

RECENT PROGRESS IN SUGAR COLOURANTS: GC-MS STUDIES AND EXTRACTION TECHNIQUES

MA GODSHALL

Sugar Processing Research Institute, Inc., 1100 Robert E Lee Blvd, New Orleans, LA 70124, United States of America

Abstract

The study of colour and colour precursors in cane sugar has been an important research area at Sugar Processing Research Institute for many years. Previous studies on isolating colourant precursors have relied on various liquid-liquid extraction procedures, time consuming methods requiring large amounts of sample and solvent. Four methods were examined for the identification of raw sugar colourants: Micro-extraction cartridges containing either strong anion exchange resin (SAX) or C-18, Empore-SDB membranes, liquid-liquid extraction, and methanol/ethyl acetate extraction of crystalline raw sugar. Of these methods, SAX and methanol/ethyl acetate gave the most useful information. Colourants and colourant precursors in raw sugars from various sources were examined by gas chromatography and mass spectrometry. Phenolic acids as well as sugar degradation products were observed.

Introduction

The understanding and control of colour in sugar processing is a constant challenge for the sugar industry. Over the decades, researchers have commented on the complexity of cane sugar colour. In 1947, Zerban stated, "The darkening of sugar products during processing and storage at elevated temperatures is one of the oldest problems of sugar manufacture and makes itself felt in practically all other industries that use these products." (Zerban, 1947). In 1967, Gross made the statement, "The challenges of the highly complex question of the nature of sugar colour has been with the industry for a very long time." (Gross, 1967). In 1971, Smith and Gregory wrote, "Sugar colour with its associated impurities and precursors is a complex mixture of diverse composition and is difficult to describe in practical terms." (Smith and Gregory, 1971).

Research on sugar colour has been ongoing for a long time, and it continues to be a complex subject with many ramifications. The knowledge gained has been incremental and has come from numerous sugar producing areas, encompassing all aspects of the process, starting with the colouring matter that is introduced by the cane plant and continuing through reactions that occur in the mill and the refinery and on into storage.

Sugar colourants are broadly classified as those that originate in the plant and those that are formed in the process (Clarke *et al.*, 1984). However, interactions between the two types complicate the overall picture.

Colourants that originate from the sugarcane plant include true pigments such as chlorophyll, anthocyanins and flavonoids. Chlorophyll, being water insoluble, is easily removed, producing minor brown degradation products that are found in raw sugar (Roberts, 1980). Anthocyanins, the red pigments, are also a minor component which are largely removed or destroyed in clarification, but a small amount is found in polymeric colourant in raw sugar (Godshall and Grimm, 1994). Flavonoids include a wide range of com-

pounds that are in the yellow range, and which may react further to form highly coloured polymeric colourant.

An important group of plant-derived colourants and colourant precursors includes the many polyphenolic compounds, benzoic and cinnamic acid derivatives, which range from colourless to yellow, darkening with alkaline pH and able to produce highly pigmented iron complexes. The phenolics are reactive and easily oxidized. Colour formation involving enzymatic polymerization of polyphenolics in juice is estimated to be a major source of colouring material in both cane and beet processing (Gross and Coombs, 1976a, 1976b; Paton, 1992b).

Amino acids and reducing sugars are not colourants, but their presence and the Maillard reactions they undergo contribute to colour formation in processing.

Most process-derived sugar colourant is polymeric and has a high molecular weight; it is subdivided into several categories: (a) caramels, thermal degradation products of sucrose; (b) melanoidins, Maillard reaction products of α -amino acids and reducing sugars; (c) alkaline degradation products of fructose, similar to caramels; (d) melanin, reaction products of amino acids with phenolics as well as the very dark enzymatic oxidation products of phenolics. (These tend to be more common in beet colourant than cane.)

Experimental

Raw sugars were obtained from sponsoring companies of the Sugar Processing Research Institute, Inc. The geographic origin, colour and date of production of the six sugars examined are listed.

