

EVALUATION OF THE SMRI JUICE COLOUR ANALYSIS METHODS AND LABORATORY CLARIFICATION TESTS

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Abstract

Raw sugar colour is an increasing sugar quality concern, not because of a decline in the quality of South African very high pol raws but rather due to rapid improvements in the raw house colours in the rest of the world. Technically, raw sugar with lower colour has direct implications for the refiner's effort and cost of processing that sugar. Enormous benefits could be derived if colourants could either be prevented from entering the factory or be removed early in the process, e.g. during clarification where insoluble impurities are already being settled out.

The method for analysing juice colours in South Africa is based on the ICUMSA methods for sugar and molasses colours. This method was evaluated on both mixed juice and clear juice. Once repeatability limits were established, the SMRI laboratory clarification settling test could be evaluated for reproducibility in terms of colour changes. It was also ascertained that the effect of freezing of the mixed juice on the analysis and on the clarification procedure was negligible for at least six weeks of freezing.

Keywords: colourants, ICUMSA colour, analysis, mixed juice, clear juice, clarification

Introduction

The Sugar Milling Research Institute (SMRI) uses a laboratory procedure that simulates as closely as possible the clarification process in a sugar factory (Burgess *et al.*, 1962). Lionnet and Ravnö (1976) assessed a similar setup in terms of initial mud settling rate, intermediate and final mud volumes and the sludge volume index, and found the experimental reproducibility of the method to be adequate. The same method, using modernised equipment, is used for evaluation of juices, impurities, chemicals, liming procedures and flocculants and for general evaluation of the overall juice clarification process, *viz.* heating, pH change and settling. To gain a better understanding of the dynamics of the laboratory procedure and the repeatability limits associated with the analysis, an in-depth method evaluation exercise was embarked on, with special emphasis on the effect on the resulting colour as analysed at pH 4, 7 and 9. The use of three different pH levels traditionally allows for some insight into the types of colourants that are affected by the various treatments (Schoonees-Muir and Gwegwe, 2008).

Analytical method to determine ICUMSA colour of juice

The method used by the SMRI to determine colour of juices is essentially a combination of the raw sugar and molasses colour methods that have *Approved* status as part of the International Commission for Uniform Methods of Sugar Analysis (ICUMSA) methods book. Since the application of these methods to factory juices and syrups are not part of ICUMSA's

scope, they have not been evaluated on an international level, although they have formed an important part of the South African Sugar Technologists' Association (SASTA) recommended methods since the 1970s. SMRI results have always been expressed as ICUMSA 420 colour and in ICUMSA units (IU). A thorough evaluation of the juice colour method was therefore included in this evaluation.

Experimental

Analytical method evaluation: ICUMSA colour for juices

A sample of fresh mixed juice (MJ) from a diffuser factory, preserved with mercuric chloride (HgCl_2), was divided into 60 subsamples and processed using three different analysts (20 subsamples each) over three consecutive days using the SMRI method (TM021) for ICUMSA colour analysis of MJ at pH 7. Samples were stored at 5°C.

A sample of evaporator syrup was collected from the Sezela factory and stored at 5°C. On nine consecutive days a portion of the syrup was diluted to 12°Bx and subsampled into 5-10 samples of deemed clear juice (CJ). (It is acknowledged that the colour types in diluted syrup will not be identical to those in clear juice.) The samples were analysed by three different analysts totalling 25 samples per analyst using the SMRI method (TM020) for ICUMSA colour analysis of CJ at pH 4, 7 and 9.

Verification of the colour repeatability limits

Six samples of MJ from random factories and different times of the season were analysed in duplicate to verify the suitability of the proposed repeatability limits of the method.

Seven samples of CJ obtained from random laboratory clarification tests were analysed in duplicate to verify the suitability of the proposed repeatability limits of the method.

Evaluation of the effect of freezing on colour analysis

Samples of fresh MJ and CJ were collected from a diffuser factory and preserved (HgCl_2). Subsamples were prepared, three of which, respectively, were immediately analysed for colour at pH 4, 7 and 9. The rest of the subsamples were quick-frozen in alcohol at -40°C and stored at -20°C until used.

Three samples each of MJ and CJ were removed from the freezer on a weekly basis over six weeks and analysed for colour.

