FULL PAPER

COMPARISON OF LEADED AND UNLEADED DIRECT ANALYSIS OF CANE, BASED ON DIFFERENT CANE VARIETIES

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Abstract

Despite lead being hazardous to human health, it is used as lead (II) acetate in the clarification process for the determination of Pol (apparent sucrose) in the sugar industry. There is a need to explore safer alternatives without lead. This work details the comparison of conventional leaded and leadless Direct Analysis of Cane (DAC) methods for determining Pol and the Estimated Recoverable Crystal (ERC) % cane on thirteen commercial sugarcane varieties grown by the Zimbabwe Sugar Industry (ZSI). With the leaded method, sugarcane juice was clarified by using lead (II) acetate, and Pol was determined on a polarimeter at 589 nm. For the leadless method (Near Infrared (NIR) Polarimetry), the sugarcane juice was filtered by using celite 577 to reduce the juice turbidity and the Pol was determined at 880 nm on a polarimeter. The ERC% cane was calculated from the Brix, Pol and fibre values. There was a strong correlation between the leaded and unleaded determinations, with R^2 ranging from 0.977 to 0.995 for the Estimated Recoverable Crystal % cane (ERC% cane) and 0.953 to 0.998 for the Pol values. The difference between leaded and unleaded ERC% cane was found to be within a tolerance of ± 0.9%. On average, the leaded values were slightly higher than the unleaded values, although the statistical analysis indicated no significant difference between the methods, with a p-value of 0.344.

Keywords: Lead (II) acetate, Pol, NIR, Polarimetry, ERC% cane, leaded, leadless

Introduction

Pol (the apparent sucrose), a value that is obtained from polarimetry, forms the basis of all international sugar sales (van Staden and Mdlalose, 2000). Since 1842, the sugar industry has relied on polarimetry to measure the sucrose content in crops, factories and refinery products (Schoonees and Alborough, 2005). Polarimetry is rapid and precise, but it indirectly quantifies the amount of sucrose in a sample. It also helps to monitor and control factory processes, budgeting, cane maturity tests and the selection of breeding cane varieties. Its speed, ease of use and the reproducibility of Pol readings have supported its continued use in the sugar industry (Player *et al*., 2000). However, polarimetry is affected by other optically-active components, such as the glucose, fructose and dextran that is present in the sample (Bradbury *et al*., 1986; Schoonees, 2003).

In order to do away with the interference of optically-active substances in polarimetric sucrose determination, lead sub-acetate is applied to complex non-sucrose components and colourants, forming a precipitate. The precipitate, therefore, entraps and absorbs all the possible solids in the juice (Thai, 2013). Upon filtration, a clear transparent solution is obtained, which can be analysed in the polarimeter with reduced interference (Schlumbach *et al*., 2017), leaving behind the lead waste.

As a result of the perceived occupational health risks, in addition to the problems and costs associated with the safe disposal of lead waste worldwide, alternative methods of Pol analysis are being investigated by the sugar industry (Schoonees and Alborough, 2005). The alternatives that have been tried include OctaPol, bismuth chloride, XYZ-clarification agent, various aluminum reagents, combined calcium and aluminum compounds, as well as zinc salts. While some of the clarification agents showed potential, none of them could clarify better than lead (II) acetate (Schoonees, 2003). The filtrates are often not transparent enough for measurements with a polarimeter at the standard wavelength of 589 nm (Schoonees, 2003). The International Commission for Uniform Methods of Sugar Analysis (ICUMSA) has introduced another wavelength, at which coloured samples can be reliably measured (De Whalley, 2013). These samples are too dark to be measured with a visible wavelength (589 nm), but they are transparent enough to be determined by a near-infra-red wavelength of 880 nm (De Whalley, 2013).