Origin	Date Produced	pH	Colour
Fiji	9/95	5,91	2 227
Louisiana	11/95	6,55	2 812
Guatemala	8/94	5,97	2 685
Dominican Republic	6/94	6,10	2 978
Mozambique	9/94	5,52	6 667
Brazil	11/94	4,65	11 972

Solid phase extraction. Three solid phase extraction media were examined:

- (1) Maxi-Clean® (Alltech) solid phase extraction cartridges containing a reverse phase packing (C18);
- (2) Maxi-Clean® (Alltech) solid phase extraction cartridges containing a strong anion exchange packing (SAX);
- (3) Empore®-SDB membranes embedded with styrenedivinylbenzene.

The hydrophilic C18 packing is expected to extract moderately polar to non-polar compounds from a polar matrix, and the SAX packing is expected to remove acidic compounds from an aqueous solution. The Maxi-Clean® cartridge bed size was 600 mg, rated as being able to remove up to 6 mg of material. The completeness of extraction, however, depends on how strongly a compound is retained and the presence of other components in the mixture. The Empore®-SDB mem-

brane is composed of an aromatic matrix, which has an affinity for aromatic, hydrophilic compounds.

Extraction procedure for SAX cartridges: 3 g sugar was dissolved in 20 ml water, the solution was filtered on a bed of analytical filter aid, and passed through a SAX cartridge. It was not necessary to condition the SAX cartridges. The solution was passed sequentially through two cartridges. If a significant amount of colour remained in the solution after passage through two cartridges, a third cartridge was used. (Developmental work had shown that 94-95% of all low molecular weight material was extracted in two cartridges, regardless of the amount of colour remaining.) Each cartridge was then washed of sugar and salts with 30 ml water. The acids were eluted from each cartridge by 3 ml 3N formic acid followed by 3 ml water. The pooled eluates were evaporated to dryness. The dried extracts were redissolved in pyridine and an aliquot taken for derivatisation as the trimethylsilyl (TMS) derivative using Tri-Sil Concentrate® (product of Pierce Chemical Company), using trehalose as the internal standard.

Extraction procedure for C18 cartridges: 5 g raw sugar was dissolved in 20 ml water, prefiltered on analytical filter aid and passed sequentially through two methanol/water conditioned C18 cartridges. Each cartridge was washed with 20 ml water and adsorbed compounds eluted with 3 ml methanol followed by 2 ml ethyl acetate. Pooled eluates were evaporated to dryness and the extracts prepared as described above for GC/MS. The effect of acidifying the sugar solution prior to extraction was also examined.

Extraction procedure for Empore-SDB membrane: 25 g raw sugar was dissolved in 50 ml water, filtered on a bed of analytical filter aid, and passed through a conditioned membrane. The membrane was conditioned with methanol followed by water and kept wet. Sugar was washed off the membrane with about 100 ml water. Colour was eluted with 3 ml methanol followed by 3 ml 50% aqueous methanol. The extract was evaporated to dryness and prepared for GC/MS in the manner described above.

Solvent extraction

Liquid/liquid extraction. 25 g raw sugar was dissolved in 100 ml water and the pH adjusted to 2.5 or not adjusted, as required. The solution was extracted with three 50 ml aliquots of ethyl acetate. The extract was dried over anhydrous sodium sulfate overnight, filtered, and evaporated to dryness, and the sample prepared for GC/MS as the TMS derivative.

Methanol/ethyl acetate extraction. The procedure used was a modification of the procedure described earlier (Larrahondo, et al., 1995; Giraldo, 1995). 25 g raw sugar was stirred with 100 ml methanol for 4 hours and then left to soak overnight. The methanolic extract was filtered on a sintered glass filter and evaporated to dryness. The dried residue, which contained a lot of sucrose, was redissolved in 10 ml water, the pH adjusted to 2.5, and extracted with three 20 ml aliquots of ethyl acetate. The ethyl acetate extract was prepared for GC/MS in the manner described above for liquid/liquid extraction.

