# THE ORIGIN OF COLOUR IN RAW SUGAR

By
N.H. (NANCY) PATON
*CSR Limited, Central Laboratory*

## Introduction

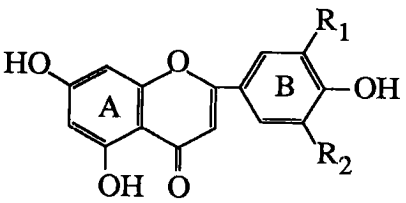
In sugar mills juice is extracted from cane, then clarified, concentrated and crystallised into raw sugar. This process also extracts other compounds from cane, some of which are coloured while others are colourless, and converts a considerable proportion of them to complex colour in raw sugar. A recent research project, "Phenolic Colourants in Sugarcane", has given a better understanding of some of the colour changes that occur in the production and storage of raw sugar (Paton *et al.*, 1989, 1990, 1991). The current knowledge of the origin of colour in raw sugar will be discussed in this paper, particularly the role of phenolics.

## Colourants and colour precursors in cane

The main colourants in cane are common plant constituents such as chlorophylls and flavonoids. Chlorophylls are the green pigments in leaves and stalks; they are not soluble in water, are prone to chemical change and degrade easily (Harborne, 1984), and there is little evidence of them in filtered cane juice.

Flavonoids are phenolic compounds with a $C_6C_3C_6$ backbone and OH substituents on the A and B ring. Anthocyanins are one type of flavonoid; they are intensely coloured and responsible for the coloured rind of some cane varieties. Anthocyanins are unstable in neutral or alkaline solutions, and are also decomposed by heat (Harborne, 1984) and they do not survive mill clarification.

Flavones are another type of flavonoid; they are yellow compounds and present in all cane varieties but their colour is usually masked by chlorophyll or anthocyanins. The basic structure of the flavones in sugarcane is shown as:




| | | |
|---|---|---|
| apigenin | $R_1 = H$ | $R_2 = H$ |
| luteolin | $R_1 = OH$ | $R_2 = H$ |
| tricin | $R_1 = OCH_3$ | $R_2 = OCH_3$ |

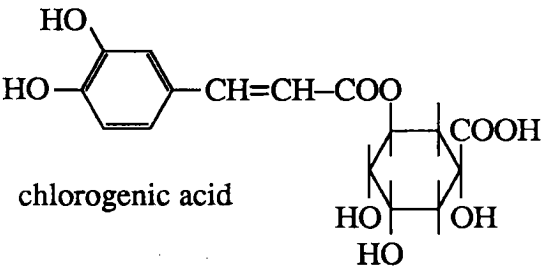
The flavones are present as glycosides in cane, that is, they have sugar units attached to the above structure. The flavone glycosides are generally stable in mill clarification and further processing.

Flavones are quite pale in acid solution but in alkaline solution are yellow or yellow green. This effect is the basis of the indicator value of flavone colourants, and it accounts for much of the difference in colour of sugar samples between pH 4 and pH 9. Indicator value or IV is the colour at pH 9 divided by the colour at pH 4, where colour is measured by the standard method. The IV of methanol extracts of cane is over 30 for some varieties.

**KEYWORDS: Cane, Chlorogenic Acid, Colour, Enzymic Browning, Flavonoids, Raw Sugar**

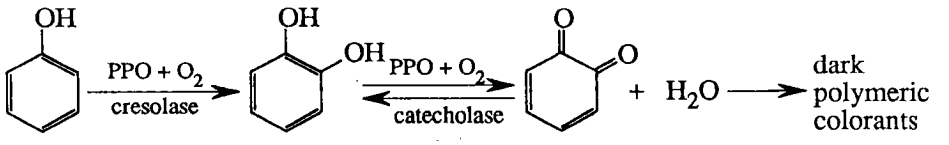
The colour precursors in cane, that is, the compounds which form colour in processing, include other phenolic compounds, reducing sugars and amino acids.