NIR Polarimetry allows for simultaneous sample preparation for Brix and Pol determination and reduces the cost of analysis, as the use of a lead sub-acetate is avoided. NIR polarimetry should not be confused with Near Infra-Red Spectroscopy, which uses an instrument that is different from the common DAC polarimeter. NIR polarimetry uses the ability of optically-active substances to rotate a plane of polarised light, while NIRS is a type of vibrational spectroscopy that corresponds to a wavelength range of 700 to 2500 nm (López *et al*., 2017; Harris *et al*., 2018). NIR light waves are able to travel through highly-coloured solutions and are thus able to eliminate the need for colour removal in polarimetry. NIR polarimetry was also reported to be applicable in the analysis of other sugars elsewhere (van Staden and Mdlalose, 2000). In this work, the results of conventional leaded polarimetry at 589.44 nm are compared to those of NIR polarimetry at 880 nm. The subsequent calculations of ERC were compared, as determined by leaded and NIR polarimetry.

Method and Materials

Sample collection

A total of thirteen different sugarcane variety samples (ZN1, ZN2, ZN3, ZN4, ZN5, ZN6, ZN7, ZN8, ZN10, N14, NCO376, CP72-1312 and CP72-2086) were obtained from the Zimbabwe Sugar Association Experiment Station (ZSAES) Centre Pivot plot for analysis. Samples were collected and analysed from planted cane from the same field at the end of each month, between June and September 2019.

Calibration of the polarimeter and refractometer

A quartz plate was used to calibrate the polarimeter with a required standard deviation of ± 0.03. The refractometer was calibrated by using four sucrose standards with a concentration of 3 °Bx, 4,5 °Bx, 5.50 °Bx, and 7.50 °Bx

Preparation of samples for the unleaded method of Pol determination

The cane juice sample for each variety was divided into ten 150 ml portions. The juice was filtered by using 2 g of celite 577 (filter aid) to remove the juice turbidity and filtered by gravity stemless funnels, using Whatman No 91-filter paper with a diameter of 18 cm. Pol was determined at a NIR wavelength of 880 nm using a polarimeter (AP880 Automatic Saccharimeter) with a 20 cm Pol tube.

Preparation of samples for the leaded method of Pol determination

Cane juice samples for each variety were divided into ten 150 ml portions. The cane juice was clarified (colour removal), by using 1 g of lead acetate, and filtered by gravity stemless funnels, using Whatman No 91-filter paper with a diameter of 18 cm. Pol was determined at 589 nm using a polarimeter (AP880 Automatic Saccharimeter) with a 20 cm Pol tube.

The leaded and unleaded analyses were carried out one after the other for each variety. Brix was also determined for each variety. The Brix, together with the Pol reading, gives the Estimated Recoverable Crystals % cane (ERC) values.

$$ERC\%\ cane = aP - bN - cF \qquad (1)$$

Where 'a', 'b' and 'c' are the ERC factors

a = a measure of the unit fraction of the recovery of Pol in sugar,

b = the loss of Pol in molasses per unit of non-Pol in cane,

c = the loss of Pol in bagasse per unit of fibre in cane, and

P *= Pol % Cane,* ***N*** *= Non-Pol % Cane and* ***F*** *= Fibre % Cane.*

Statistical analysis of data

The student's t-test was used, when comparing the data for the two samples. The data were reported as mean values ± standard deviation.

Results and Discussion

A total of ten samples for each variety were analysed in June 2019, using the ICUMSA leaded and unleaded NIR polarimetry methods. The mean Pol and ERC% cane results for the two methods were regressed and the R^2 values were noted. The average Pol difference between the leaded and NIR polarimetry was 0.21 and that between the leaded and NIR Polarimetry ERC% cane was 0.22. An R^2 of 0.8628 and 0.0014 were observed for the ERC% cane and Pol, respectively. The low R^2 value on Pol readings could be caused by the immature cane samples collected during that period. The immature samples were collected before drying off and they were still undergoing vegetative growth. Vegetative growth is associated with the presence of reducing sugars which in turn interfere with sucrose determination on polarimetry. It is the stress caused by the cold winters and lack of irrigation water that causes cane to mature and accumulate more sucrose. Low Pol values were also observed for the unleaded NIR polarimetry, supposedly due to the non-removal of fructose, as no clarification is being done in the method. Although the correlation for Pol values was weak, it was not so in ERC which gave a significantly higher R^2 value of 0.8628, as shown in Figure 1 below.