GC/MS

Separation and identification was accomplished using a Hewlett Packard Series II Model 5890A gas chromatograph coupled to a Hewlett Packard 5972A mass selective detector (MSD). The MSD was equipped with a hyperbolic quadrupole mass filter and an electron impact ion source. The column was 30 m x 0.25 cm with 0.25 µm film thickness, 5% phenyl methyl silicone phase. Various temperature program-

ming regimes were used, depending on the nature of the compounds. Retention times reported for most compounds were obtained using an initial temperature of 100°C; hold for 4 min; increase 4°C/min to 250°C and hold for 10 min.

Compounds were identified on the basis of their mass spectral patterns and retention times. A commercial mass spectral library from Wiley was used for comparison and identification of spectra, along with an SPRI library of standards.

Results and discussion

What is a sugar colourant? The definition of sugar colourant can be extremely broad, with almost any component in a raw sugar that is not sucrose, reducing sugar, or inorganic salt falling into one or another category of colourant. In many instances, an identified compound is not coloured, but it may have the potential of entering into colour-forming reactions or to act as a catalyst for colour-forming reactions.

Most of the compounds identified in this study are not strictly colourants. They are either not coloured or have light shades of yellow. However, because of their nature and abundance, they constitute a source from which colour reactions can occur. The highly coloured components of sugar colourant (dark brown, golden and reddish) have molecular weights >1000 daltons. These high molecular weight colourants cannot be studied by our GC/MS system, which is limited to molecular weights not exceeding approximately 1000 daltons.

The SPRI mass spectral library. Many preliminary identifications were suggested by the Wiley mass spectral commercial library, which contains 138 000 spectra. However, many compounds remained unidentified, and some of the matches obtained with the library spectra were poor. As an adjunct to better identification of components, SPRI created its own library of spectra of known compounds.

As many as possible of the compounds already identified in sugar processing in the literature were entered into the library, as well as chemically related compounds. This has greatly enhanced the certainty of component identification and has had the added advantage of providing retention times, another identification aid. The SPRI mass spectral library now has a little over 200 spectra, which include a wide range of carboxylic acids, sugars (sugar alcohols, monosaccharides, disaccharides, trisaccharides, special derivatives), aldehydes, phenolic acids, many caramel-type flavoring compounds, volatiles, lipids, esters, hydrocarbons, etc. In addition, compounds of fructose degradation and partially characterised compounds found in cane juice, have been entered into the library for purposes of identifying the source of certain compounds and to follow them through the process. The library is an ongoing enterprise, and new compounds are entered whenever they become available and are of interest.

Comparing extraction procedures. The extracts discussed in this study yielded more than 100 individual compounds, including carboxylic acids, polyhydroxyphenolic acids, lactones, alcohols, lipids, furans, glycosides, amino acids, and at least one inorganic acid (phosphoric). Of these, 64 compounds were tentatively or definitely identified, or partially characterised, by their mass spectra.

Empore-SDB membranes extracted most of the sugar colour, but only about half of the retained colour could be recovered. Despite extensive washing, sucrose remained a major interference in the extracts. Except for some beta-sitosterol, the Empore membrane procedure did not yield small molecular weight compounds amenable to GC/MS. The results indi-

cate that these membranes are better suited to examination of high molecular weight colourant, and are not further discussed in this paper.

Tables A and B in the appendix summarise the extraction results. Table A shows the results of five extraction procedures. Table B compares six raw sugars by the methanol/ethyl acetate extraction procedure.

The modified methanol/ethyl acetate method (Larrahondo *et al.*, 1995; Giraldo, 1995), shown in the first two columns of Table A, may be considered a surface extraction procedure. That is, the procedure is more likely to strip the colourants out of the first few layers of the sucrose crystal than accessing the entire crystal. However, since the method calls for stirring the sugar with a magnetic stirring bar for four hours, the sugar crystals are broken into much smaller particles by the procedure. The breaking of the crystals makes more of the in-crystal colourant accessible to the methanol. At the end of the procedure at least two-thirds of the colour has been extracted into the methanolic layer.