The phenolic compounds are usually derivatives of cinnamic acids, and their basic structure is similar to the B ring of the flavones. They are present in the plant as esters, when the phenolic acid is bound to an alcohol, or as glycosides when the acid is bound to a sugar unit such as glucose. The most common phenolic ester in cane is chlorogenic acid, an ester of caffeic acid with quinic acid (a cyclic compound with alcoholic and acidic substituents).




chlorogenic acid

Phenols may be oxidised to dark coloured polymers by enzymic oxidation or non-enzymic oxidation, and these polymers contribute to the colour of raw sugar. When plant tissue is cut and exposed to the air the phenols may undergo enzymic oxidation leading to the formation of quinones and then dark coloured polymers. Enzymic browning is common in fruit and vegetables, such as apples and sweet potatoes, and occurs when sugar cane is crushed. The enzymes involved in browning are polyphenol oxidases (PPO) and the reactions are summarised below.




monophenol o-diphenol quinone

In some plants the enzyme catalyses both reactions, whereas in others it only catalyses the formation of a quinone from an o-diphenol. Quinones react with compounds such as another quinone, an o-diphenol, or an amino acid to form polymers.

Chlorogenic acid is an o-diphenol present in cane and it is a substrate for PPO activity when cane is crushed. The reaction from chlorogenic acid to the quinone is reversible; when chlorogenic acid quinone oxidises some compounds then chlorogenic acid is regenerated. In 1978 Goodacre and Coombs estimated that over half the colour in cane juice originated from enzymic reactions.

The activity of PPO may differ from year to year depending on temperature and rainfall. Varietal factors are important in most crops as the rate of enzymic oxidation that leads to browning depends not only on the concentration of phenols and enzyme activity but also on the nature of the phenols; some phenols react faster than others (Macheix *et al.*, 1990).

Phenols such as chlorogenic acid may also be oxidised by chemical means; the rate of the reaction is increased by increasing pH and temperature. Oxidation reactions occur under storage conditions, even at low temperature; this reaction can be appreciable during prolonged periods of storage (Cilliers and Singleton, 1989).

The other significant colour precursors in cane are reducing sugars and amino acids. When solutions of reducing sugars are heated in alkaline conditions then coloured polymers are formed, known as alkaline degradation products. However, when high brix solutions of reducing sugars and amino acids are heated then coloured polymers are formed, known as melanoidins. Furthermore, if ash constituents are present, as in sugar processing, they may be incorporated in the polymer. Melanoidins also form during the storage of raw sugar, mainly on the syrup film surrounding the crystal. The alkaline degradation products and melanoidins are sometimes called the factory colourants.

In a study of these reactions with a series of model solutions which reflected the composition of mill streams it was found that alkaline degradation products were likely to form in evaporator supply juice, and melanoidins in higher brix solutions such as molasses (Paton and McCowage, 1987).

The factory colourants have a higher molecular weight (MW) than the natural colourants, possibly up to 50 000 compared to protein standards. In contrast to the natural cane colourants which may have an IV up to 33, the factory colourants show only a small increase in colour when the pH is increased from 4 to 9, and the indicator value of colourants in model solutions is 1–2.5.

It should be noted that agricultural practices affect the level of amino acids in cane. Excessive applications of nitrogenous fertiliser are stored by the plant as amino acids, resulting in a higher than normal concentration of these acids in juice. This in turn influences the extent of colour formation during processing and storage of raw sugar.

## Colour of cane extracts

As part of the research into phenolics in cane the concentration of chlorogenic acid and flavonoids was determined in 18 commercial varieties of cane in the 1989 and 1990 seasons. This was done by extracting cane billets with methanol to minimise enzymic oxidation, removing the methanol, and determining the concentration of chlorogenic acid, neutral phenolics and total flavonoids by high performance liquid chromatography (HPLC). In addition the colour of the extracts was measured at pH 4, 7 and 9. The range of results is given in Table I.