Laboratory clarification procedure

A subsample (1 L) of the MJ was transferred to a stainless steel container with a heating element and constant magnetic stirring (Figure 1a). Phosphoric acid (H_3PO_4) (diluted to 25% m/m PO_4) was added at a level of 50 mg/kg juice. The MJ was heated to 95°C and milk of lime (MOL; 10% $\text{Ca}(\text{OH})_2$) was added dropwise until a pH of 6.7 (95°C) was reached. The limed juice (LJ) was boiled for 30 seconds to expel any dissolved air and immediately decanted into three pre-heated 250 cm³ volumetric cylinders already containing the flocculant LT027 (3 mg/kg juice) at 95°C. Care was taken to ensure that no air was entrained during the

transfer which would disturb the forming flocs and subsequent settling process. The cylinders were placed in a 95°C water bath for 30 minutes to allow complete settling of the solid mud particles (Figure 1b). The volume of mud was recorded as the average percentage of the volume of mud to total liquid in each of the cylinders. The clarified portions of the juice were decanted and combined.

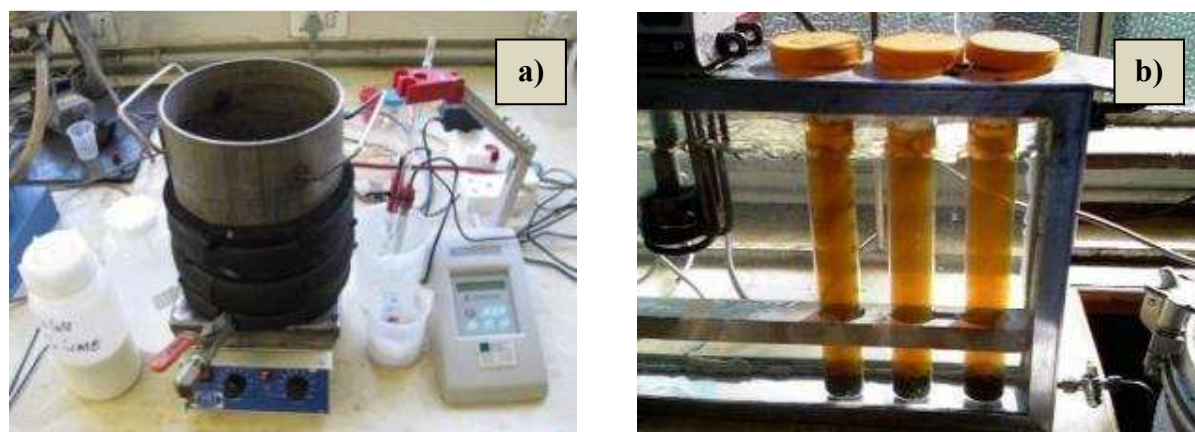


Figure 1: a) Stainless steel laboratory clarification heating pot, and b) cylindrical settling tubes in a water bath at 95°C.

Evaluation of the repeatability of the laboratory clarification procedure and the effect of freezing on the same

A 40 L sample of MJ was obtained from a diffuser factory and preserved (HgCl_2). Five 1 L subsamples were subjected to the laboratory clarification procedure in immediate succession. The CJ samples and two subsamples of the original MJ were stored at 5°C overnight and analysed for colour at pH 4, 7 and 9.

The remainder of the MJ was subsampled into 1 L sachets and frozen (-20°C). Five sachets were removed on a weekly basis, combined and subsampled into five samples which were subjected to the laboratory clarification procedure as above.

Statistical evaluation

All statistics were done using MS Office Excel 2007 with the Analysis Toolpack add-on. Outliers were detected using the method of Hawkins (1981) to avoid skewing of the mean and repeatability limit. The repeatability limits were calculated using the British Standard 5497 method as described by Fisher (1984).

Results and Discussion

Analytical method evaluation: ICUMSA colour

MJ

Basic statistics of the MJ colour results at pH 7 are shown in Table 1. Values were rounded to the nearest 100 IU. Analysis of variance (ANOVA) showed no significant difference between the analysts ($p=0.05$).

Table 1: Basic statistics for MJ colour at pH 7.

