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# The relationship between the coloured compounds present in the pressed liquor of cane sugar manufacture and those formed in Maillard reactions, in alkaline degradation of sugars, and in caramelisation

**Javad Keramat* & Harry E. Nursten**

*Department of Food Science and Technology, University of Reading, Whiteknights, PO Box 226, Reading, UK, RG6 2AP*

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The coloured compounds of pressed liquor (PL) have been compared with those formed in Maillard reactions from alanine and glucose (MAG) and alanine and fructose (MAF), in the alkaline degradation of glucose (ADG) and of fructose (ADF), and in caramelisation of sucrose (CAR) under conditions simulating those obtaining in carbonation. The coloured compounds were isolated by adsorption on non-ionic resins (Amberlite XAD-7 and XAD-16, in series) at pH 2·5, the columns being washed with 0·01 N HCl, and eluted with 0·01 N HCl in methanol. The coloured extracts were evaporated to dryness and analysed by spectrophotometry and TLC. Distribution of absorbance units measured at 420 nm between unabsorbed material and XAD-7 and XAD-16 extracts showed CAR to be closest to PL, but the recovery of absorbance units measured at the maximum in the range 300–320 nm for the XAD-7 extracts was PL, 54%; MAG, 62%; and MAF, 20%; ADG, ADF, and CAR have no maximum in this range. The XAD-7 extracts of ADG and ADF were exceptional in exhibiting maxima at 440 nm. On TLC of the XAD-7 extracts in BAW, PL gave 10 bands, of which seven appeared also in MAF and in MAG and four in ADF, in ADG and in CAR. Overall it seems that the coloured compounds in PL bear most resemblance to those formed in Maillard reactions. These studies are being greatly extended by HPLC analysis of extracts and their fractions.

## INTRODUCTION

Coloured compounds have always provided special difficulties for the sugar industry, particularly in sugar refineries, where refining itself causes formation of part of the colour. This work was carried out to provide more information about the nature and mechanisms of formation of the coloured compounds involved. At the last Maillard symposium, we reported on classification of sugar colorants and some of their properties (Keramat & Nursten, 1990). We now report in more detail on the isolation of sugar colorants via adsorption onto non-ionic resins and on their comparison with 'synthetic' colorants, isolated similarly, by spectrophotometry and TLC.

## MATERIALS AND METHODS

### Pressed liquor

Collected from Tate & Lyle Co., Thames Refinery, London, UK.

### Melanoidins

*(a) From alanine and glucose (MAG)*
Alanine (1 mole) and glucose (1 mole) were heated in water (1 litre) at 80–85°C for 60 min, after adjusting the pH to 10·2 with NaOH. The final pH was 8·5. The solution was immediately cooled to 20°C and the pH adjusted to 7·0 with HCl.

*(b) From alanine and fructose (MAF)*
The same procedure was followed, replacing glucose by fructose.

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* Present address: Department of Food Science, College of Agriculture, University of Technology, Esfahan, Iran.

### Alkaline degradation products

*(a) From glucose (ADG)*

Glucose (1 mole) was heated in water (1 litre) at 80–85°C for 60 min, after adjusting the pH to 10·9 with NaOH. The pH was not allowed to drop below 8·3. The solution was immediately cooled to 20°C and the pH adjusted to 7·0 with HCl.

*(b) From fructose (ADF)*

The same procedure was followed, replacing glucose by fructose.

### Caramelisation of sucrose (CAR)

Sucrose was dissolved in water (1 litre) to give a solution of 65°Bx, which was heated at 80–85°C for 60 min, after adjusting the pH to 10·8 with NaOH. The final pH was 9·6. The solution was immediately cooled to 20°C and the pH adjusted to 7·0 with HCl.