The effect of including an acidification step in the methanol/ethyl acetate procedure is especially marked in the extraction of aconitic acid, where 46 ppm was extracted from the acidified solution, but less than 1 ppm was extracted from the same sugar when the solution was not acidified. This effect was confirmed by repeating the extraction of the Fiji raw sugar as well as two other sugars. Other carboxylic acids, such as lactic, malic, succinic, citraconic, etc, showed similar trends. Neutral components (lactic acid lactate, lactones) and less acidic components (ferulic acid, fatty acids) were extracted to about the same extent by both methods.

The results of the acidified ethyl acetate liquid/liquid extraction (third results column of Table A) can be used to interpret how much additional material was inside the crystals. The results showed no new compounds were found exclusively inside the crystal. Several compounds were present in higher concentrations than in the acidified methanol extract, indicating that they were partially occluded in the crystal. Among these were lactic acid, aconitic acid, the unknown (m/z 145) at 7,35 min, citraconic acid, the fructose degradation product at 10,38 min (FDP-1), ferulic acid, 5-hydroxymethyl-2-furancarboxylic acid, resorcinol, and p-hydroxybenzoic acid. The difference in lactic and aconitic acids by the two procedures, however, was not very great. It should be noted that the compounds found inside the crystal also have a tendency to form more colour or to be involved in degradation reactions.

While many constituents were present in approximately the same concentration by both acid liquid/liquid extraction and the surface extraction, a few others were present in considerably lower concentration in the liquid-liquid extract; for example, 2-furan-carboxylic acid, malonic acid, glyceric acid, and erythronic acid. A possible explanation for this is that the concentration of sugar relative to the extracted material by each method was quite different, facilitating better extraction from the surface procedure than the liquid/liquid procedure. In an earlier study, the concentration of sugar was found to be a factor in the efficiency of extraction (Godshall, 1975).

The last two columns of Table A compare solid phases in microcartridges -- a strong anion exchange resin (SAX) and a hydrophobic silica-based reverse phase packing (C18). These extractions also represent the colourant in the entire crystal. The SAX cartridge had a high affinity for most of the carboxylic and fatty acids, with the notable exception of malonic, fumaric and aconitic acids. SAX had little affinity for the

phenolic acids nor for neutral compounds. The yield of quinic acid (23,40 ppm) greatly exceeded that of the other procedures.

Table 1 compares the SAX extract of the Mozambique and Fiji raws with the acidified methanol/ethyl acetate surface procedure for selected compounds. The compounds that are extracted in significantly higher quantity by SAX include malic, aspartic, quinic, palmitic, stearic, citric and gluconic acids as well as glucono-delta-lactone and 5-oxo-proline.

Table 1
Comparison of SAX extraction and acidified methanol/ethyl acetate procedure on selected compounds in two raw sugars (reported as ppm on sugar.)

Compound	Mozambique raw		Fiji raw	
	MeOH/EtAC	SAX	MeOH/EtAC	SAX
Malonic acid	3,14	0,85	2,18	0,50
Succinic acid	5,29	3,08	2,00	1,38
Glyceric acid	2,35	1,20	0,71	2,63
Malic acid	6,33	15,74	5,07	8,69
Aspartic acid	0,11	5,51	0	2,71
5-oxo-proline	1,40	2,95	2,23	2,52
Citric acid	Fru interferences	18,07	Fru interferences	11,43
Aconitic acid	19,07	3,88	45,9	5,77
Quinic acid	1,46	53,25	1,67	23,40
Palmitic acid	2,50	1,29	0,76	1,28
Stearic acid	1,59	3,65	0,60	2,92
Glucono-delta-lactone	0	11,84	0	2,91
Gluconic acid	0,22	25,99	0	6,87

The large concentration of glucono-delta-lactone and gluconic acid obtained by the SAX extraction and the absence of these in the other extraction procedures was noted. We wondered if this was an artefact of the extraction procedure, possibly being formed by the action of formic acid on residual sugars in the extract, as the formic acid was being evaporated off. Several control experiments were conducted. Refined sugar was passed through SAX cartridges and worked up in the usual manner. Neither glucono-delta-lactone nor gluconic acid were observed. In a second control experiment, sugarcane indigenous polysaccharide (ISP) was treated with formic acid in a manner similar to the extracts, also with negative results.

