Control of reducing sugars in sugarcane bioenergy industries by colorimetric methods: Is it always possible?

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ABSTRACT

Interferents influence analytical results of colorimetric methods. This work evaluated the level of analytical influence by metallic ions (K^+, Ca^{2+} and Fe^{3+}) on reducing sugars analysis. The difference on the reducing sugars (RS) content was analyzed by the 3,5-DNS, Somogyi-Nelson, Lane-Eynon and ion chromatography (IC) methods. The RS contents were compared to each other, using sugarcane juice samples. The same sample presented, generally, Somogyi-Nelson with higher contents, while 3,5-DNS lower. Therefore, mixed solutions with 1.5 g L^{-1} (proportion 1:1, v/v) of glucose and fructose were prepared with increasing doses of K^+, Ca^{2+} and Fe^{3+} ions (2.6; 5.2; 7.8; 10.4; 13.0 mmol L^{-1}). K^+ underestimated the results of 3,5-DNS. Nevertheless, Ca^{2+} overestimated the 3,5-DNS and Lane-Eynon results. While, Fe^{3+} overestimated the RS content in 3,5-DNS and underestimated in Somogyi-Nelson. Regardless of the metallic ion added, ion chromatography was more stable and showed the most accurate and exact methodology. Lastly, the use of colorimetric methods can interfere with sugarcane process monitoring, especially fermentable yield.

KEYWORDS: Colorimetric methods. Cane juice analysis. Fructose. Glucose. Ion chromatography.

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INTRODUCTION

Analytical methods are widely applied in food quality control and colorimetric methods associated to oxidation-reduction are most used (SHAO and LIN, 2018). Practicality, simplicity, portability and low cost are some factors highlighted in other methods (XIA *et al.*, 2015).

In sugarcane mills the reducing sugars analyses are most important to evaluate the ethanol fermentation or crystal sugar quality. The methods Somogyi-Nelson (KING and GARNER, 1947; SOMOGYI, 1952; HATANAKA and KOBARA, 1980), 3,5-dinitrosalicylic acid (3,5-DNS) (SUMNER and GRAHAM, 1921; MILLER, 1959; MOHUN and COOK, 1962) and Lane-Eynon (LING and CARTER, 1930; OUGH and LLOYD, 1965; IWAIDA *et al.*, 1973) are widely used in the analysis of reducing sugars (RS). In the cane industry, Lane-Eynon is the official method regulated by International Commission for Uniform Methods of Sugar Analysis (ICUMSA, 2017; CONSECANA, 2015) and there were recommendations in Brazilian sugarcane industry to use Somogyi-Nelson to monitor the sugarcane process due its greater agility compared to Lane-Eynon (ISEJIMA *et al.*, 2002). In addition Somogyi-Nelson (PRADO *et al.*, 2014; AGUIAR *et al.*, 2015; PERRONE *et al.*, 2016), as well 3,5-DNS (MARTÍNEZ *et al.*, 2017; MARTÍNEZ *et al.*, 2018), have been used constantly by sugarcane researchers.

Recently, studies have shown interferences or interferents that could cause analytical errors inherent in the method used. Amino acids and metals are possible interferents and are found with abundance in sugarcane juice, especially iron, calcium and potassium, the main raw material of the crystal sugar (TAVARES *et al.*, 2010; TEIXEIRA *et al.*, 2012).

Chromatography stands out for its precision and accuracy, for example, in the glucose and fructose analysis. This is a relevant fact, as analytical errors can cause many economic losses to the industry and consumers. Besides, chromatography is capable of quantifying reducing sugars (RS) (glucose and fructose in food), ensuring versatility to the method (GALANT *et al.*, 2015; SARTORI *et al.*, 2017).

Thus, considering the high usage rate of colorimetric methods in the sugarcane industry and researches and taking into account the high amount of

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mineral in the sugarcane juice, this work evaluated metallic ion interferences (Fe^{3+}, Ca^{2+} and K^+) on the colorimeter to determine RS in four methods (3,5-DNS, Somogyi-Nelson, Lane-Eynon and also ion chromatography method).