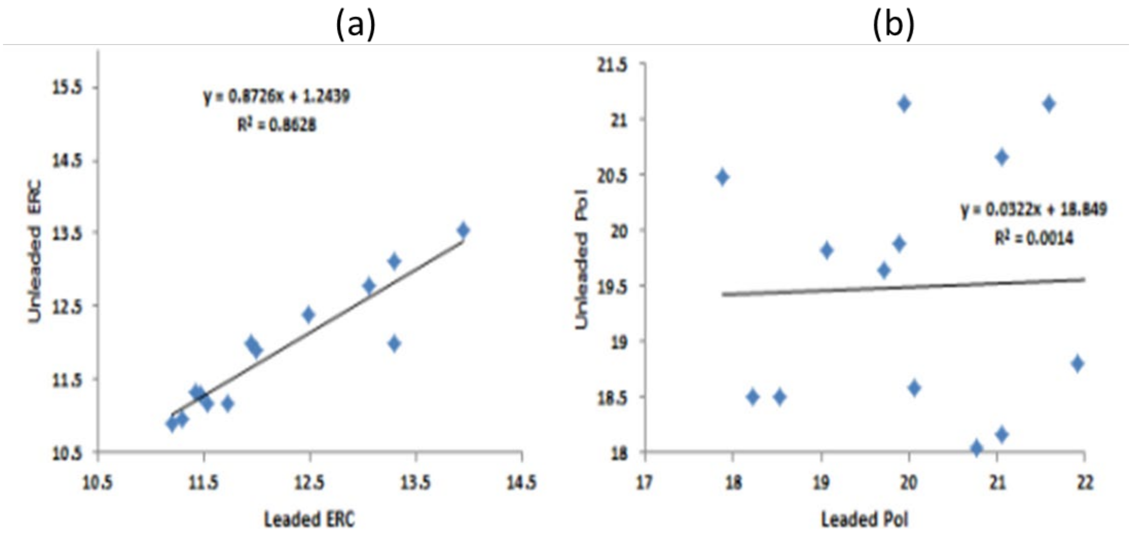

Figure 1. Graph showing the regression analysis of leaded vs. unleaded ERC (a) and leaded Pol vs. unleaded Pol (b), as at the end of June 2019

A total of ten samples for each variety were analysed in July 2019 by using the ICUMSA leaded and NIR polarimetry methods. The mean ERC values for the two methods were comparably similar; however, the Pol values for the NIR polarimetry were slightly lower than those of the leaded polarimetry. The average differences were 0.24 and 0.31 for Pol and ERC% cane, respectively. The differences were within the acceptable tolerance limit of 0.60% and 0.30% for ERC% cane (ERC) and Pol% cane (Pol), respectively, as regulated in the local inter-laboratory exchange samples proficiency testing scheme. The Coefficient of Determination values of 0.9687 and 0.9869 were observed for the ERC% cane and Pol regressions, respectively. The R^2 values for ERC and Pol had significantly improved, compared to those for the month of June. This could be because of the increased maturity, as the winter progresses. During winter, the temperatures are low and result in fewer physiological reactions in the plants, as opposed to summer. The regressed graphs of ERC and Pol are shown in Figure 2.