**TABLE I**—Chlorogenic acid, flavonoids and colour of cane extracts.

| Parameter | Range of results |
|---|---|
| Chlorogenic acid | 10– 1060 mg/kg solids |
| Neutral phenolics (as sinapoyl glucose) | 110– 620 mg/kg solids |
| Flavonoids (as sinapoyl glucose) | 170– 1340 mg/kg solids |
| Colour pH 4 | 1160– 4880 |
| Colour pH 7 | 3890– 19600 |
| Colour pH 9 | 25500–123000 |
| Indicator value (IV) | 10–33 |

After the 1989 season it was found that the colour of the cane extract at pH 9 was related to the concentration of chlorogenic acid and flavonoids. The multiple linear regression for this relationship was highly significant statistically with a coefficient of determination $r^2$ of 0.90. The result for the 1990 season confirmed this finding. The regression equation, with $r^2$ of 0.94 was:

$$\text{Colour of cane extract pH 9} = 6267 + 83.7\ \text{Flavonoids} + 18.6\ \text{Chlorogenic Acid}$$

with the concentration of flavonoids and chlorogenic acid as mg/kg sugar solids. The calculated and actual values for the 1990 season samples are shown in Figure 1.

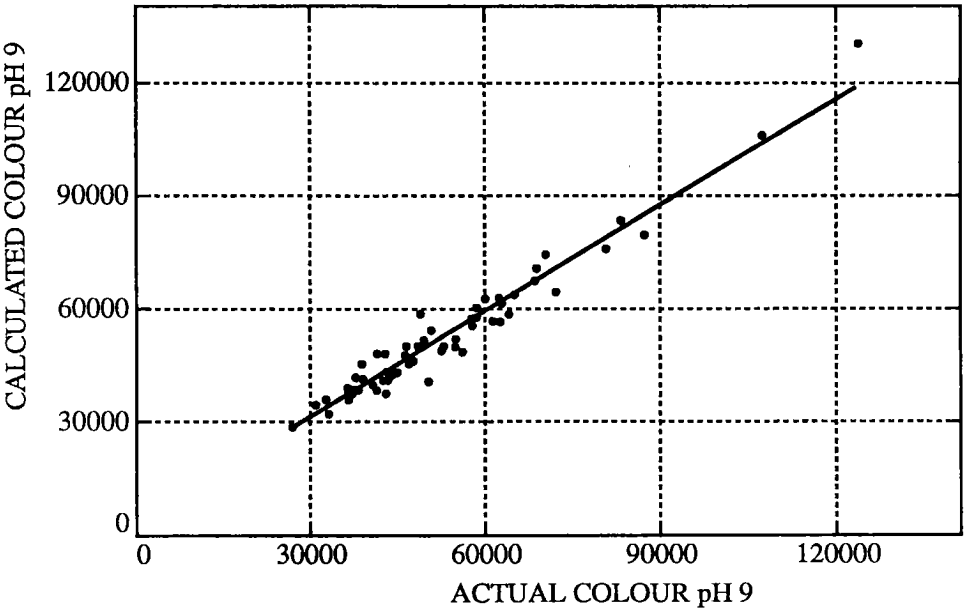



Fig. 1—Relationship between colour of methanol extract of cane and calculated colour level on the concentration of flavonoids and chlorogenic acid.

The concentration of flavonoids is the main source of colour in cane extracts. For example, from the above equation the calculated colour at pH 9 of cane extract with a typical concentration of flavonoids of 500 mg/kg and of chlorogenic acid of 200 mg/kg is 51837. A reduction in flavonoid concentration of 100 mg/kg lowers the colour of cane by 8370 whereas a similar reduction in chlorogenic acid concentration only lowers the colour by 1860. Therefore, cane with a high concentration of flavonoids would be expected to give an extract of high colour, and cane with low flavonoids would be expected to give an extract of low colour.

The colour of laboratory extracts of cane varies from pale yellow to deep yellow, and is due mainly to small colourant molecules, flavonoids and chlorogenic acid. This is shown in a size exclusion profile of cane extract colourants in Figure 2. A buffer of pH 7.0 and detection at 450 nm were used; conditions very similar to colour measurement. In this type of separation the molecules are eluted in order of size with large molecules (high MW) being eluted first; flavonoids start to be eluted at 19.5 minutes. Very little of the visible colour of the cane extract is due to polymeric colourants. However, some high MW compounds are detected in ultraviolet light at 280 nm.

## Colour changes at crushing

The colour of first expressed juice at a mill differs from the cane extract as shown in Table II for 19 sets of cane and corresponding juice from the 1989 season.