Sample	MJ colour @ pH 7
Number of analysts	3
Number of analysts retained after removing outliers	3
Number of individual test results after removing outliers	57
Mean value (IU)	25,600
Repeatability standard deviation (s_r) (IU)	700
Repeatability coefficient of variation (CV_r) (%)	3
Repeatability limit (r) [$\pm \frac{1}{2} r$] (IU)	1,800 [± 900]

CJ

Basic statistics of the CJ colour results at pH 4, 7 and 9 are shown in Tables 2, 3 and 4. ANOVA analysis showed significant differences between the analysts for the pH 4 and 9 results but not for the pH 7 result ($p=0.05$). However, the RSD values were well within the acceptable range of 5% (Horwitz, 1988) and values were rounded to the nearest 100 IU.

Table 2: Basic statistics for CJ colour at pH 4.

Sample	CJ colour @ pH 4
Number of analysts	3
Number of analysts retained after removing outliers	3
Number of individual test results after removing outliers	74
Mean value (IU)	16,900
Repeatability standard deviation (s_r) (IU)	600
Repeatability coefficient of variation (CV_r) (%)	4
Repeatability limit (r) [$\pm \frac{1}{2} r$] (IU)	1,600 [± 800]

Table 3: Basic statistics for CJ colour at pH 7.

Sample	CJ colour @ pH 7
Number of analysts	3
Number of analysts retained after removing outliers	3
Number of individual test results after removing outliers	74
Mean value (IU)	26,400
Repeatability standard deviation (s_r) (IU)	800
Repeatability coefficient of variation (CV_r) (%)	3
Repeatability limit (r) [$\pm \frac{1}{2} r$] (IU)	2,200 [$\pm 1,100$]

Table 4: Basic statistics for CJ colour at pH 9.

Sample	CJ colour @ pH 9
Number of analysts	3
Number of analysts retained after removing outliers	3
Number of individual test results after removing outliers	68
Mean value (IU)	63,200
Repeatability standard deviation (s_r) (IU)	1,000
Repeatability coefficient of variation (CV_r) (%)	2
Repeatability limit (r) [$\pm \frac{1}{2} r$] (IU)	2,800 [$\pm 1,400$]

Verification of the colour repeatability limits

Duplicate colour results for six samples of MJ from random factories at different times of the season are shown in Table 5.

Table 5: Duplicate MJ colour results at pH 7.

Factory	MJ Colour at pH 7 (IU)					
	A	B	C	D	E	F
Replicate 1	22,300	20,100	18,800	27,900	35,800	22,600
Replicate 2	21,300	21,100	18,600	28,100	36,200	22,100
<i>Average</i>	<i>21,800</i>	<i>20,600</i>	<i>18,700</i>	<i>28,000</i>	<i>36,000</i>	<i>22,400</i>
<i>Difference</i>	<i>1,000</i>	<i>1,000</i>	<i>200</i>	<i>300</i>	<i>400</i>	<i>500</i>

Differences between the duplicate samples were well within the repeatability limit of 1,800 IU for MJ colour at pH 7.

Duplicate colour results for seven samples of CJ from random factories at different times of the season are shown in Table 6.

Table 6: Duplicate CJ colour results at pH 4, 7 and 9.

Factory		CJ Colour (IU)				Average or Difference		
		pH 4	pH 7	pH 9		pH 4	pH 7	pH 9
A	Replicate 1	11,500	20,300	71,800	Average	11,600	20,800	72,300
	Replicate 2	11,800	21,300	72,900	Difference	300	1,000	1,100
B	Replicate 1	9,200	19,700	70,500	Average	8,800	19,500	70,000
	Replicate 2	8,500	19,300	69,400	Difference	700	500	1,100
C	Replicate 1	11,000	17,700	59,300	Average	10,800	18,100	63,600
	Replicate 2	10,500	18,500	67,800	Difference	400	800	8,500
D	Replicate 1	9,700	19,600	59,200	Average	8,700	20,400	57,600
	Replicate 2	7,800	21,100	56,000	Difference	1,900	1,500	3,200
E	Replicate 1	7,100	13,600	58,900	Average	7,000	14,000	61,400
	Replicate 2	7,000	14,400	63,900	Difference	200	800	4,900
F	Replicate 1	7,300	15,300	50,200	Average	7,500	14,700	49,500
	Replicate 2	7,700	14,100	48,700	Difference	380	1,200	1,500
G	Replicate 1	7,000	15,100	50,200	Average	7,200	15,200	49,300
	Replicate 2	7,400	15,400	48,400	Difference	360	300	1,900

Bold (red) values indicate differences outside the repeatability limits

Differences between the duplicate results for colour at pH 7 were well within the repeatability limit of 2,200 IU; only one of the differences for colour at pH 4 was higher than the repeatability limit of 1,600 IU which is acceptable at the 95% confidence interval; and three of the differences for colour at pH 9 were well outside the repeatability limit of 2,800 IU. Colour at pH 9 should therefore be viewed with caution, and must be rounded to the nearest 500 units (\therefore repeatability limit = 3,000 IU).