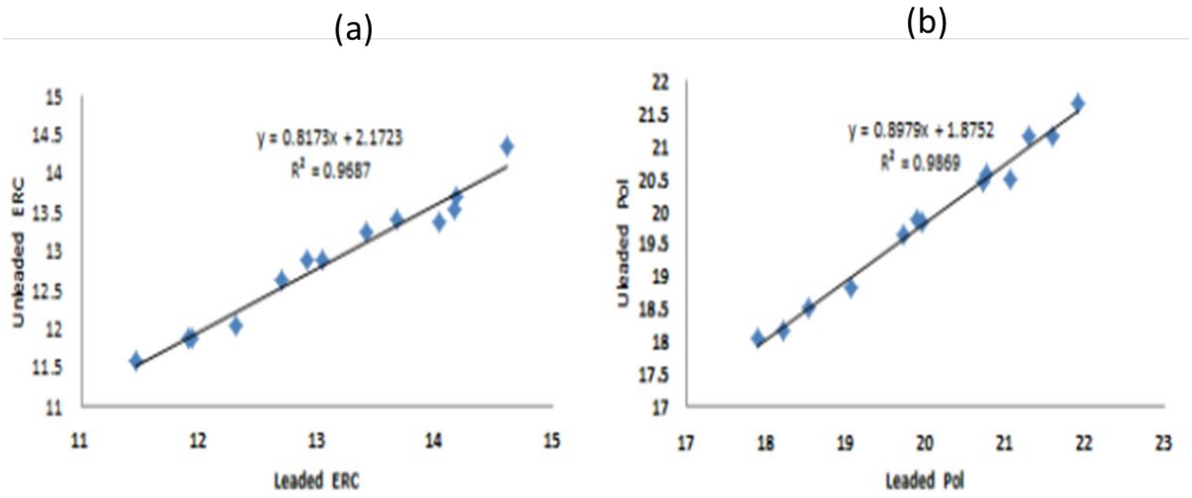

Figure 2. Graph showing the regression analysis of leaded ERC vs. unleaded ERC (a) and leaded Pol vs. unleaded Pol (b), as at the end of July 2019

There was a significant increase in the correlation of unleaded and leaded Pol results, giving an R^2 value of 0.9701 in the month of August. A total of ten stalks for each variety were combined into a sample and analysed by using the ICUMSA leaded and NIR polarimetry methods. The mean ERC values for the two methods were comparably similar; however, the Pol values for the leadless method were slightly lower; they were the same as those observed during the month of July. It is suspected that the lower Pol values emanate from the presence of fructose, which is conventionally eliminated by clarification when sub-lead acetate is used. In the leaded method, lead acetate complexes and removes about 11% of the fructose. Sucrose is a dextrorotatory, optically-active compound, thus it rotates the plane of a passing polarised light wave to the right. Glucose and dextrans turn the plane clockwise (dextrorotatory), and fructose turns it anti-clockwise (levorotatory). The direction of the polarisation of fructose therefore largely interferes with that of sucrose in the unleaded juice.

An analysis of a large number of samples would also help to come up with a correctional factor that can be used to compensate for the effect of fructose (Schoonees and Alborough, 2005). The average difference was 0.33 for Pol and 0.22 for ERC. Correlations of 0.87 and 0.97 were observed for the ERC and Pol, respectively, as shown in Figure 3.

