

SEPARATION AND IDENTIFICATION OF SUGAR COLOURANT

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Abstract

Unrefined sugar contains organic colour material originating in the sugarcane or formed during the extraction and purification processes. In this work, a cost effective analytical technique to separate colourants from sugar through a sucrose packed, medium pressure chromatographic column was investigated. Three-dimensional perspective plots of wavelength/absorbance/time were developed to provide insight into the nature of the sugar colourants and to provide a means of investigating various decolourisation systems. In addition to the above experiment a procedure was developed to remove colourant species from unrefined sugar samples and from samples taken during the refining process for chemical analysis. In this work only one technique – gas chromatography-mass spectroscopy was used to identify the species.

Keywords: colour, spectroscopy, chromatography

Introduction

Unrefined sugar contains organic colour material originating in the sugarcane or formed during the extraction and purification processes. Organic colour material removed in the production of white sugar, is a complex mixture of phenolic compounds, flavonoids, caramels, alkaline degradation products and melanoidins. Iron, pH and other impurities have an effect on the colour of phenolic and flavonoid pigments, which are natural products found in sugarcane. Heating sucrose or acid/base catalysed degradation of sucrose can lead to the formation of coloured polymers known as caramels (Shore *et al.*, 1984). Melanoidins are brown nitrogen containing pigments produced in a reaction between a reducing sugar and an amino compound.

Various techniques have been used to study sugar colourant removal including gel permeation chromatography (Godshall, 1992), high performance liquid chromatography (HPLC) and electrophoresis. Bento (1993,1994) described a technique to separate sugar colourants using a sucrose packed medium pressure chromatographic column. Sugar colourants were separated through the column using eluents graded in polarity. The absorbances of the eluted colourants were determined at 280 nm (Bento, 1993) and in the range 220 to 500 nm (Bento, 1994) as a function of time. The profiles obtained indicated the ultraviolet-visible properties of the colourant and could be used for optimisation of the sugar refining process. The profiles could also be used to test different decolourisation systems (carbonatation, acrylate/styrene-based resins, activated carbon etc.) and to establish which colourants have a higher affinity for the sugar crystals. In this work a simpler technique was developed, following Bento's work, to produce cost effective and rapid profiles of the ultraviolet-visible properties of sugar colourants. Three dimensional perspective plots, similar in concept to those of Bento, were obtained.

The Bento technique does not allow for identification of the species causing the colouration. A method was developed

in this work to remove organic colourants from sugar samples for analysis using gas chromatography-mass spectroscopy (GC-MS).

Experimental

Equipment

The equipment used included a Varian DMS 300 ultraviolet-visible spectrophotometer; a CKB Bromma 2132 Microperpex peristaltic pump; a 33 cm length and 1 cm internal diameter glass column. Surfer graphics software. A Hewlett Packard (HP) 5890 Series II Gas Chromatograph and a HP 5971 Series Mass Selective Detector (Electron Impact). The GC column used was a HP-5 cross linked 5% phenylmethylsilicone fused capillary column, i.d.: 250 μ m, length: 25 m.

Stationary phase

The glass column was packed using 'first boiling' white sugar of a crystal size which ranged between 0,425 mm and 0,600 mm. Approximately 20 g of white sugar was packed to a height of 28 cm in the column. The sugar placed in the column was washed first with methanol:acetonitrile (60:40, 0,5% H₂SO₄ 1M) for one hour at 0,5 ml/minute flow rate, followed by propanol:pentanol (75:25, 0,3% NH₄OH 2M) for 30 minutes. The column was refilled with new white sugar for each mill sample, and a 0,45 μ m membrane filter (Millipore) was placed in the bottom of the column.

Mobile phase

Five solvents of increasing polarity were used as eluents, each with a low solubility for sucrose, and transparent in the range 220 to 500 nm. Mixtures of the solvents were used to increase gradually the polarity of the eluent through the column (see Table 1).