**TABLE II**—Colour of cane extracts and first expressed juice.

| Parameter | Cane extract | First expressed juice |
|---|---|---|
| Colour pH 4 | 1160– 4440 | 3560–10100 |
| Colour pH 7 | 4600–14600 | 5400–15700 |
| Colour pH 9 | 32900–90000 | 17600–42600 |
| IV | 15–29 | 3.1–6.4 |

The colour of juice at pH 4 and the visible colour increase considerably, the colour at pH 7 increases slightly, and the colour at pH 9 and IV decrease considerably. These changes are the effects of chlorogenic acid, other phenolics and flavonoids reacting to form enzymic browning/oxidation colourants, and the latter having a low IV. A significant characteristic of browning colourants in cane juice is their solubility in a sugar solution whereas many browning colourants, such as those in beet juice and grapes, are insoluble.

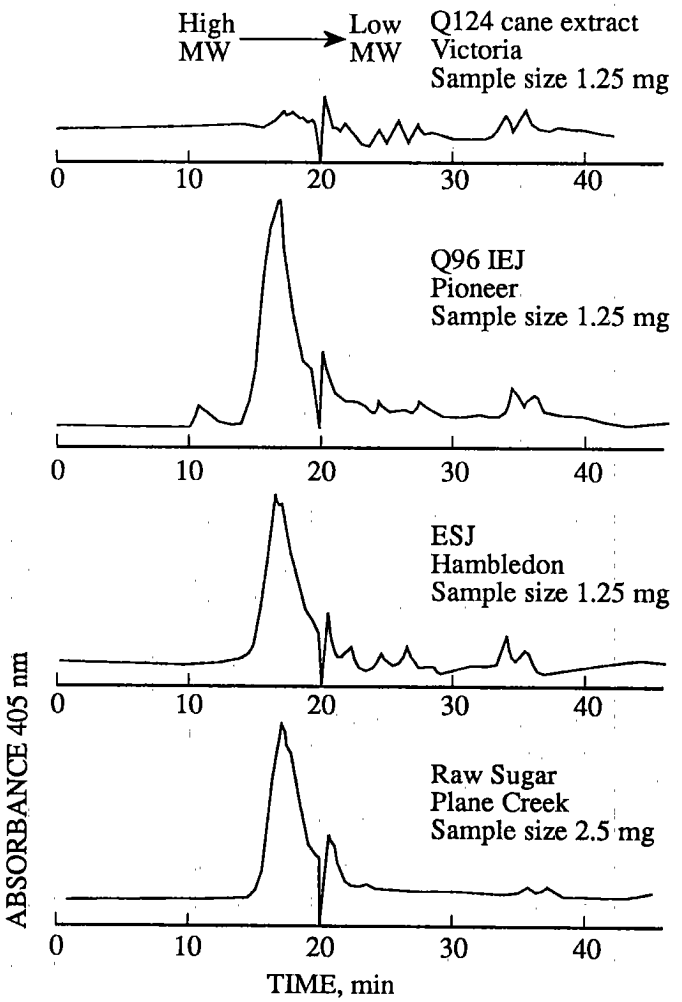



Fig. 2—Size exclusion profiles of colourants in cane extract, first expressed juice, evaporator supply juice and raw sugar with detection at 405 nm.

The concentration of phenolics in Q123 cane extract and corresponding first expressed juice is given in Table III. The concentration of all types of phenolics was much lower in juice than in cane; note that some chlorogenic acid was converted to isomers upon crushing.

The changes in neutral phenolics and flavonoids in Q123 cane and juice are illustrated in Figure 3, which shows the colourant profiles with the same sample size on sugar solids basis and the same sensitivity. The peaks C, E and G contain

flavonoids with an o-diphenol group and they were very much smaller in juice than in cane. Other flavonoids with a monophenol group also decreased, and neutral phenolics, such as peaks 12 and 18 almost disappeared as a result of crushing.

The results concerning the contribution of flavonoids and phenolics to enzymic browning do not preclude a contribution from other compounds, such as amino acids, to this reaction.