The C18 cartridge had very little affinity for carboxylic acids and minimal affinity for phenolic acids, but it did extract comparable amounts of palmitic and stearic acids. It also extracted two steroids, stigmasterol and sitosterol (not quantitated). The C18 procedure was repeated several times on these and other sugars with similar results each time. Acidifying the sugar solution prior to extraction enhanced extraction, and it is the acidified results that are shown in Table A. Of the five procedures listed, the C18 cartridge gave the least satisfactory results and provided no additional information to supplement the other methods.

Comparison of colourants in six raw sugars. Six raw sugars were compared using the acidified methanol/ethyl acetate extraction procedure. Results are shown in Table B of the Appendix. This method, while putatively a surface-extracting procedure, gave the widest range of components in the highest over-all yields (cf, Table A, Appendix).

The first thing to note is the general over-all qualitative similarity among the sugars. Although they represent a diversity of origins, many of the same compounds were found in

each, the main differences being in relative quantity. The Guatemala, Fiji, Dominican Republic (DR) and Louisiana raws had low colours, in the range of 2 000-3 000 ICU. The Brazil raw had very high colour, low pH and high reducing sugars and had been difficult to decolourise. The Mozambique raw had a moderately high colour but had not been particularly difficult to refine.

Aconitic acid was low in the Brazil raw, and could have been degraded. Aconitic acid in raw sugar can be degraded by heat, with subsequent colour formation. However, the concentration of aconitic acid in cane juice is a function of cane maturity (it is higher in immature cane), and a low concentration does not necessarily indicate its involvement in colour formation. Aconitic acid was also low in the DR sugar, which did not have a colour problem.

The Louisiana raw was generally higher than the other sugars in phenolic acids, especially ferulic, caffeic and chlorogenic acids. It was also higher in some of the plant metabolic acids, such as aconitic, malic, fumaric, and 5-oxo-proline. This may be a reflection of Louisiana's short growing season and the use of varieties that are selected for cold tolerance and early maturation.

A hexanedioic acid ester (the nature of the ester moiety was not determined) was noted in the Guatemala, DR and Louisiana raws. An earlier extract of a very low colour Louisiana raw sugar produced in 1991 (1 306 ICU) had also shown a prominent hexanedioic acid ester peak. The Fiji and Mozambique raw had measurable concentrations of the triphenyl ester of phosphoric acid. The Brazil raw had 0,11 ppm of each. Peaks such as these may be characteristic of certain locations, but more study would be needed.

The effect of heat on raw sugar colourants. The effect on selected compounds in the Fiji raw upon heating for 4 hours at 120°C is shown in Table 2. Colour increased from 2 201 to 8 608 ICU. Notable changes were the large increase in FDP-1 (m/z 273) at 10,38 min, from 0,68 ppm to 37,17 ppm, and the loss of much of the aconitic acid, from 45,9 ppm to 10,6 ppm. Other sugar degradation products that increased significantly were 2-furancarboxylic acid, 3-hydroxy-propanoic acid, glyceric acid, citraconic acid, FDP-2 (m/z 271), lactic acid lactate, 5-hydroxy-methylfurancarboxylic acid, and the lactones at 11,71, 14,14 and 14,20 min. Lactic, malic and erythronic acids remained unchanged.