Table 1
Eluent system fed onto the column at a flow rate of 0,5 ml/min

Time (minutes)	Eluent		
25	pentanol:propanol	1:3	0,3% NH ₃ OH 2M
25	propanol		0,3% NH ₃ OH 2M
10	propanol:ethanol	1:1	0,3% NH ₃ OH 2M
25	ethanol		0,3% NH ₃ OH 2M
10	ethanol:methanol	1:1	0,5% H ₂ SO ₄ 1M
25	methanol		0,5% H ₂ SO ₄ 1M
10	methanol:acetonitrile	1:1	0,5% H ₂ SO ₄ 1M
30	acetonitrile		0,5% H ₂ SO ₄ 1M

Sample preparation

The following procedure was used in the preparation of the raw sugar sample for injection onto the sucrose packed column, following the procedure of Bento (1994).

- Ten g of the unrefined (raw) sugar sample was weighed into a beaker.
- The unrefined sugar was dissolved in 5 g of Milli-Q water.
- One g of fructose was added to this solution.
- Ten ml of propanol:H₂O:HCl (50:49:1 v/v/v) was added.
- Ten ml of 30 g/litre of NaCl was added.

The solution was then filtered through a 0,8 µm membrane filter.

Running

After the white sugar stationary phase in the column had been washed, a prepared 200 µL raw sugar sample was injected onto the column into the eluent flow, and the run was started. Fractions of the colourant eluted from the column were collected in five minute intervals, and ultraviolet-visible spectra in the range 220 to 500 nm were obtained. The absorbance/wavelength spectrum obtained for each fraction was used in the generation of the three dimensional perspective plots on Surfer graphics software.

Colour removal

Amberlite IRA 958S was used for removal of colourants from unrefined sugar and from sugar samples obtained from the refinery for GC-MS investigation. Amberlite IRA 958S is a macroporous strongly basic anion exchange resin exhibiting quaternary ammonium functionality in a cross linked acrylic polymer matrix.

A raw sugar concentration of 68° brix (90,7 g/100 ml) was prepared in a closed double walled glass flask, and to that solution 15 g of anion exchange resin was added. The solution was stirred for four hours and maintained at 80°C by circulating heated water through the walls of the flask. A thermometer was inserted into the solution through a rubber bung in the top of the flask. The brix and temperature were based on refinery conditions (Kirkiridis, 1992). The solution was filtered and the retained resin was washed with warm water a number of times to remove as much sucrose as possible, before the resin was regenerated using a 10% NaCl solution. The colourant was thus removed from the resin and the resultant solution was collected by filtration. The colourant solution was then gently boiled on a waterbath to precipitate cubic NaCl crystals. This step was repeated a number of times until no crystals were observed to ensure that all of the salt was removed. The solution was then carefully boiled to dryness on a waterbath to ensure that none of the chemicals was destroyed.

The brown colourant was dissolved in methanol and injected onto the GC-MS for compound identification. ICUMSA 420 colour values were determined for all raw sugar solutions before and after decolourisation as an indication of the efficacy of the decolourisation technique (see Table 2).

Table 2
ICUMSA 420 colour of the mill and refinery samples before and after colour removal

	ICUMSA 420 (before)	ICUMSA 420 (after)
Sezela	3274	670
Maidstone	2848	724
Saturator supply	4279	696
Brown liquor	1304	308
Secondary liquor	217	179

Results and Discussion

Samples of the unrefined sugar used in the investigation were obtained from two sugar mills: Sezela and Maidstone. Two samples from each mill were obtained for the weeks ending (w/e) 08/10/94 and 15/10/94. Ultraviolet-visible spectra of the fractions of eluted colourant were obtained, and used in the generation of three dimensional plots to provide absorbance/wavelength/time profiles as shown in Figures 1 to 4. It was intended that the techniques could provide a simple, cost effective and reproducible analytical methods to optimise the refining process, to test different decolourisation systems and to determine which colourants had the greatest affinity for the sugar crystals.