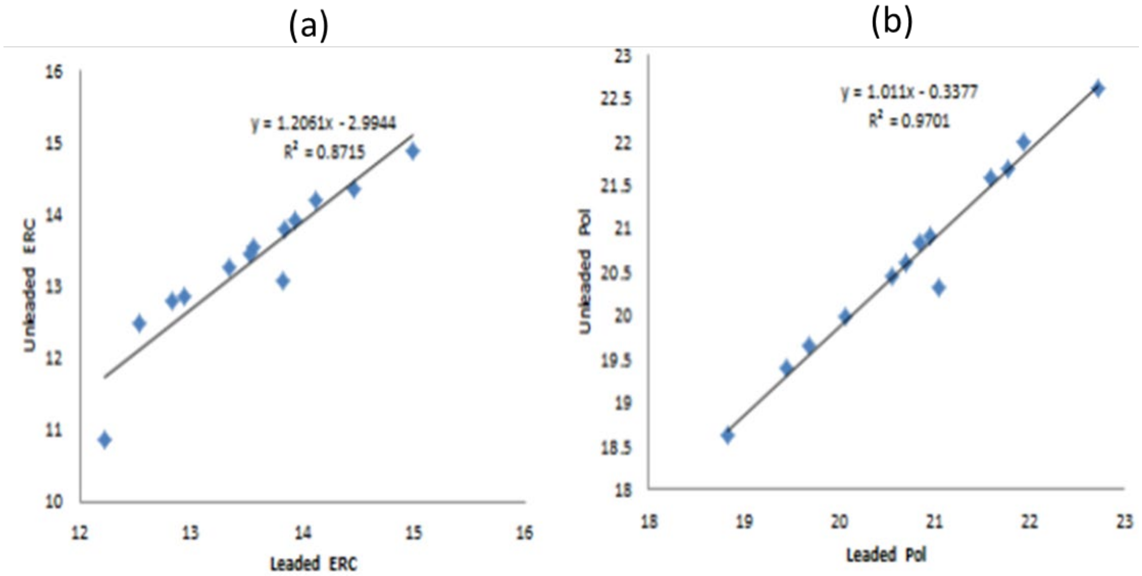

Figure 3. Graph showing regression analysis of leaded ERC vs. unleaded ERC (a) and leaded Pol vs. unleaded Pol (b), as at the end of August 2019

At the maturity point in September, there was a strong positive relationship between the leaded and unleaded Pol and ERC, giving R^2 values of 0.95 and 0.91, respectively. As expected, the sucrose concentration increased with time and with the cane maturing. Higher differences between the leaded and unleaded Pol and ERC cane values were experienced in younger cane, compared to matured cane, as shown in Figure 5. Younger cane bears more simple sugars (fructose and glucose) than mature cane, as the vegetative growth needs the reducing sugars for respiration. However, the simple sugar concentration depletes towards maturity, as it will be converted to sucrose. For this reason, the differences between the two methods appeared to decrease as the cane approached maturation. Fructose is the major cause of the differences, as it causes the plane of polarised light to rotate in an anti-clockwise direction (levorotatory.) The average difference was 0.20 for Pol and 0.14 for ERC. The regressed graphs for the leaded and unleaded methods for the determination of ERC and Pol are shown in Figure 4.

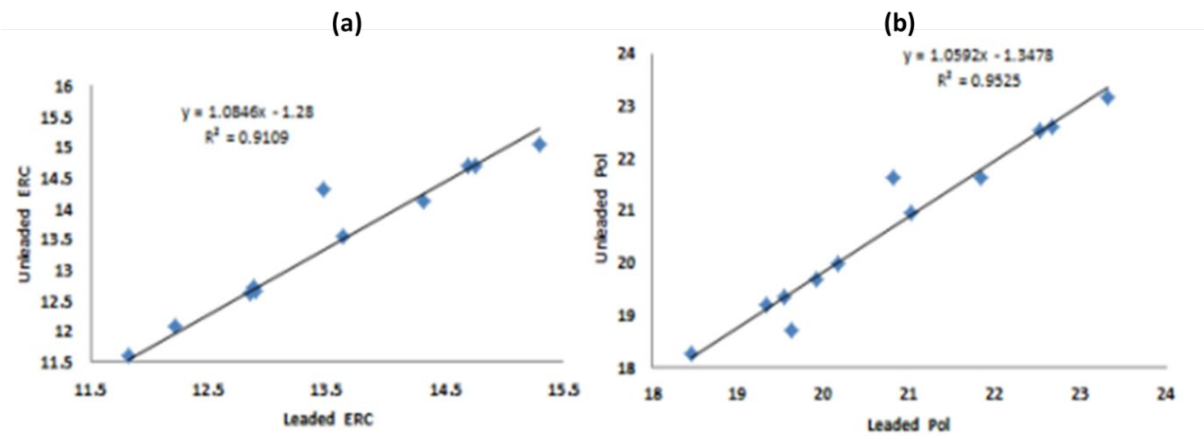

Figure 4. Graph showing regression analysis of leaded ERC vs. unleaded ERC (g) and leaded Pol vs. unleaded Pol (h), as at the end of September 2019