MATERIAL AND METHODS

SUGARCANE SAMPLES AND JUICE EXTRACTION: AS A STUDY MODEL

Sugarcane were harvested randomly from five vases (V0, V1, V2, V3 and V4) and kept in greenhouses under controlled conditions (25.5 ºC and humidity at 58%) for 180 days at Experimental Station of University of São Paulo (Piracicaba city; Latitude 22° 43' 30'' S and Longitude 47° 38' 51'' W). The sugarcane juice used in the experimental tests was obtained from healthy sugarcane plants (mature phase). The plants were disintegrated in forage mill machine and extracted by milling for 1 min at 250 kgf cm^{-2}. The sugarcane juice was sifted (200 mesh) to remove insoluble impurities, sand and fibers. The juice was immediately stored at - 18 °C until the analyses.

DETERMINIG REDUCING SUGARS CONTENTS IN SUGARCANE JUICE

The RS concentration was quantified by three colorimetric methods: 3,5-DNS, Somogyi-Nelson, Lane-Eynon and the results were compared against results from ion chromatography. The results were expressed as the mean ± standard deviation (g L^{-1}).

PREPARATION OF PURE SOLUTIONS OF GLUCOSE AND FRUCTOSE WITH CATIONS

Solutions with known concentrations of glucose and fructose were used in the RS content analysis. Mixed solutions at 1.5 g L^{-1} (ratio 1:1, v/v) of glucose and fructose were prepared and increasing doses of K^+, Ca^{2+} and Fe^{3+} were added. Each sample was analyzed by the same methods used in RS quantification in the sugarcane juice. The concentrations were determined in mole of charge per liter (mol_c L^{-1}), once the number of charges was not the same between monovalent, bivalent and trivalent cations. Potassium (K^+) was used as reference, based on close

values present in sugarcane juice (Table 1). For calculation, 1 mol_c of K^+ was considered equivalent to 1/2 mol_c of Ca^{2+} and 1/3 mol_c of Fe^{3+}.

Table 1 – Conversion of ion concentretions from mg L^{-1} to $mmol_c$ L^{-1}

Concentration $mmol_c$ L^{-1}	KCl mg L^{-1}	$CaCl_2.2H_2O$ mg L^{-1}	$FeCl_3.6H_2O$ mg L^{-1}
2.6	100.0	191.1	235.2
5.2	200.0	382.2	459.5
7.8	300.0	573.4	702.8
10.4	400.0	764.5	946.1
13.0	500.0	955.6	1623.0

Source: The authors (2022)

COLORIMETRIC ANALYSIS OF REDUCING SUGARS BY THE 3,5-DINITROSALICYLIC ACID METHOD (3,5-DNS)

The 3,5-DNS method consists of the RS reaction with 3,5-dinitrosalicylic acid, reducing it to 3-amino-5-nitrosalicylic, while aldehyde group of RS is oxidized at aldonic acid (MILLER, 1959). One mL of samples containing RS was pipetted into a graduated test tube and 1 mL of 3,5-DNS reagent was added. The mixture was homogenized in vortex and taken to boiling water bath for 5 min. Then, the tubes were cooled in running water and 10.5 mL of distilled water was added. Finally, the absorbance was read at 540 nm in spectrophotometer Mod. UV-Vis Mini1240 (Shimadzu Co., Japan). The results were expressed in g L^{-1} with mean ± standard deviation of three repetitions against the calibration curve of glucose and fructose (HPLC grade).

COLORIMETRIC ANALYSIS OF REDUCING SUGARS BY SOMOGYI-NELSON

The Somogyi-Nelson method is based on the reduction of cupric ion to cupric oxide by heating RS in an alkaline medium. RS changes to enodiols, which is responsible for the reduction of cupric ion. In sequence, the oxide cupric reduces to arsenic-molybdenum, which presents a bluish color as the RS quantity increases (SOMOGYI, 1952). Thus, the Somogyi-Nelson method allows determining RS concentration through the quantification of the oxidizing agent that is reduced by the solution of the same sugar (SILVA *et al.*, 2003). One mL of samples containing RS was pipetted in a graduated test tube and 1 mL of Somogyi reagent. The tubes were shaken and taken to water bath (100 ºC for 10 min). Then, the samples were

cooled in running water and 1 mL of Nelson reagent was added with 9.5 mL of distilled water. Finally, absorbance was read at 520 nm in spectrophotometer Mod. UV-Vis Mini1240 (Shimadzu Co., Japan). The results were expressed in g L^{-1} with mean ± standard deviation of three repetitions against the calibration curve of glucose and fructose (HPLC grade).