Table 2

Effect of heat on selected components in a Fijian raw sugar. Method of extraction was acidified methanol/ethyl acetate (reported as ppm)

R.t.	Compound	Not heated	Heated
3.50	Lactic acid	18.02	20.25
3.85	Unk (m/z 73,147,205)	2.92	11.56
4.04	2-furancarboxylic acid	0.13	0.58
4.22	3-OH-propanoic acid	0.70	1.65
8.11	Glyceric acid	0.71	1.59
8.22	Fumaric acid	0.35	3.25
8.36	Citraconic acid	0.13	2.96
10.38	Fructose degrd. product (m/z 273) (FDP-1)	0.68	37.17
11.10	Fructose degrd. product (m/z 271) (FDP-2)	0.03	0.48
11.52	Lactic acid lactate	0.17	1.41
11.71	Lactone (m/z 147, 189, 261)	0.63	4.16
12.49	5-OHMe-2-furan-carboxylic acid	0.25	1.23
14.14	Xylonic acid lactone	0.25	1.25
14.20	2C-methyl ribonic acid lactone	1.40	2.32
16.90	Aconitic acid	45.9	10.60

Discussion of selected groups of compounds

This study has shown that many compounds are present in raw sugar, and the significance of this information poses a question. Why should we be interested in the trace components of raw sugars? The presence of numerous compounds in raw sugar emphasises the complexity of the challenge faced by the sugar refiner. There are many compounds to be removed, each with its own kinetics for removal in process, a tendency to increase or decrease in process, with concomitant colour formation, and affinity for inclusion in the sugar crystal.

The following sections discuss several groups of compounds that are of particular interest in the study of sugar colour.

Fructose degradation products. The degradation reactions of fructose in both acid and alkaline conditions have been studied extensively (Hodge, 1953; Isbell *et al.*, 1969; McWeeny, 1973; Newth, 1951; Shallenberger and Birch, 1975; Shallenberger and Mattick, 1983). The absence of 5-hydroxymethyl-2-furfural (HMF), a major degradation product of fructose under acid conditions and also produced under alkaline conditions, in all the sugars studied and by all extraction methods, was noted. HMF was easily extracted into ethyl acetate from alkaline and acid-degraded fructose solutions prepared in the laboratory, so its absence was not an artefact of the extraction procedures used.

(1) *FDP-1 (Mass 273 compound at 10,38 min).* This compound was present in all but the Mozambique raw sugar. The same compound was noted as a very minor peak in acid degraded fructose prepared in the laboratory and as a moderately large peak in alkaline degraded fructose. Its concentration in the Fiji raw increased more than 50-fold upon heating.

The mass spectrum of FDP-1 (TMS) is shown in Figure 1. A tentative structure is partially hydrated 5-hydroxymethyl-2-furancarboxylic acid, C₆H₈O₄, which would have a TMS molecular weight of 288; the loss of a methyl group (15 amu) would give the prominent m/z 273 peak.

The relative abundance of the major mass fragments were: 73 (100), 101 (12), 116 (6), 129 (9), 133 (7), 147 (29), 155 (22), 183 (23), 217 (2), 245 (5), 273 (51), 288 (4). (The first number is the molecular weight of the mass fragment and the number in parenthesis is the relative abundance as a percentage of the fragment relative to the most abundant mass.)

(2) *FDP-2 (Mass 271 compound at 11,10 min.)* This compound was present in trace quantities in the Guatemala, Brazil, Fiji and Dominican Republic raws and absent in the Mozambique and Louisiana raws. The same compound was noted as a minor peak in both acid and alkaline degraded fructose prepared in the laboratory. Its concentration increased in the heated Fiji raw from 0,03 ppm to 0,48 ppm.

The mass spectrum of the TMS derivative is shown in Figure 2. The TMS molecular weight of this compound is probably 286; the loss of a methyl group (a common pattern in MS of TMS derivatives) from the parent ion would account for the prominent m/z 271 fragment. The empirical formula for this compound is probably C₇H₁₀O₃, but the structure is not known at this time.

The relative abundance of the major mass fragments were: 73 (19), 117 (1), 128 (3), 133 (4), 147 (4), 169 (2), 183 (1), 199 (7), 271 (100), 272 (23), 273 (11), 286 (0,4).