Evaluation of the effect of freezing on colour analysis

Analysis of variance for the analyses of colour of subsamples of MJ and CJ in triplicate on the day of collection and thereafter on a weekly basis after being frozen for a total of six weeks, showed no significant differences in results due to freezing of the samples. To ensure the evaluation was complete, no outliers were removed.

Results for the fresh MJ samples (triplicate) were well within the analytical repeatability limit determined for the analyses of colour at pH 7 (1,800 IU), confirming the suitability of this value.

Results for the fresh CJ samples (triplicate) were well within the analytical repeatability limits determined for the analyses of colour at pH 4 and 7 (1,600 and 2,200 IU), respectively, confirming the suitability of these values. Some of the pH 9 colour values were not within the expected repeatability limit (3,000 IU); the pH adjustment to 9, stability of these solutions and potential use of buffers need to be considered.

The results show that MJ and CJ samples can be frozen for at least six weeks when intended for colour analysis without having a detrimental effect on the results.

Evaluation of the laboratory clarification procedure and the effect of freezing on the same

The laboratory clarification was done five times on fresh MJ and thereafter on the same MJ each week after freezing for one to six weeks. The technician followed an exact procedure without exception. Results for the analyses of colour at pH 4, 7 and 9 of the five clarified juice samples on fresh juice and after freezing for one to six weeks are shown in Figures 2-4. To ensure that the evaluation was complete, no outliers were removed. The first clarified juice sample (CJ 1) after two weeks of freezing was not analysed (reason unknown).

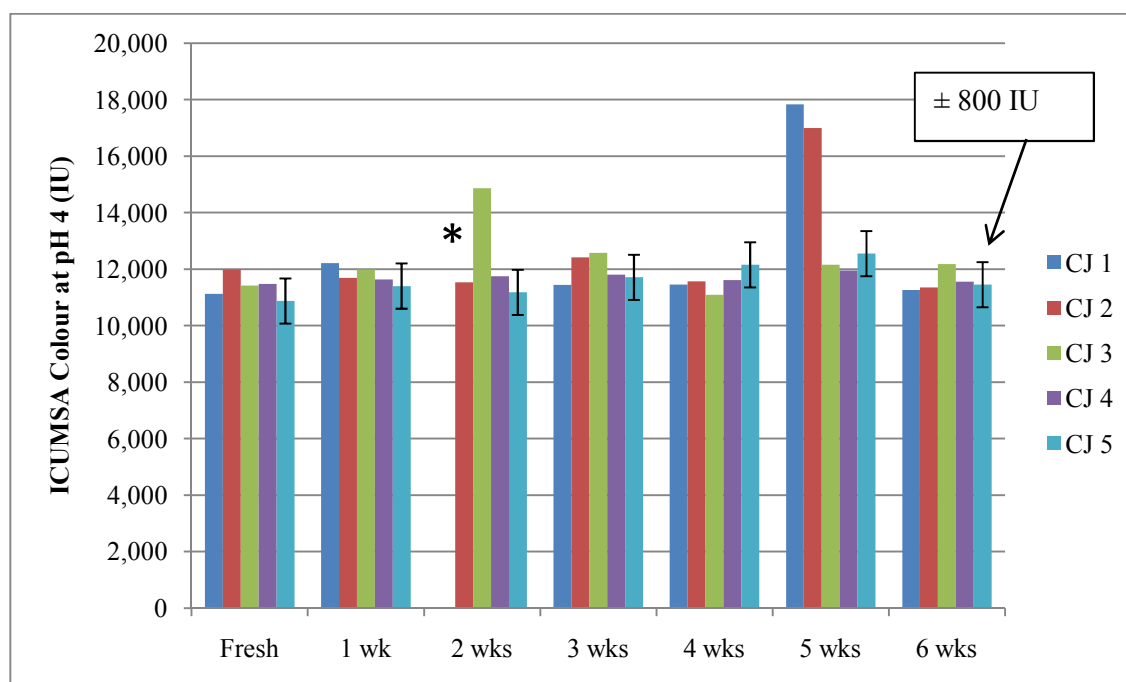


Figure 2: Colour results of laboratory clarified juice at pH 4 – fresh and after freezing for one to six weeks (* not analysed).