**TABLE III**—Concentration of phenolics in Q123 cane and juice.

| Sample | Chlorogenic acid* | Chlorogenic acid isomers* | Total neutral phenolics** | Flavonoids** |
|---|---|---|---|---|
| Cane | 710 | 90 | 310 | 1070 |
| Juice (IEJ) | 180 | 120 | 110 | 380 |

* as mg/kg sugar solids ** as mg sinapoyl glucose/kg sugar solids

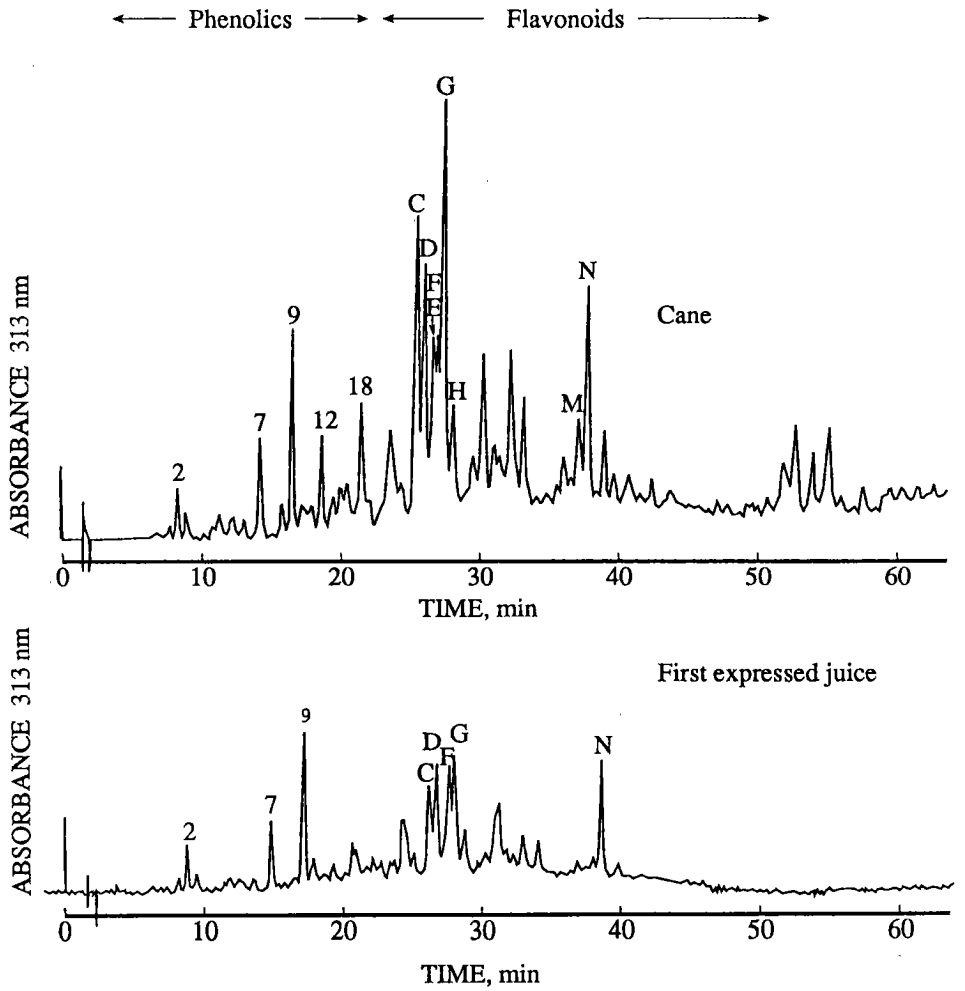



Fig. 3—HPLC profiles of neutral phenolics and flavonoids in Q123 cane and first expressed juice.

**Contribution of browning colourants to the colour of first expressed juice**

Using the colour data for cane and first expressed juice, and assuming that (1) no browning colourants were in the cane extract, and (2) the indicator value of the browning colourants was 1.0, then the amount of colour in cane at pH 4 that was converted to browning colourants may be calculated and hence the browning colourants in juice and percent contribution to colour. With these calculations the mean results for the 1990 season were:

84% of the colour of first expressed juice at pH 4 was due to browning;
53% of the colour of first expressed juice at pH 7 was due to browning.

