of water W_w . The mixture is allowed to stand and then sieved to allow the free liquid only to run off. The run-off mass, W_r , is obtained. This is represented by the mass balance.

$$W_w + W_c = W_s + W_r \quad \dots (4)$$

where W_s is the mass of the wet cane, at equilibrium.

Equation 4 may be re-written as

$$W_w = (W_s - W_c) + W_r$$

or $W_w = W_h + W_r$ (5)

where W_h is the water held by the cane.

Equation 5 cannot be used directly because the brix balance shown by equation 6 must also be satisfied.

$$\frac{W_c \cdot B_c}{100} = \frac{W_s \cdot B_s}{100} + \frac{W_r \cdot B_r}{100} \quad \dots (6)$$

If cane sub-samples of known, equal masses are used and different known masses of water are added to each, equation 4 shows that a plot of W_w versus W_r should be linear. The value of W_w when $W_r = 0$ gives W_h the amount of water held by the cane when there is no run-off.

Then, $W_n = W_h / W_c$ (7)

Experimental Procedure

Equation 3 shows that the independent variable is 1/W. The masses of cane were chosen to yield five equidistant points on the 1/W axis. The quantities used were 2 000 cm³ of water and 400, 500, 600, 800 and 1 000 g of cane, shredded in a Jeffco cutter-grinder. With this preparation, equilibrium was found to have been reached after about 40 minutes. However 60 minutes were used in all the tests.

The equipment has been described in a previous paper.¹⁰ All the tests were done at 80°C and at a pH of 6,2 — 6,4, measured at 80°C.

The following constituents were selected for analysis:-

- Pol and brix % cane which also allows comparison with the DAC results;
- Total phenols by the Folin-Ciocalteu method, since phenols are a class of compounds which has been associated with colour problems in juices and sugar;
- Amino-nitrogen by the ninhydrin method. Amino-nitrogen is involved in Maillard type reactions which can cause colour problems;
- Optical absorbance in the visible region of the spectrum (420 nm) which gives a direct indication of colour. Two pH levels were used, namely 4 and 9, to allow the calculation of Indicator Values (IV). Australian workers¹¹ have shown that plant pigments have IV's of 7-13 while enzymatic and other colorants have IV's of 1-5.
- Optical absorbance in the UV (280 nm) was also measured since a number of colour bodies and colour precursors absorb in the UV.

The analytical results are reported as follows:-

Absorbances : Absorbance units on cane, given by

$$\frac{\text{Absorbance} \times \text{Dilution factor}}{\text{Cell length (cm)}}$$

 Total phenols : ppm caffeic acid on cane
 Amino nitrogen : ppm aspartic acid on cane

Results and Discussion

Fit of the model

The linearity of the plot of 1/C_∞ versus 1/W was checked for a number of constituents. Typical results, with the number of observations being 5 in each case, are shown in Table 1.

Table 1

Correlation coefficient for the plots of 1/C_∞ versus 1/W, from equation 2.

Analysis	Corr. Coefficient
Brix	0,999
Pol	0,999
Total phenols	0,982
Amino-nitrogen	0,986
Abs. 420 nm, pH 4	0,942
Abs. 420 nm, pH 9	0,996
Abs. 280 nm, pH 4	0,987

The excellent linearity confirms the validity of the approach.

Since the method yields brix and pol % cane, a comparison may be done with the values obtained by DAC. This has been done for 31 samples in Figure 1.

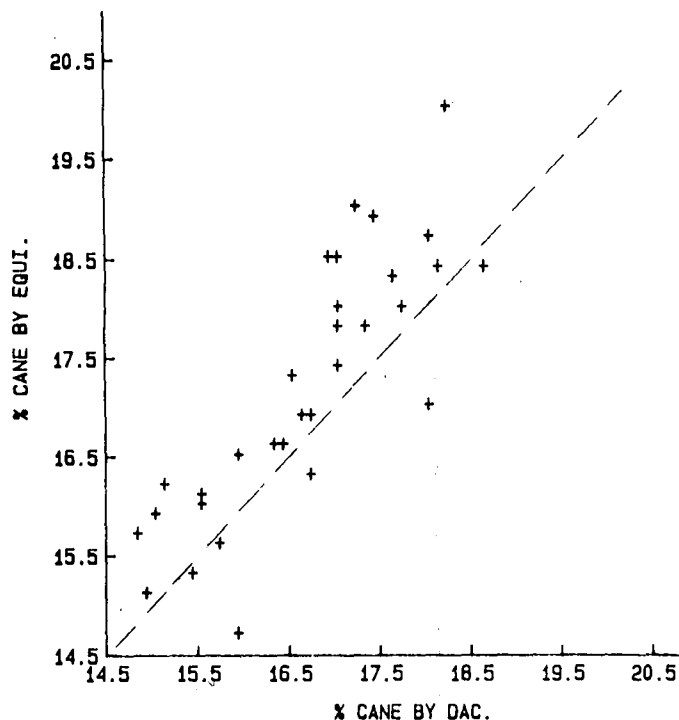


FIGURE 1 Brix and pol % cane by the DAC and equilibrium methods. (Equivalence line shown).