COLORIMETRIC ANALYSIS OF REDUCING SUGARS BY LANE-EYNON

The Lane-Eynon method is based on the reduction of copper salts at high temperatures, in a tartaric solution (Fehling solution) by aldoses and ketoses, which forms copper salts that precipitate, losing the initial bluish color. The principle used in this methodology is titration. The sugar samples are titrated in the reactive and the volume spent during titration indicates the sugar content (CONSECANA, 2015).

The volume spent in the analysis was used to determine RS concentration in the samples, through the Equation 1 (CONSECANA, 2015):

$$Reducing\ sugar = \frac{(f*5.2096-0.2625)*0.00052*L_{Pb}*V}{V*(0.00431*B+0.99367)} \qquad (1)$$

Where: f: dilution factor; Lpb: Saccharimetric reading; V: spent volume corrected by the factor "f" of the Fehling liqueur (f = 1.0256; in this work); B: sugarcane juice brix.

REDUCING SUGARS ANALYSIS BY ION CHROMATOGRAPHY

The glucose and fructose concentrations were determined by ion chromatography Mod. 930 Compact IC Flex (Metrohm Co., Switzerland) equipped with an amperometric detector (Metrohm). It was used 100 mmol L^{-1} of sodium hydroxide as the mobile phase. At first, samples were filtered in Millipore (0.45 µm) membranes. A Metrosep Carb 1 (150/4.0) column was kept at 35°C with flux of 1.0 mL min^{-1} in a 9 min run. The injection volume was 0.25 µL. Before the quantitative analysis, standard solutions of glucose and fructose were prepared to elaborate the calibration curve (10 to 500 mg L^{-1}) (METROHM, 2015).

STATISTICAL DESIGN

The statistical design used to determine the RS contents in sugarcane juice was a randomized block design with one repetition. Each vase was considered a block and the analysis was done in triplicate. The results were submitted to the Analysis of Variance (ANOVA) and Tukey test with 99% confidence. The analysis of pure solutions of glucose and fructose with interferents used the completely randomized design with replicates per sample. The results were submitted to the ANOVA, Tukey test (95% and 99% confidence) and regression analysis. The hypothesis tested was that cations influenced the RS concentrations measured by the methods, except for ion chromatography.

CASE STUDY – IMPACT OF METHODS DEVIATION IN THE FERMENTATION YIELD

Using the average concentrations of RS for 3,5-DNS, Somogyi-Nelson, Lane-Eynon and ion chromatography for the same sugarcane juice samples, it was calculated the theoretical fermentation yield (in mL of ethanol/L of sugarcane juice) expected for each method and thus identify the one with the smallest analytical deviation for use in the theoretical calculation of the ethanol content based on the Gay-Lussac equation (Equation 2) was used to calculate the yield.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \tag{2}$$

Stoichiometrically, 360 kg of glucose yield 51.1 kg of ethanol and 48.9 kg of CO2. Considering the density of ethanol (789 kg m^{-3}), the theoretical volume of ethanol to be produced is 64.75 mL, then the fermentation yield was obtained using Equation 3.

$$Fermentation\ Yield\ \left(\frac{mL}{L}\right) = (RS_{concentration}\left(\frac{g}{L}\right) * 64.75)/360 \tag{3}$$

The results of RS in sugarcane juice and pure solutions, it was expected to conclude which method had the lowest deviation due to interfers. Thus, it was chosen official methods.

RESULTS AND DISCUSSION

ANALYSIS OF REDUCING SUGARS IN SUGARCANE JUICE AND FERMENTATION YIELD

Each method has a reaction mechanism (SILVA *et al.*, 2003; GALANT *et al.* 2015) while there were significant differences between the averages of the RS concentration in the vases and sugarcane samples. This means that the same sample presented different RS concentrations depending on the method used (Table 2).