Furthermore, the colour at pH 4 that was converted to polymeric colour was enhanced by a factor of about 5. It is thought that the enhancement was due to participation of flavonoids in the browning reaction.

The large contribution of high molecular weight colourants to the colour of first expressed juice was confirmed by size exclusion profiles as shown in Figure 2. About 70 percent of the colour of juice at pH 7 was from compounds with molecular size greater than flavonoids.

All juices from the milling train contain flavonoid colourants and colour precursors, and maceration has been found to be a very effective way of extracting colour; in fact the colour:sugar ratio increases along the milling train.

Thus the concentration of flavonoids, in particular the luteolin based flavonoids with an o-diphenol group, are important as they indicate the potential to form enzymic browning colourants. Varieties with a high concentration of both chlorogenic acid and luteolin based flavonoids have the potential to form colour on crushing. It was found that varieties with a high level of luteolin flavonoids also have a high level of flavonoids and therefore a high colour juice such as Q96, whereas varieties with a low level of luteolin flavonoids such as Q124 have a lower colour juice.

**Colour changes at incubation and clarification**

Further colour changes occur during incubation and clarification. It is thought that enzymic oxidation continues until the enzyme is inactivated by heat. The other colour changes that occur are due to the effect of heat and/or slight alkalinity.

Eleven sets of mixed juice (MJ), corresponding evaporator supply juice (ESJ) and raw sugar from 5 mills, were analysed for phenolics and colourants in 1990. Average results are given in Table IV with phenolics calculated on solids basis.

**TABLE IV**—Phenolics and colour in mixed juice, evaporator supply juice and raw sugar.

| Sample | Chlor. acid mg/kg | Cinn. acids mg/kg | Neut. phen. mg/kg | Colour | | | IV |
|---|---|---|---|---|---|---|---|
| | | | | pH 4 | pH 7 | pH 9 | |
| MJ | 60 | 50 | 200 | 9930 | 16200 | 45300 | 4.6 |
| ESJ | 160 | 110 | 130 | 6490 | 14100 | 44900 | 6.9 |
| Raw sugar | 16 | 9 | 23 | 1410 | 2330 | 6430 | 4.6 |

The changes in phenolics showed a consistent trend. Firstly, the concentration of chlorogenic acid in evaporator supply juice was much higher than in mixed juice; this increase was thought to be due to the regeneration of chlorogenic acid from an oxidation product, or other chlorogenic acid complex. Secondly, the concentration of cinnamic acids such as caffeic, p-coumaric and ferulic, was higher in evaporator supply juice than in mixed juice, and the concentration of some neutral

phenolic derivatives was lower. These changes indicated some hydrolysis of phenolic esters to free acids during clarification.

During incubation and clarification a small percentage of factory colourants could form such as polymers from the alkaline degradation products of reducing sugars, and oxidation products of phenolics. However, clarification also removed some of the high molecular weight colourants. The net result of incubation and clarification was that the colour of evaporator supply juice was always lower at pH 4 than the corresponding mixed juice and it had a higher indicator value. Despite the reduction of high molecular weight colourants in clarification this type of colourant still contributed over 60 percent of the colour of typical evaporator supply juice as shown in Figure 2.

**Colour changes at crystallisation**

As the brix increases during evaporation more significant colour formation occurs due to the formation of melanoidins from the Maillard reaction between amino acids and carbonyl compounds such as reducing sugars. Colour formation increases with increase in temperature, and factors such as time, amino nitrogen concentration, pH and percent reducing sugars also influence the reaction. Colour formation from oxidation of phenolic compounds should continue during evaporation, although this type of oxidation is considered to be very much slower than enzymic oxidation.

The crystallisation step partitions the colourants between crystal and liquor, and it is an efficient means of removing colour. It was found that colourants were included in the crystal to varying degrees. The partition coefficient or ratio of the colour of massecuite:colour of sugar or the ratio colour of liquor:colour of sugar indicates the extent of inclusion; a high partition coefficient indicates low inclusion in the crystal.

Using the same principle the partition coefficient was calculated for the main phenolic and flavonoid peaks in HPLC profiles of evaporator supply juice and corresponding raw sugar. The acidic phenolics such as chlorogenic acid had the highest partition coefficient, then flavonoids, and the neutral phenolics had the lowest coefficient.