It is evident that the equilibrium method shows a consistent bias of between 0,5 and 1 unit on both pol and brix values. The higher values are probably due to two main factors. Firstly some evaporation must have taken place from the stirred vessels used in the equilibrium method although precautions were taken to minimise it. Secondly, the higher temperature used for the equilibrium method could result in higher extractions, particularly as far as the impurities are concerned. Generally, however, the agreement is such that it can be concluded that the equilibrium method yields meaningful results.

The effect of tops and trash

Tops and trash were added, in known quantities, to clean cane and the mixtures were analysed for the selected components. The results obtained are shown in Table 2.

Table 2

Changes in concentrations due to the addition of 10% by mass of tops or trash to clean cane

Analysis (on cane)	Clean cane	Concentration in	
		Clean cane + 10% tops	Clean cane + 10% trash
Brix	18,8	18,3	18,2
Pol	16,9	15,9	15,5
Total phenols	1 150	1 286	1 624
Amino-nitrogen	125	128	138
Abs. 420 nm pH 4	0,58	1,14	0,94
Abs. 420 nm pH 9	7,8	10,6	10,6
Abs. 280 nm	36,0	45,6	47,3

In all cases tops and trash have adverse effects on cane quality since they reduce the level of pol (Table 2) and increase the impurities.

Tops and trash have very different compositions and are best compared by using their dry matter content as reference. This has been done in Table 3 where the effects are expressed as a percentage of the clean cane value due to the addition of 1% dry matter from tops or trash.

Table 3
Differences due to addition of tops or trash

Analysis	Difference as a % of clean cane value due to the addition of 1 % dry matter from	
	Tops	Trash
Brix	-0,3	-0,2
Pol	-0,6	-0,4
Total phenols	+1	+2
Amino-nitrogen	+0,3	+0,6
Abs. 420 nm pH 4	+10	+3
Abs. 420 nm pH 9	+4	+2
Abs. 280 nm	+3	+2

On a comparable basis, tops introduce more impurities than trash. This was not apparent on the mass basis.

On a mass basis, the South African industry processes cane with about 2 % tops and 4 % trash. Using the results obtained here, this quantity of extraneous matter has reduced the pol in cane by nearly 5 % and has increased the total phenols by about 15 %, the amino-nitrogen by 5 % and the absorbances by about 20 %. The effects of tops and trash on the colour present in the juice is thus not negligible.

The effect of cane deterioration

The equilibrium method has been used to investigate the effect of cane deterioration, due to delays, on the cane quality. Detail results have been given elsewhere¹² and it was concluded that the deterioration of healthy cane has little or no effect on the levels of colour impurities in the cane. Diseased cane, however, showed very high levels of amino-nitrogen.

Burning versus trashing

In the course of the various experiments, a number of samples of burnt and unburnt cane, of the same or similar origin, was analysed. It was therefore possible to study the effect of burning on the levels of colour impurities in clean, whole stalk sugarcane.

The results obtained are shown in Table 4.

Table 4

The effect of burning on the levels of colour impurities in clean cane

No. of Samples	Analysis	Average concentration in cane	
		Burnt	Unburnt
2	Abs. 420 nm pH 4	1,2	0,6
	Abs. 420 nm pH 9	10,0	7,5
3	IV	8	12
	Abs. 420 nm pH 4	1,9	1,2
	Abs. 420 nm pH 9	14,0	10,8
10	IV	7	9
	Abs. 420 nm pH 4	1,9	1,0
	Abs. 420 nm pH 9	13,2	9,6
30	IV	7	10
	Abs. 420 nm pH 4	1,0	0,8
	Abs. 420 nm pH 9	11,0	9,1
	IV	11	11
	Total phenols	1 125	1 040
	Amino-nitrogen	88	56
	Abs. 280 nm	45,0	42,2

Although there are no large differences, the burnt cane always shows higher absorbances and higher levels of the selected impurities. IV values from the burnt cane are slightly lower than those of the unburnt cane. This is due to the fact that the increase in the absorbance at pH 4 is larger than the increase in absorbance at pH 9. Burning thus seems to cause the pH 4 absorbance to increase significantly.

Similar results have been reported by Australian workers.¹³

It thus appears that burning could increase the levels of colour but it must be stressed that the comparison given here is based on perfectly clean cane. As shown in the previous section, only small percentages of tops and trash are sufficient to cause much larger increases in the levels of impurities and this would outweigh the effect of burning.