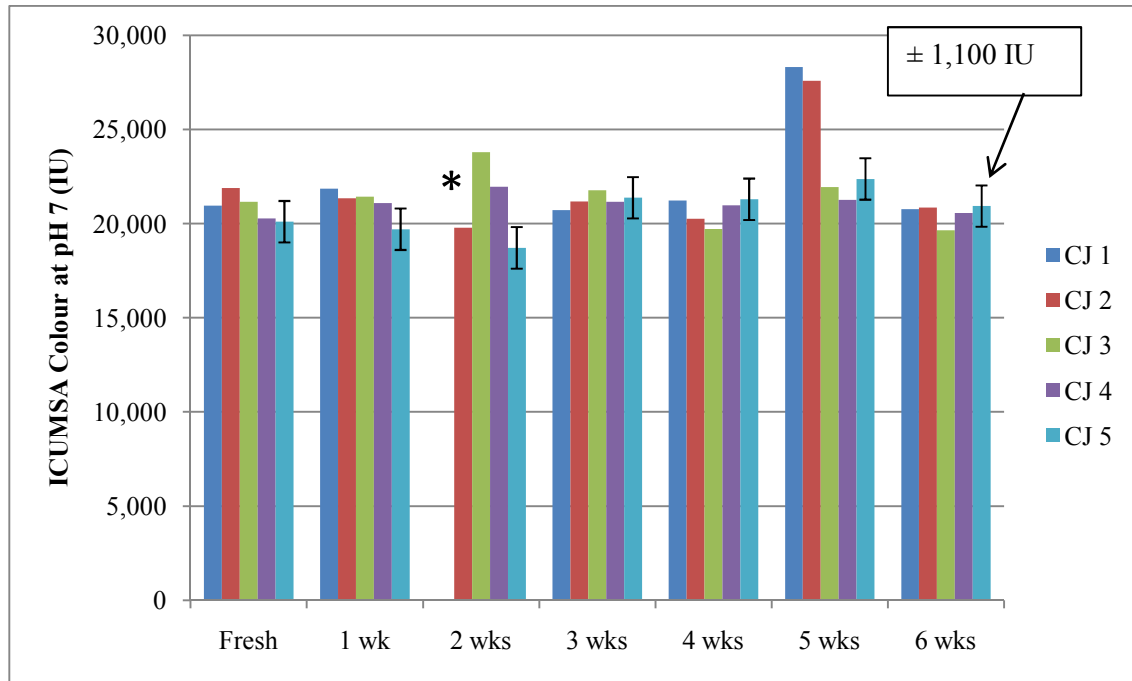


Figure 3: Colour results of laboratory clarified juice at pH 7 – fresh and after freezing for one to six weeks (* not analysed).