Table 2 – Glucose and fructose concentrations in sugar cane mg L^{-1} to $mmol_c$ L^{-1}

Methods	Reducing Sugars (g L^{-1})				
	Vessel #0	Vessel #1	Vessel #2	Vessel #3	Vessel #4
3,5-DNS	24.70 ± 0.91 [D]	40.36 ± 1.44 [C]	31.50 ± 0.23 [D]	38.01 ± 1.24 [C]	25.07 ± 0.85 [D]
Somogyi-Nelson	38.02 ± 1.13 [B]	63.90 ± 0.75 [A]	44.86 ± 1.30 [D]	58.83 ± 0.00 [A]	38.82 ± 1.89 [A]
Lane-Eynon	27.84 ± 0.19 [C]	41.61 ± 0.47 [C]	34.33 ± 1.61 [C]	36.33 ± 0.14 [D]	28.25 ± 0.67 [C]
IC[a]	45.91 ± 0.73 [A]	52.51 ± 0.75 [B]	40.82 ± 0.45 [B]	43.79 ± 0.80 [B]	32.91 ± 0.55 [B]

[a]Ion chrommatography; Means followed by the same capital letter within a column are not significantly different according to the Tukey test at p ≤ 0.01.
Source: The authors (2022)

Considering that the oxide-reduction mechanisms are subjected to different interferents and to mineral and organic complexity of the sugarcane juice, the deviation from real value was distinct for each method. Generally, the contents by the Somogyi-Nelson method were higher, while the values of 3,5-DNS were lower (Table 3).

Table 3 – Average concentrations of reducing sugars in sugar cane juice for the 3,5-dinitrosalicylic acid, Somogyi-Nelson, Lane-Eynon and ion chromatography methods

Method	(g L^{-1})
3,5-DNS	31.92 ± 7.20[D]
Somogyi-Nelson	48.88 ± 11.83[A]
Lane-Eynon	33.67 ± 5.74[C]
IC[a]	43.19 ± 7.17[B]

[a]Ion chrommatography; Means followed by the same capital letter within a column are not significantly different according to the Tukey test at p ≤ 0.01.
Source: The authors (2022)

However, only the variation results in sugarcane juice did not allow identifying which methodology was more accurate. Thus, analyses were carried out with pure solutions of glucose and fructose with interferents, using known concentrations.

ANALYSIS OF REDUCING SUGARS IN PURE SOLUTIONS OF GLUCOSE AND FRUCTOSE

In 3,5-DNS, the samples with potassium added tended to decrease the RS concentrations as the cation concentrations increased. The F test (1% of significance) indicated statistical differences between the control (without K^+) and all other samples. The decrease stabilized between 7.8 and 13.0 $mmol_c$ L^{-1} of K^+. On the other hand, the increase of K^+ concentration did not influence the RS contents by the Somogyi-Nelson, Lane-Eynon and ion chromatography methods (Tables 4 and 5).

Table 4 – The F test for difference of reducing sugars concentration averages in pure solutions of glucose and fructose with increasing K^+ concentrations, analyzed by the 3,5-DNS, Somogyi-Nelson, Lane Eynon methods and ion chromatography (divided into fructose and glucose)

Source of Variation	Degree of Freedom	Sum of Squares	Means of Squares	F
3,5-DNS	5	0.00529	0.00106	63.5333 *[a]
Residue	12	0.00020	0.00002	
Total	17	0.00549		
Source of Variation	**Degree of Freedom**	**Sum of Squares**	**Means of Squares**	**F**
Lane-Eynon	5	0.00032	0.00006	0.1163 ns[b]
Residue	12	0.00653	0.00054	
Total	17	0.00685		
Source of Variation	**Degree of Freedom**	**Sum of Squares**	**Means of Squares**	**F**
Somogyi-Nelson	5	0.00098	0.00020	0.5531 ns
Residue	12	0.00427	0.00036	
Total	17	0.00525		
Source of Variation	**Degree of Freedom**	**Sum of Squares**	**Means of Squares**	**F**
IC[c] - Glucose	5	4.73486	0.94697	0.0193 ns
Residue	12	589.71384	49.14282	
Total	17	594.44870		
Source of Variation	**Degree of Freedom**	**Sum of Squares**	**Means of Squares**	**F**
IC - Fructose	5	4.27951	0.85518	0.0045 ns
Residue	12	2291.63933	190.96994	
Total	17	2295.91523		

a. Significative at 1% level; b. Not significant at 1% level; c. Ion chrommatography.*Represents those results were significant and ns: not significant.