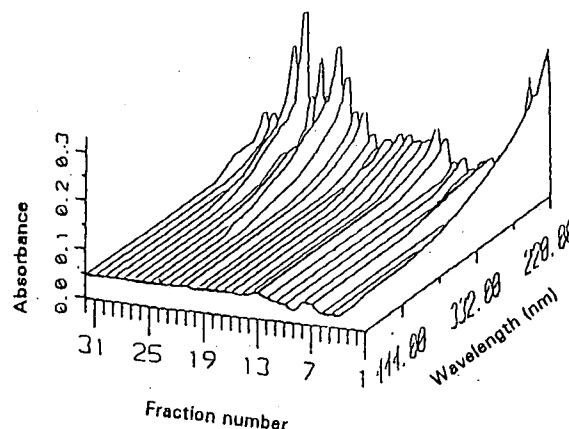


FIGURE 1: Sezela w/e 08/10/94

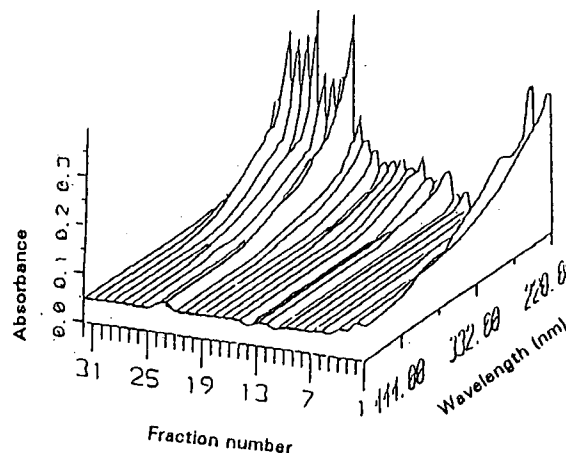


FIGURE 2: Sezela w/e 15/10/94

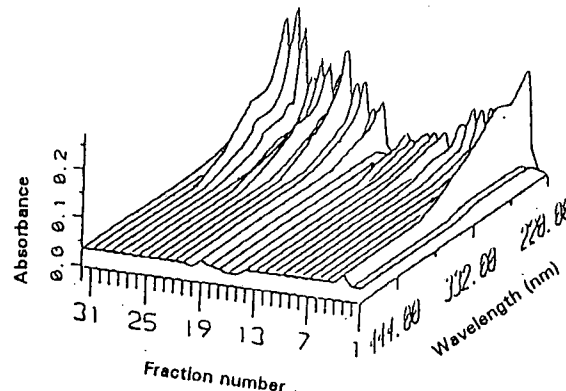


FIGURE 3: Maidstone w/e 08/10/94

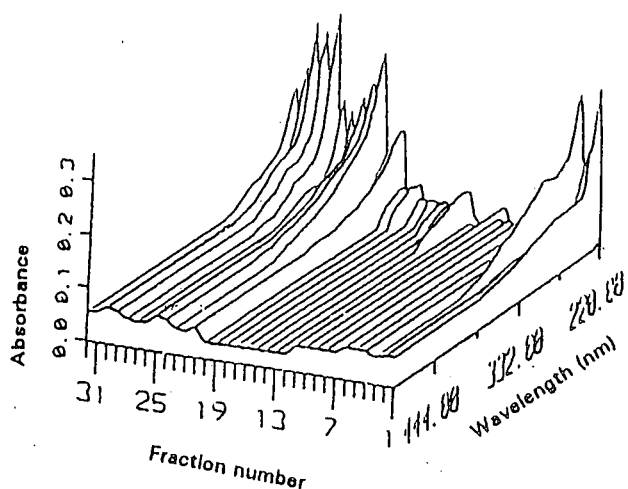


FIGURE 4: Maidstone w/e 15/10/94

It appears that the colourants elute predominantly in two main groups. The first appears to elute in the first 15 minutes (3 fractions) and has an affinity for the low polar medium, being eluted with a propanol/pentanol solvent mixture. The second group of compounds appears to elute after 100 minutes (25 fractions) in the high polar medium. It is possible however, that the second group could be further resolved into more groups if the elution time for each solvent were lengthened, or if the solvents were graded in polarity using a computer operated intelligent pump. The plots of all four samples are very similar in profile which could indicate that similar colourants originate at each mill at this time of the season. It is evident that Maidstone w/e 08/10/94 has less of the low polarity compounds due to fractions 1 and 2 having very low absorbance compared to the other samples. Maidstone w/e 08/10/94 also contains less colourant than the other samples due to a lower absorbance across the spectrum. It is possible that a relationship exists between the absorbance of a group of compounds and the difficulty experienced in removing the colourant at the refinery. Analysis of raw sugar samples before and after decolourisation (e.g. carbonatation, phosphatation) could provide information on the efficacy of the decolourisation method.