### Isolation of coloured compounds

Adapting the method of Williams and Bhardwaj (1988), a pair of non-ionic resins, a polyacrylic one (Amberlite XAD-7) and a polystyrene one (Amberlite XAD-16), was used in series. Resin (220 ml) of each type was transferred to a separate column (120 × 2·4 cm i.d.) and classified by back-washing with water, each expanding by at least twice its volume. Fine particles were removed concomitantly. Each column was washed with 0·001 N HCl in methanol and then back-washed with 0·01 N aqueous HCl, before the columns were connected. Pressed liquor (4 litres, 63·5°Bx) was diluted to 15·9°Bx and the pH adjusted to 7·0. The solution was filtered through a 0·45-$\mu$m filter and degassed under vacuum in an ultrasonic bath. The absorbance at 420 nm of an appropriately diluted sample was measured and its spectrum scanned (600–190 nm). The pH was then adjusted to 2·5 with HCl and the solution applied to the first column at 2 ml min$^{-1}$. The effluent from the second column (XAD-16) was collected as unabsorbed colour. When all the sample had been applied, the columns were washed with 0·01 N HCl and the effluent was also collected. The two effluents were combined, the total volume was measured, the pH adjusted to 7·0, the solution degassed after filtering, the absorbance measured at 420 nm and the spectrum scanned (600–190 nm).

The solutions of the 'synthetic' mixtures were treated similarly. The volume of each was measured after filtering, using kieselguhr on glass filter paper, and degassing. The absorbance at 420 nm was measured after appropriate dilution (MAG, MAF, ×40; ADG, ×30; ADF, ×10; CAR, ×4).

The adsorbed colour was desorbed by back-washing each column separately with 0·01 N HCl in methanol. Each eluate was adjusted to pH 7·0 and degassed. Its absorbance was measured at 420 nm and its spectrum scanned (600–190 nm).

**Table 1. The number of TLC bands separated from XAD-7 extracts using different solvents**

| Solvent system[a] | Sample | | |
|---|---|---|---|
| | PL | MAG | ADG |
| TAW (6 : 7 : 3) | 5 | 0 | 2 |
| TAD (22 : 1 : 6) | 6 | 1 | 2 |
| TCA (8 : 5 : 7) | 4 | 2 | 0 |
| TMA (2 : 11 : 1) | 3 | 2 | 2 |
| AAW (70 : 29 : 1) | 2 | 1 | 2 |
| BAW (4 : 1 : 5) | 10 | 7 | 4 |
| BAW (4 : 1 : 0) | 7 | 4 | 2 |
| BAW (5 : 1 : 5) | 7 | 3 | 2 |
| PTBAW (4 : 2 : 1 : 2) | 5 | 5 | 2 |

[a] TAW: toluene : acetic acid : water (Paton, 1978, who used benzene not toluene). TAD: toluene : acetic acid : dioxan (Farber & Carpenter, 1971). TCA: toluene : chloroform : acetone (Farber & Carpenter, 1971). TMA: toluene: methanol : acetic acid (Farber & Carpenter, 1971). AAW: anisole : acetic acid : water (Paton, 1978). BAW: butanol : acetic acid : water (Linecar *et al.*, 1978). PTBAW: isopropanol: t-butanol : acetic acid : water (Paton, 1978).

Each eluate was concentrated on a rotary evaporator (<30°C), the methanol-free residue being freeze-dried, weighed, dissolved in methanol (200 ml) and stored at −21°C.

### TLC

Type of plate and solvent system were investigated. Silica gel gave better separation than cellulose. The greatest number of bands was observed with BAW (4 : 1 : 5) (see Table 1).

## RESULTS AND DISCUSSION

### Spectrophotometry

Table 2 gives the total colour in each sample in units of AU.L, i.e. the absorbance at 420 nm as determined in a 1-cm cell, multiplied by the volume in litres. It also lists the distribution of the colour, as defined, between the

**Table 2. Total colour of samples as measured at 420 nm and its distribution on resin treatment**

| Sample | Total colour in sample (AU.L) | Proportion of total colour (%) | | | |
|---|---|---|---|---|---|
| | | Eff | XAD-7 | XAD-16 | Irreversibly adsorbed |
| PL | 1·96 | 4 | 14 | 5 | 77 |
| PL[a] | 1·84 | 10 | 43 | 8 | 39 |
| MAG | 5·96 | 7 | 24 | 32 | 37 |
| MAF | 3·10 | 4 | 51 | 33 | 12 |
| ADG | 2·73 | 17 | 42 | 16 | 25 |
| ADF | 4·00 | 17 | 52 | 24 | 7 |
| CAR | 0·29 | 7 | 16 | 7 | 70 |

[a] XAD-7 and XAD-16 resins had already undergone adsorption/desorption with the previous batch of PL.