Source: The authors (2022)

Table 5 – Concentrations of glucose and fructose in samples with K⁺ added and analyzed by 3,5-DNS, Somogyi-Nelson, Lane Eynon methods and ion chromatography

K⁺	3,5-DNS	Somogyi-Nelson	Lane-Eynon	Ion Chromatography	
mmol$_c$ L⁻¹		Glucose + Fructose (g L⁻¹)		Fructose (g L⁻¹)	Glucose
0.0	1.59 ± 0.03 [A]	1.49 ± 0.01 [A]	1.42 ± 0.03 [A]	0.72 ± 0.00 [A]	0.75 ± 0.00 [A]
2.6	1.55 ± 0.03 [B]	1.49 ± 0.01 [A]	1.41 ± 0.01 [A]	0.72 ± 0.00 [A]	0.75 ± 0.01 [A]
5.2	1.53 ± 0.03 [C]	1.50 ± 0.04 [A]	1.41 ± 0.01 [A]	0.72 ± 0.01 [A]	0.75 ± 0.00 [A]
7.8	1.54± 0.00 [BC]	1.49 ± 0.01 [A]	1.42 ± 0.01 [A]	0.72 ± 0.01 [A]	0.75 ± 0.01 [A]
10.4	1.55 ± 0.00 [B]	1.49 ± 0.00 [A]	1.43 ± 0.03 [A]	0.72 ± 0.01 [A]	0.75 ± 0.00 [A]
13.0	1.54 ± 0.04 [BC]	1.50 ± 0.04 [A]	1.42 ± 0.01 [A]	0.72 ± 0.03 [A]	0.75 ± 0.01 [A]

Means followed by the same capital letter within a column are not significantly different according to the Tukey test at p ≤ 0.01.

Source: The authors (2022)

Unlike the other methods, ion chromatography stood out because it allowed quantifying glucose and fructose separately; in addition, no other compound was detected, besides the RS. The correlation between K⁺ and RS concentration was linear (Table 6). However, for all methods, including 3,5-DNS that was significant in the Tukey test (at 1% level of significance), the values P >F indicated that equation y = a + bx did not fit the experimental data. Once the estimates of the b coefficient were between a negative and positive value, there was 99% of probability that b equaled zero. Thus, y = a is obtained in the end.

There were statistical differences between the control and the samples added with Ca²⁺ analyzed by the 3,5 DNS method (Tables 6 and 7). The RS content increased as the concentration also increased. The solution with 13.8 mmol$_c$ L⁻¹ of calcium presented higher RS concentration. The influence of calcium was cited by Teixeira *et al*. (2012), who analyzed the influence of amino acids on the RS analysis by the 3,5-DNS method. The samples with Ca²⁺ also presented variation compared to the control in the Lane-Eynon method. The F test (Table 6) was significant and the Tukey test (Table 7) showed a statistical difference between the control and Ca²⁺ concentrations tested.

Table 6– The F test for difference of reducing sugars concentration averages in pure solutions of glucose and fructose with increasing Ca²⁺ concentrations, analyzed by the 3,5-DNS, Somogyi-Nelson, Lane Eynon methods and ion chromatography (divided in fructose and glucose)

Source of Variation	Degree of Freedom	Sum of Squares	Means of Squares	F
3,5-DNS	5	0.03616	0.00723	130.1800 *[a]
Residue	12	0.00067	0.00006	
Total	17	0.03683		
Source of Variation	**Degree of Freedom**	**Sum of Squares**	**Means of Squares**	**F**
Lane-Eynon	5	0.01778	0.00356	13.9174 *
Residue	12	0.00307	0.00026	
Total	17	0.02085		
Source of Variation	**Degree of Freedom**	**Sum of Squares**	**Means of Squares**	**F**
Somogyi-Nelson	5	0.00064	0.00013	0.8593 ns[b]
Residue	12	0.00180	0.00015	
Total	17	0.00244		
Source of Variation	**Degree of Freedom**	**Sum of Squares**	**Means of Squares**	**F**
IC[c] - Glucose	5	51.69780	10.33956	0.2994 ns
Residue	12	414.42814	34.53568	
Total	17	466.12595		
Source of Variation	**Degree of Freedom**	**Sum of Squares**	**Means of Squares**	**F**
IC - Fructose	5	39.47818	7.89564	0.1988 ns
Residue	12	476.63597	39.71966	
Total	17	516.11415		

a. Significative at 1% level; b. Not significant at 1% level; c. Ion chrommatography.*Represents those results were significant and ns: not significant.