The fractions collected are not purely solvent and colourant, but do contain dissolved sucrose as confirmed by nuclear magnetic resonance spectra. This limits the study of these fractions for possible compound identification. As a result a method for the removal of colourant from the samples was developed in an attempt to isolate organic colourant present in the mill samples and in samples from the refinery for structural elucidation. GC-MS was selected for compound identification because it is a rapid and reliable method. The mill samples were taken from Sezela and Maidstone for the week ending 08/10/94. The refinery samples taken were the saturator supply (6:00 am), brown liquor (7:45 am) and the secondary liquor (8:15 am). Once the colourants had been removed using the resin decolourisation technique described, they were injected onto the GC-MS for separation and identification. It was imperative that as little sucrose as possible was present in the colourant samples, to ensure that no decomposition occurred in the GC system.

All of the compounds found are cyclical and polar in nature and are aromatic, or contain at least one double bond (see Table 3). The compound, 5-hydroxymethyl-2-furancarboxaldehyde, was found in all of the samples analysed and can be

formed via the action of a weak acid, or even pure water, at a temperature of 130-170°C on sucrose under pressure (Patarau, 1969). It is possible that this compound could have been formed after injection of the colourant samples onto the gas chromatograph.

Table 3
Compounds identified using GC-MS

Sample	Compound Name
Maidstone w/e 08/10/94	5-hydroxymethyl-2-furancarboxaldehyde 5,5'-oxy-dimethylene-bis-2-furaldehyde
Sezela w/e 08/10/94	5-hydroxymethyl-2-furancarboxaldehyde 5-hydroxymethyl-2-furancarboxaldehyde
Saturator supply	5-hydroxymethyl-2-furancarboxaldehyde 3-methyl-1H-pyrazol 4-amino-5-methyl-2(1H)pyrimidinone or 2-amino-3-methyl-4-(3H)pyrimidinone 6-methyl-3(2H)-pyridazinone 1H-pyrrole-2,5-dione Pulegone 5,5'-oxy-dimethylene-bis-2-furaldehyde
Brown liquor	5-hydroxymethyl-2-furancarboxaldehyde 1H-pyrrole-2,5-dione 5,5'-oxy-dimethylene-bis-2-furaldehyde
Secondary liquor	5-hydroxymethyl-2-furancarboxaldehyde 1H-pyrrole-2,5-dione 5,5'-oxy-dimethylene-bis-2-furaldehyde

It is possible that some of the other compounds identified were artefacts formed on the column, however, the nitrogen containing compounds cannot be sugar degradation products formed on the column. Adsorption of colourants and colour precursors onto the resin can take place without the exchange of anions. It has been shown that the high selectivity of anion exchange resins for colourants includes electrostatic and van der Waals' interactions (Kunin, 1978). The acrylate-based anion exchange resins, as used in this work, are aliphatic and less selective for the high molecular weight cyclic colourants (Kunin, 1979). Therefore, the use of a styrene-based resin in conjunction with an acrylate-based resin could prove far more effective in this type of investigation.

Conclusions

This work was initially conducted, following the work of Bento (1993,1994), to provide informative, simple and cost effective colour profiles as an aid to optimisation of the refining process. The technique provides information on: the ultraviolet-visible properties of the colourant to distinguish raw sugar samples, the affinity of the colourant for sucrose and the efficacy of various decolourisation systems. It was extended in an attempt to identify the colour compounds present in the samples.

It was found that crystalline sucrose was an unsuitable chromatographic phase for separating colourants for further analytical analysis. The work developed here indicated that it could be more effective to use a combination of ion-exchange resins (acrylate and styrene-based) to remove colourant from raw sugar samples first. Thereafter, the colourant could be fractionated on a silica gel column to produce wavelength/absorbance/time plots similar to those presented here, to eliminate the problems experienced due to trace amounts of

sucrose complicating structural analysis. The procedure developed to identify the colourants could be extended to include other analytical techniques such as infra-red and nuclear magnetic resonance spectroscopy. The various refining processes including carbonation, phosphatation, ion exchange resins and activated carbon could be investigated for colour removal efficacy. A possible long term project would be to investigate the variation in the nature of the colourant with the region (e.g. Northern Natal, Eastern Transvaal), the climatic conditions and the harvesting season.

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