Rind, node and internode

Twenty-three stalks of burnt, healthy NCo 376, 22 month-old cane were peeled by hand. The rind, nodes and internodes were weighed, shredded and analysed separately. Because of the limited quantity of sample available, the usual 5 point approach could not be used. The results could thus be less precise but are still comparable, and are shown in Table 5.

Table 5

Concentrations of the selected constituents in the rind, nodes and internodes of healthy cane

Analysis	Concentration in		
	Rind	Nodes	Internodes
Brix %	18,6	20,4	20,7
Pol %	15,4	18,1	18,1
Total phenols	1 360	950	560
Amino-nitrogen	190	175	155
Abs. 420 nm pH 4	1,5	0,6	0,6
Abs. 420 nm pH 9	9,3	6,7	3,0
IV	6	11	5
Abs. 280 nm	56,0	33,8	22,5
% by mass	29	20	51

An identical exercise was repeated but with severely drought stressed cane. The results are shown in Table 6.

Table 6

Concentrations of the selected constituents in the rind, nodes and internodes of drought stressed cane

Analysis	Concentration in		
	Rind	Nodes	Internodes
Brix %	13,6	14,2	13,7
Pol %	8,6	9,7	8,5
Total phenols	1 550	1 205	880
Amino-nitrogen	410	345	220
Abs. 420 nm pH 4	2,1	1,8	1,0
Abs. 420 nm pH 9	14,3	14,1	7,5
IV	7	8	8
Abs. 280 nm	64,4	50,0	31,9
% by mass	40	19	42

These results show clearly that the rind of the cane stalk contains more impurities than the rest of the stalk. Furthermore, the marked effect of drought both on the concentration of impurities and on the increase in the proportion of rind to total stalk is very evident.

In view of the marked influence of drought on the level of colour bodies in cane, more comparisons were done, as described in the next section.

Effect of water stress

Two cane samples were taken from the same field, one from a low-lying area where soil humidity was high and the other from a hilltop where water stress was likely to occur. This was done at two different fields.

The clean stalks were then shredded and analysed by the equilibrium method. The results are shown in Table 7.

Table 7
The effect of water stress on the levels of impurities in cane

Analysis	Concentration in cane			
	Test 1 (NCo 376, 22 months)		Test 2 (NCo 376, 18 months)	
	Normal	Stressed	Normal	Stressed
Purity	92,2	88,8	88,0	80,1
Total phenol	6 265	6 165	4 295	5 730
Amino-nitrogen	245	345	490	1 215
Abs. 420 nm pH 4	0,7	1,8	1,3	2,0
Abs. 420 nm pH 9	8,6	12,5	10,2	14,9
IV	12	7	8	7
Abs. 280 nm	7,6	10,8	12,2	9,1

These results confirm those obtained in the previous section and show that water stress results in significant increases in the content of colour bodies. The increase in amino-nitrogen is particularly pronounced and could result in serious colour problems during processing since amino-nitrogen is linked to Maillard type reactions.

Partition coefficient

The equilibrium method uses a partition coefficient K given by equation 2, which can be calculated.

Average values of K, based on more than fifty determinations are shown in Table 8.

Table 8
Values of the partition coefficient K

Analysis	Mean value of K
Brix %	1,00
Pol %	1,01
Total phenols	0,92
Amino-nitrogen	0,86
Abs. 420 nm pH 4	0,89
Abs. 420 nm pH 9	0,79
Abs. 280 nm	0,88

These results indicate that K is equal to 1 for pol and brix. This is a prerequisite for any method, such as DAC, which is based on a mass balance calculation. The equilibrium method thus shows that the DAC approach is correct for pol and brix but not for the other constituents which would be underestimated by 10 to 20% if the mass balance approach were used.

The equilibrium method requires more equipment and is tedious but offers a number of advantages not found in the

usual methods of cane analysis. It is not seen as a replacement for DAC or pressing techniques but rather as complementary to the existing techniques.

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APPENDIX 1

Nomenclature

- B_c Brix % cane
- B_s Brix % swollen pulp
- B_r Brix % run off
- C_∞ Concentration (g·cm⁻³) at infinite time
- K Partition coefficient (g·cm⁻³)
- V Volume (cm³) of water added to the cane pulp
- V_n Volume of liquid taken up by unit mass of cane (cm³·g⁻¹)
- W Mass of cane (g)
- W_r Mass of run-off (g)
- W_s Mass of swollen cane (g)
- W_n Mass of water held by cane (g)
- X_c Mass fraction of constituent in cane (g/g)
- Y Mass of soluble constituents extracted per unit mass of cane
- ρ_w Density of water (g·cm⁻³)
- ρ Density of extract (g·cm⁻³)