Source: The authors (2022)

Table 7 – Concentrations of glucose and fructose in samples added with Ca²⁺ analyzed by 3,5-DNS, Somogyi-Nelson, Lane Eynon methods and ion chromatography

Ca²⁺ mmol_c L⁻¹	3,5-DNS	Somogyi-Nelson	Lane-Eynon	Ion Chromatography	
	Glucose + Fructose (g L⁻¹)			Fructose (g L⁻¹)	Glucose
0.0	1.59 ± 0.00 [D]	1.49 ± 0.01 [A]	1.37 ± 0.01 [Cc]	0.75 ± 0.00 [A]	0.75 ± 0.00 [A]
2.6	1.62 ± 0.01 [C]	1.49 ± 0.00 [A]	1.38 ± 0.02 [BC]	0.75 ± 0.01 [A]	0.75 ± 0.01 [A]
5.2	1.64 ± 0.01 [C]	1.50 ± 0.00 [A]	1.43 ± 0.04 [A]	0.75 ± 0.01 [A]	0.75 ± 0.00 [A]
7.8	1.67 ± 0.00 [B]	1.49 ± 0.00 [A]	1.43 ± 0.01 [A]	0.75 ± 0.01 [A]	0.75 ± 0.00 [A]
10.4	1.70 ± 0.01 [A]	1.50 ± 0.00 [A]	1.42 ± 0.01 [AB]	0.75 ± 0.01 [A]	0.75 ± 0.01 [A]
13.0	1.71 ± 0.01 [A]	1.50 ± 0.01 [A]	1.46 ± 0.01 [A]	0.75 ± 0.00 [A]	0.75 ± 0.00 [A]

Means followed by the same capital letter within a column are not significantly different according to the Tukey test at p ≤ 0.01.

Source: The authors (2022)

The RS content increased up to 5.2 mmol$_c$ L^{-1} of calcium. Afterward, the RS concentrations stabilized in the samples with 5.2 and 13.0 mmol$_c$ L^{-1} of Ca^{2+}. However, there were no statistical differences, regardless of the Ca^{2+} amount added to the samples in the Somogyi-Nelson and ion chromatographic methods.

No compounds were detected, besides glucose and fructose in ion chromatography chromatogram. There was a linear relation between the addition of Ca^{2+} and RS concentration measured (y = a + bx). The P >F of Somogyi-Nelson indicated that equation y=a + bx did not fit the experimental data; the angular coefficient estimated for b was a value between negative and positive, then, there was 95% probability that this value equaled zero and y=a. Ion chromatography also presented y = a (Table 9). The 3,5-DNS and Lane-Eynon method presented 0.0096 and 0.0063 of angular coefficient, respectively. An increase in Ca^{2+} concentration resulted in an increase of the RS content; however, both values indicated a low effect (Table 8).

Table 8 – Estimation of the linear equation parameters related to the reducing sugars and Ca^{2+} concentration

Methods	a Coefficient Angular	b Coefficient Linear	R^2	F	P >F
3.5–DNS	1.5929 (1.581 to 1.605)	0.0096 (0.008 to 0.011)	0.9843	315.4	0.0001
Somogy-Nelson	1.4900 (1.481 to 1.499)	0.0004 (0.000 to 0.002)	0.3333	3.5	0.1347
Lane-Eynon	1.3743 (1.341 to 1.408)	0.0008 (0.002 to 0.011)	0.7590	16.8	0.0149
IC[a]	1.5000	0	-	-	-

a. Ion chrommatography.