An increase in ERC was noted over time when using the leaded method, as shown in Figure 5 below. For the ZN1, ZN3, ZN5, ZN6 and CP72-1312 varieties, the ERC values were highest in the month of September when the cane had matured after being in the field for 14 months. In varieties like ZN2, ZN4, ZN7, ZN8 and CP72-2086, the highest ERC values were observed in the month of August. In Zimbabwe, the cold spell normally lasts from May to August and could thus have contributed to the accumulation of more sucrose at the expense of the reducing sugars, due to limited physiological activity during this period. ZN10 and NCO376 were the only ones that had their highest ERC in July. These trends were exhibited in both the leaded and unleaded DAC. The results for ZN10 and NCO376 show that more studies are needed to prove their persistence, otherwise it could be attributed to the early maturity of these varieties. A graph of the ERC values for different cane varieties, for the months of June, July, August and September, are shown on Figure 5.

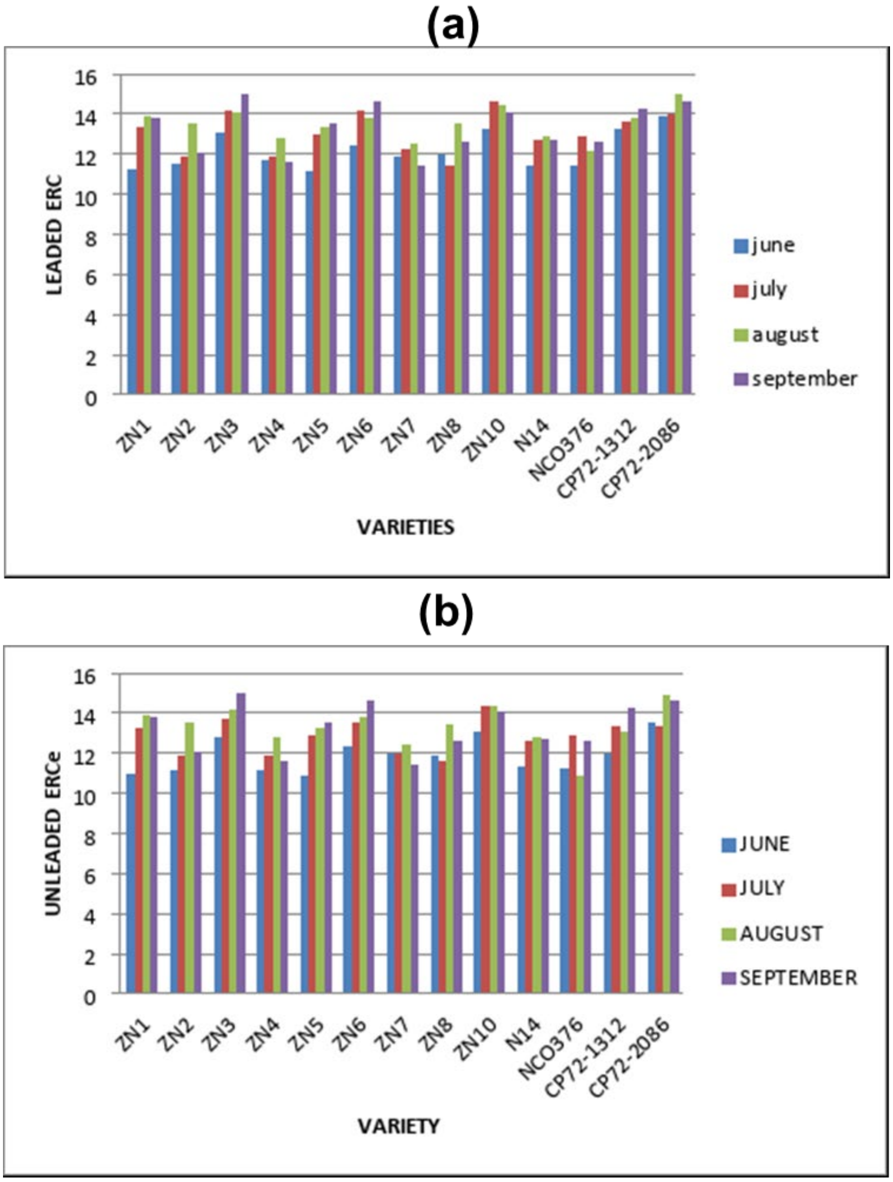

Figure 5. Showing the changes in leaded ERC (a) and unleaded ERC (b) from the month of June to September for all 13 varieties used in the study