The partition of phenolics and flavonoids between syrup and crystal was confirmed when a high DI raw sugar was centrifuged at high speed. The HPLC profiles of phenolics and flavonoids in the sugar and syrup showed that the syrup had over half the chlorogenic acid, cinnamic acids and flavonoids but only 30 percent of the neutral phenolics. The syrup was approximately 2 percent by mass of the original raw sugar.

Model crystallisation tests showed that enzymic browning colourants were included in the crystal more readily than flavonoids and acidic phenolics. Previously it had been found that synthetic alkaline degradation products and melanoidins had higher partition coefficients than cane juice colourants (Paton *et al.*, 1991). Furthermore, in the beet industry most of the colour of the massecuite is attributed to melanoidins and the partition coefficient is high, which is evidence of low inclusion of these colourants in the crystal. If the high molecular weight colourants are classified as neutral or acidic, and they follow the trend of phenolics, then it is deduced that neutral polymeric colourants are included in the raw sugar crystal more readily than acidic polymers.

The indicator value of raw cane sugar is always lower than that of the massecuite and this shows that a higher proportion of high molecular weight colourants is in raw sugar than in the massecuite. The net result is that about 70 percent of the colour of raw sugar when made is from high molecular weight colourants as shown in Figure 2.

## Colour changes on storage of raw sugar

Australian raw sugar may be stored at a terminal for up to seven months before shipment, and it darkens with time. Colour formation during storage is accompanied by a loss of amino nitrogen and is attributed mainly to the Maillard reaction; the amount of colour formation depends on several factors such as temperature and storage time. In a review of raw sugar in storage Petri and Carpenter (1979) stated that the colour of a poor quality raw stored at 30 °C may double in a year, whereas high quality raws only increase about 50 percent under the same conditions.

Laboratory storage trials of raw sugar showed that colour formation also involved all types of phenolics and was influenced by relative humidity. A typical set of results, for trials done at 35 °C to accelerate colour formation, is in Table V.

**TABLE V**—Phenolics and colour in raw sugar before and after storage at 35 °C.

| Sample | Chlor. acid mg/kg | Cinn. acids mg/kg | Neut. phen. mg/kg | Flav. mg/kg | Colour | | | IV |
|---|---|---|---|---|---|---|---|---|
| | | | | | pH 4 | pH 7 | pH 9 | |
| Before storage | 23 | 12 | 27 | 42 | 2520 | 3740 | 9220 | 3.7 |
| 20 weeks 52% RH | 16 | 6 | 22 | 36 | 4060 | 5320 | 10900 | 2.7 |
| 10 weeks 63% RH | 1 | 1 | 33 | 24 | 4700 | 5880 | 9880 | 2.1 |

The concentration of acidic phenolics, particularly chlorogenic acid and the cinnamic acids decreased significantly with colour formation; flavonoids with an o-diphenol group such as peaks G and H decreased to a lesser extent. The neutral phenolics showed only small changes but several new peaks appeared after storage at 63 percent relative humidity to give an increase in this type of phenolic. The rate of colour formation was influenced considerably by humidity and was much faster at 63 percent relative humidity than at 52 percent.

The oxidation of phenolics is much slower at lower temperatures. For example raw sugar stored at Central Laboratory at ambient temperature for 2 years still had 17–23 mg total chlorogenic acid/kg sugar. However, any colour formation in storage will produce more high molecular weigh colourants in raw sugar.

## Conclusions

Colour formation of various types occurs throughout sugar processing. It involves the formation of high molecular weight colourants from compounds in the cane plant such as phenolic acids, flavonoids, amino acids and reducing sugars. The reactions leading to colour formation include enzymic browning, oxidation of phenolics and the Maillard reaction. High molecular weight colourants are formed initially when cane is crushed and they contribute most of the colour of cane juice, mill process streams and raw sugar.

### Acknowledgements

The author wishes to thank CSR Limited for permission to publish this paper, the Sugar Research and Development Corporation for financial support, and mill staff who provided samples. The contributions of colleagues at Central Laboratory to various aspects of this project are gratefully acknowledged.

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