**Table 3. Distribution of absorbance (%) as measured at different absorption maxima (nm) on resin treatment**

| Sample | Eff<br>190–210 | Eff<br>260–80 | XAD-7<br>190–210 | XAD-7<br>260–80 | XAD-7<br>300–20 | XAD-16<br>190–210 | XAD-16<br>260–80 | XAD-16<br>290–300 |
|---|---|---|---|---|---|---|---|---|
| PL | 44 | 24 | 18 | 36 | 54 | 9 | 14 | — |
| MAG | 31 | 23 | 76 | 57 | 62 | 17 | 19 | 13 |
| MAF | 35 | 29 | 24 | 27 | 20 | 27 | 28 | 27 |
| ADG | 67 | 77 | 12 | 12 | 1[a] | 14 | 17 | — |
| ADF | 60 | 51 | 25 | 18 | 2[a] | 22 | 20 | — |
| CAR | 33 | 22 | 3 | 5 | — | 3 | 6 | — |

[a]Maximum at 440 nm.

**Table 4. Comparison of $R_f$ values from TLC of the bands of XAD-7 extract of pressed liquor with those of other samples**

| Band No. | Sample | | | | | |
|---|---|---|---|---|---|---|
| | PL | MAG | ADG | MAF | ADF | CAR |
| 1 | 0·10–0·14 | 0·08–0·14 | 0·08–0·13 | 0·10–0·13 | 0·10–0·12 | 0·11–0·15 |
| 2 | 0·16–0·19 | 0·16–0·20 | — | — | — | — |
| 3 | 0·25–0·28 | 0·26–0·29* | — | — | — | — |
| 4 | 0·30–0·34* | 0·36–0·38 | — | 0·34–0·37* | — | — |
| 5 | 0·41–0·44 | — | — | — | — | 0·40–0·42* |
| 6 | 0·45–0·48* | — | — | 0·45–0·48* | — | — |
| 7 | 0·50–0·56* | 0·54–0·57 | 0·53–0·56* | 0·51–0·57* | 0·52–0·56* | — |
| 8 | 0·58–0·65* | 0·61–0·66 | 0·59–0·64* | 0·59–0·65 | 0·59–0·64 | — |
| 9 | 0·66–0·72 | 0·69–0·74 | 0·66–0·73 | 0·67–0·72 | 0·66–0·74 | 0·67–0·70 |
| 10 | 0·74–0·78* | — | — | 0·76–0·80* | — | 0·74–0·76* |

*Bands are visible, except those asterisked, which required UV radiation for observation.

effluent (unabsorbed material) and the XAD-7 and XAD-16 extracts (desorbates). A large proportion of colour is not accounted for in some cases, e.g. 77% for PL. Much of such lost material presumably remains adsorbed on the resins. This is corroborated by the fact that the desorbed resins are still coloured and by an experiment in which resin used for PL was re-used for a second sample of pressed liquor, when the material not accounted for dropped to 39% (see Table 2). The results given in Table 2 show CAR to behave most like PL.

The spectral scans, 600–190 nm, provided a vast amount of additional information, difficult to present in a digestible form. Table 3 is an attempt to do so and is concerned with the per cent absorbance, not at 420 nm, as above, but at maxima in the UV. The most interesting points to make are the following:

1. For absorbance at the maximum in the range 300–320 nm, the recoveries for the XAD-7 extracts were PL, 54%; MAG, 62%; MAF, 20%. ADG, ADF, and CAR do not exhibit a maximum in this range.
2. XAD-7 extracts of ADG and ADF are exceptional in having maxima at 440 nm.
3. ADG and ADF are also unusual in the high proportions of absorbance found in the effluent (at 190–210 nm, 67 and 60%; at 260–280 nm, 77 and 51%, respectively).
4. The XAD-16 extracts of MAG and MAF are unusual in constituting a sizable proportion of the absorbance at a maximum in the range 290–300 nm (13 and 27%, respectively).

## TLC

Table 4 presents the TLC results in more detail. It can be seen that the 10 PL bands can be matched approximately by the seven bands each from MAG and MAF and the four bands each from ADG, ADF, and CAR. If appearance under normal light is taken into account, MAF gives the most extensive match of six bands, followed by MAG with four.

## CONCLUSION

From the results presented, it appears that the behaviour of the coloured compounds in PL on adsorption/desorption with resin columns, spectrophotometry, and TLC overall, resembles Maillard compounds more than caramel and alkaline sugar degradation products least. These results are being followed up with a detailed HPLC examination of the extracts and their fractions.

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