A graphical presentation of the differences in ERC between the leaded and unleaded DAC shows a gradual decrease, with the increase of time. Thus, as the cane matures, the fructose content decreases, and therefore the difference in ERC, as determined by the two methods, decreases. The decrease in the differences can be attributed to the maturation of the cane.

The average difference was highest in June, which recorded 0.31%, and it was the lowest in September, with an average difference of 0.14% (Figure 6)

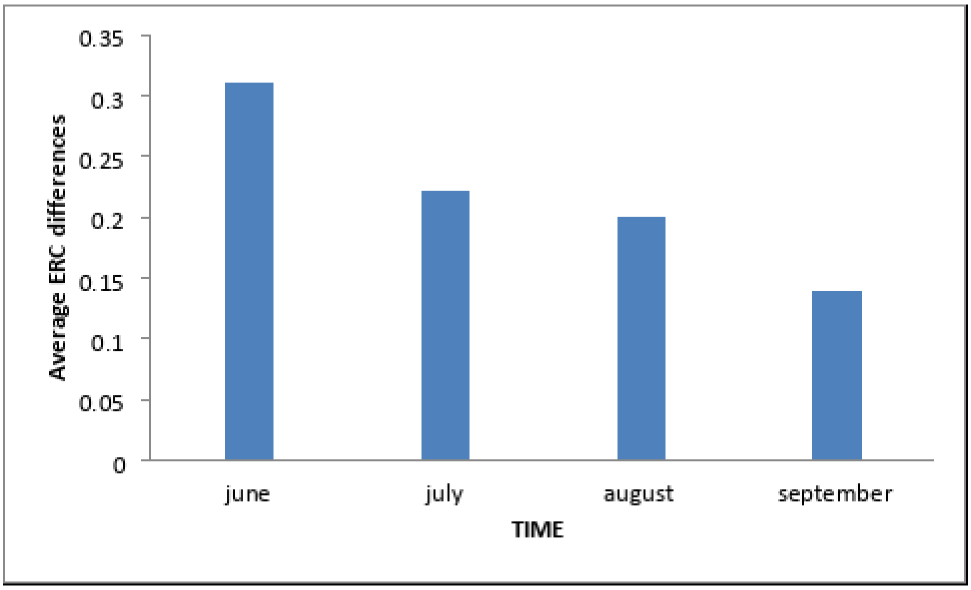

Figure 6. The decrease in ERC differences between the leaded and unleaded DAC methods for the period June to September 2019, due to cane maturation

Conclusion

There was a strong relationship between the leaded and NIR polarimetry-determined ERC. The NIR polarimetry values were generally lower than those of the leaded polarimetry, in all 13 varieties. The differences between the two methods are reduced as the cane matures. NIR polarimetry was proved to be user-friendly and cheap, as a toxic lead sub-acetate is not used in the process. This difference might need to be corrected, and a factor may need to be determined, after more thorough studies.

Recommendations

More analyses should be conducted to generate data for the validation and determination of correctional factors, in order to compensate for the differences that were observed. The Sugar Industry will be encouraged to adopt the leadless DAC for the cane and sugar analysis, once it is validated.

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