Source: The authors (2022)

The F test for pure samples with Fe^{3+} analyzed by 3,5-DNS was significant at 1% level. The RS concentration measured did not change between 0.0 and 7.8 mmol$_c$ L^{-1} of Fe^{3+}, but there was an increase from 10.4 mmol$_c$ L^{-1} to Fe^{3+}. Samples with Fe^{3+} analyzed by Somogyi-Nelson were also significant at 1% level. There was an increase from 7.8 mmol$_c$ L^{-1} to Fe^{3+}. The samples analyzed by Lane-Eynon did not present significance at 1% level and in ion chromatography (Tables 9 and 10).

Table 9 – The F test for difference of reducing sugars concentration averages in pure solutions of glucose and fructose with increasing Fe³⁺ concentrations, analyzed by the 3,5-DNS, Somogyi-Nelson, Lane Eynon methods and ion chromatography (divided in fructose and glucose)

Source of Variation	Degree of Freedom	Sum of Squares	Means of Squares	F
3,5-DNS	5	0.01111	0.00222	100.0000 *[a]
Residue	12	0.00027	0.00002	
Total	17	0.01138		

Source of Variation	Degree of Freedom	Sum of Squares	Means of Squares	F
Somogyi-Nelson	5	0.03071	0.00614	12.2844 *
Residue	12	0.00600	0.00050	
Total	17	0.03671		

Source of Variation	Degree of Freedom	Sum of Squares	Means of Squares	F
Lane-Eynon	5	0.00337	0.00067	0.5211 ns[b]
Residue	12	0.01551	0.00129	
Total	17	0.01887		

Source of Variation	Degree of Freedom	Sum of Squares	Means of Squares	F
IC[c] - Glucose	5	29.53583	5.90717	0.1511 ns
Residue	12	469.15357	39.09613	
Total	17	498.68940		

Source of Variation	Degree of Freedom	Sum of Squares	Means of Squares	F
IC[C] - Fructose	5	17.69376	3.53875	0.1502 ns
Residue	12	282.74005	23.56167	
Total	17	300.43380		

a. Significative at 1% level; b. Not significant at 1% level; c. Ion chrommatography.*Represents those results were significant and ns: not significant.

Source: The authors (2022)

Table 10 – Concentrations of glucose and fructose in samples added with Fe³⁺ analyzed by 3,5-DNS, Somogyi-Nelson, Lane Eynon methods and ion chromatography

Fe³⁺	3,5-DNS	Somogyi-Nelson	Lane-Eynon	Ion Chromatography	
				Fructose	Glucose
mmol$_c$ L⁻¹	Glucose + Fructose (g L⁻¹)			(g L⁻¹)	
0.0	1.58 ± 0.00 [CD]	1.49 ± 0.01 [A]	1.40 ± 0.01 [A]	0.73 ± 0.00 [A]	0.75 ± 0.00 [A]
2.6	1.58 ± 0.01 [D]	1.43 ± 0.02 [AB]	1.40 ± 0.02 [A]	0.73 ± 0.00 [A]	0.75 ± 0.00 [A]
5.2	1.58 ± 0.00 [D]	1.43 ± 0.04 [AB]	1.40 ± 0.04 [A]	0.73 ± 0.00 [A]	0.75 ± 0.00 [A]
7.8	1.59 ± 0.01 [C]	1.41 ± 0.01 [BC]	1.40± 0.01 [A]	0.73 ± 0.00 [A]	0.75 ± 0.00 [A]
10.4	1.63 ± 0.01 [B]	1.39 ± 0.01 [BC]	1.38 ± 0.01 [A]	0.73 ± 0.00 [A]	0.75 ± 0.00 [A]
13.0	1.65 ± 0.01 [A]	1.36 ± 0.01 [C]	1.36± 0.01 [A]	0.73 ± 0.00 [A]	0.75 ± 0.00 [A]

Means followed by the same capital letter within a column are not significantly different according to the Tukey test at p ≤ 0.01.

Source: The authors (2022)

There was linear relation between Fe^{3+} and the RS concentration. In the 3.5-DNS, the linear coefficient 0.0056 indicated positive effect in the RS content, as the Fe^{3+} dose increased. The Lane-Eynon and Somogyi-Nelson linear coefficient were -0.0087 and -0.0029, respectively, and indicated a negative effect; however, both coefficients were low. Finally, in the ion chromatography, y was equal to a, once the linear coefficient was zero (Table 11).