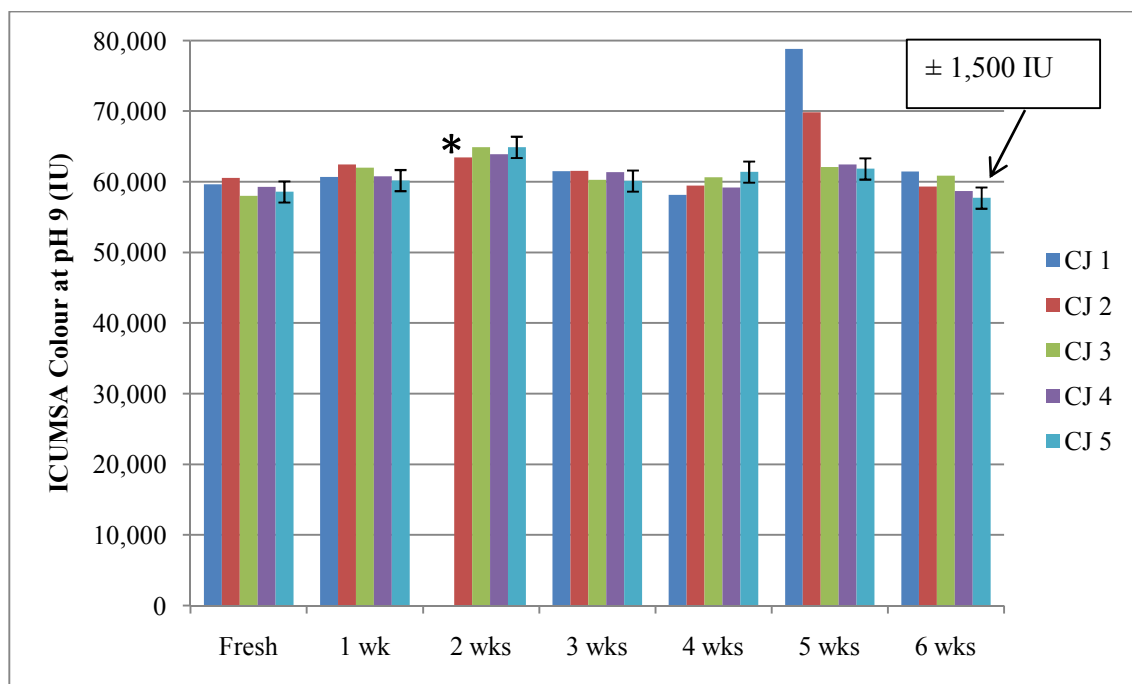


Figure 4: Colour results of laboratory clarified juice at pH 9 – fresh and after freezing for one to six weeks (* not analysed).

Three outlier samples were noticed for colour results at pH 4, 7 and 9 (Figures 2-4): one after two weeks of freezing (excluding pH 9) (CJ 3) and two after five weeks of freezing (CJ 1 and CJ 2), respectively. Since colour values at all three pH levels were affected in the same way (bar pH 9) the samples were most likely different due to unintentional deviation from the clarification procedure and not due to analysis. Apart from these, it is quite clear that the results of the laboratory clarification tests were not affected by freezing. Averages and

standard deviations of the sample sets, with the three outliers removed, showed that all results were within the expected repeatability limits (where applicable). MJ can therefore be frozen for up to six weeks prior to laboratory clarification tests.

Conclusions

This study confirmed that the adjusted ICUMSA colour methods can be used for analysis of sugarcane juices and evaporator syrup. Repeatability limits for the analysis of sugarcane MJ and CJ were established as follows:

- a. MJ at pH 7: ± 900 IU
- b. CJ at pH 4: ± 800 IU
- c. CJ at pH 7: $\pm 1,100$ IU
- d. CJ at pH 9: $\pm 1,500$ IU

Juice (and syrup) colour results at pH 4 and 7 should be rounded to the nearest 100 units, and at pH 9 to the nearest 500 units. The pH 9 analysis needs some additional attention.

The investigation confirmed that CJ and MJ may be frozen for up to six weeks without having a detrimental effect on either colour analysis or laboratory scale clarification results.

The SMRI laboratory clarification test was repeatable as well as reproducible with no effect observed due to freezing of the MJ for up to six weeks. The occurrence of occasional outliers, possibly due to unintentional deviations from the laboratory clarification procedure, needs to be elucidated.

Acknowledgements

The author would like to acknowledge the tremendous effort with experimental work by the following SMRI staff members: P Ramsuraj, MG Ngema, N Memela, V Pillay and N Mdlaka. Also, Stephen Davis and Stephen Walford from the SMRI are acknowledged for their valuable input into the experimental design and data evaluation.

REFERENCES

- Burgess IG, Beardmore RH, Fortescue GE and Davis CW (1962). Development and application of a laboratory clarification test. *Proc Int Soc Sug Cane Technol* 11: 920-928.
- Fisher BV (1984). Statistics in Chemistry. *Anal Proc* 21: 443-448.
- Hawkins DM (1981). *Identification of Outliers*. Chapman and Hall, London.
- Horwitz W (1988). Protocol for the design, conduct and interpretation of collaborative studies. *Pure & Applied Chem* 60: 855-864.
- Lionnet GRE and Ravnö AB (1976). Flocculant evaluation using a portable batch settling kit. *Proc S Afr Sug Technol Ass* 50: 176-178.
- Schoonees-Muir BM and Gwegwe BMM (2008). The use of polyaluminium coagulants for the removal of colour during clarification. *Proc S Afr Sug Technol Ass* 81: 160-164.