Table 11 – Estimation of the linear equation parameters related to RS and Fe^{3+} concentration

Methods	a Coefficient Angular	b Coefficient Linear	R^2	F	P >F
3.5–DNS	1.5622 (1.534 to 1.597)	0.0056 (0.002 to 0.010)	0.7417	15.36	0.0173
Somogy-Nelson	1.4748 (1.447 to 1.503)	-0.0087 (-0.012 to 0.005)	0.9009	46.46	0.0024
Lane-Eynon	1.4086 (1.388 to 1.430)	-0.0029 (-0.005 to 0.000)	0.6122	8.89	0.0406
IC[a]	1.4800	0	-	-	-

a. Ion chrommatography.

Source: The authors (2022)

CASE STUDY – IMPACT OF DEVIATION IN FERMENTATION YIELD

It was calculated the fermentation yield using the average concentrations of reducing sugars for each method (g L^{-1}) (Table 3). Then, for the same sugarcane juice samples, the fermentation yield was 5.74; 8.79; 6.06 and 7.77 g ethanol L^{-1} for 3,5-DNS, Somogyi-Nelson, Lane-Eynon and ion chromatography, respectively. Considering ion chromatography as the better method due to non-interference in their results, the colorimetric achieved worrying values. 3,5-DNS and Lane-Eynon underestimated the real value in 74 and 78%, respectively. While Lane-Eynon overestimated in 13%. The major problem in overestimate the RS concentration is that the expected fermentable yield is higher than a possible value to achieve. In the sugarcane industry, fermentation efficiency below 88% (g of ethanol per g of fermentable sugar) is considered bad yields (Lavarack, 2003). Then, the efficiency loss can be an analytical problem and not a disorder in the process.

CONCLUSIONS

If the ion chromatography as the base of RS quantification, because it is the unique without coloring agents that provides some interference in the results; the 3,5-DNS and Lane-Eynon underestimated the IC value in 74 and 78%, respectively. While Lane-Eynon overestimated in 13%. The major problem in overestimate the RS concentration is that the expected fermentable yield is higher than a possible value to achieve, i.e., the efficiency loss can be an analytical problem and not a disorder in the process.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

Controle de açúcares redutores em indústrias de bioenergia da cana-de-açúcar por métodos colorimétricos: sempre é possível?

RESUMO

Os interferentes influenciam em resultados analíticos de métodos colorimétricos. Este trabalho avaliou o nível de influência analítica de íons metálicos (K^+, Ca^{2+} e Fe^{3+}) na análise quantitative de açúcares redutores. A diferença no teor de açúcares redutores (AR) foi analisada pelos métodos colorimétricos 3,5-DNS, Somogyi-Nelson, Lane-Eynon e a cromatografia iônica (IC). Os teores de AR foram comparados entre si, utilizando amostras de caldo de cana-de-açúcar. A mesma amostra apresentou, geralmente, maiores teores para o método de Somogyi-Nelson, enquanto 3,5-DNS os menores teores. Portanto, soluções mistas com 1,5 g L^{-1} (proporção 1:1, v/v) de glicose e frutose foram preparadas com doses crescentes de íons K^+, Ca^{2+} e Fe^{3+} (2,6; 5,2; 7,8; 10,4; 13,0 $mmol_c$ L- 1). K^+ subestimou os resultados do método colorimétrico à base de 3,5-DNS. No entanto, o Ca^{2+} superestimou os resultados de 3,5-DNS e Lane-Eynon. Enquanto, Fe^{3+} superestimou o conteúdo de AR em 3,5-DNS e subestimou em Somogyi-Nelson. Independentemente do íon metálico adicionado, a cromatografia de íons foi mais estável e mostrou ser a metodologia mais precisa e exata. Por fim, o uso de métodos colorimétricos pode interferir no monitoramento do processamento da cana-de-açúcar, especialmente no balanço de massa e no rendimento industrial (de açúcar ou da fermentação).

PALAVRAS-CHAVE: Métodos colorimétricos. Análise de caldo de cana-de-açúcar. Frutose. Glicose. Cromatografia de íons.

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