- (3) *HMF dimer*. This compound was noted as a major peak in the colourant extract of a Colombian raw sugar reported previously (Larrahondo *et al.*, 1995; Larrahondo *et al.*, 1996) and also in the extract of a standard beet liquor that had produced white sugar with high colour (Godshall, 1996). It was a major peak in acid degraded fructose prepared in the laboratory but was negative in alkaline degraded fructose. It was not found in the six raw sugars in this study.

The mass spectrum of the TMS derivative is shown in Figure 3, along with a proposed structure. The TMS molecular weight of this compound is estimated to be 452.

The relative mass abundances of the major fragments were: 73 (71), 109 (14), 147 (6), 182 (4), 271 (100), 272 (23), 273 (10), 378 (3), 452 (0,3).

- (4) *Difructose dianhydrides (DFDA)*. In acidic medium, fructose can dehydrate to form a series of at least seven non-reducing dimeric dianhydrides (Hilton, 1963). These have the trivial names diheterolevulosan I, II, III, and IV, and difructose anhydride I, II, and III, abbreviated as DHL and DFA, respectively. Alternate anomeric structures are also possible. Heat alone may produce difructose dianhydrides. The presence of DFDA in raw sugar or any other process sample is another indicator of sucrose loss, due to thermal and/or acidic conditions.

Extraction conditions in this study were not optimised for the examination of DFDA, but traces were noted in the methanolic extract of the Fiji heated sugar. Sucrose interferes with their chromatographic analysis, as it co-elutes, so either further purification steps are necessary or selected ion monitoring by GC/MS should be done in order to analyse them.

Other sugar degradation products. Although fructose is generally recognised as being more reactive than glucose and participating more readily in degradation and colour-forming reactions, glucose also undergoes oxidation and degradation in alkaline media. In the presence of air and calcium hydroxide, glucose, upon oxidation, yields carbon dioxide, formic acid, oxalic acid, arabonic acid, erythronic acid, glyceric acid, glycolic acid and glucic acid (Gortner, 1938). At the turn of the century, Nef (1907, 1914) showed that, in the presence of sodium hydroxide, glucose could react to yield an equilibrium mixture containing at least 93 different compounds.

In the present study, measurable amounts of erythronic acid (trihydroxybutyric acid) and glyceric acid (2,3-dihydroxypropionic acid) were found in the six raw sugars examined.

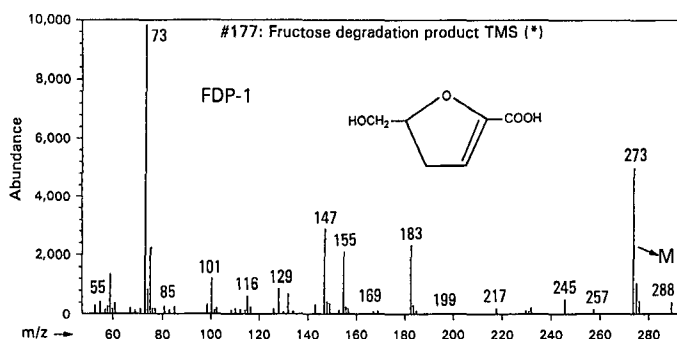


FIGURE 1: Mass spectrum of fructose degradation product (FDP-1) found in raw sugar.

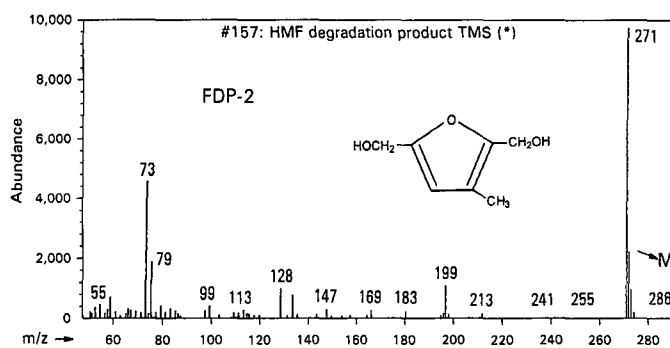


FIGURE 2: Mass spectrum of fructose degradation product (FDP-2) found in raw sugar.

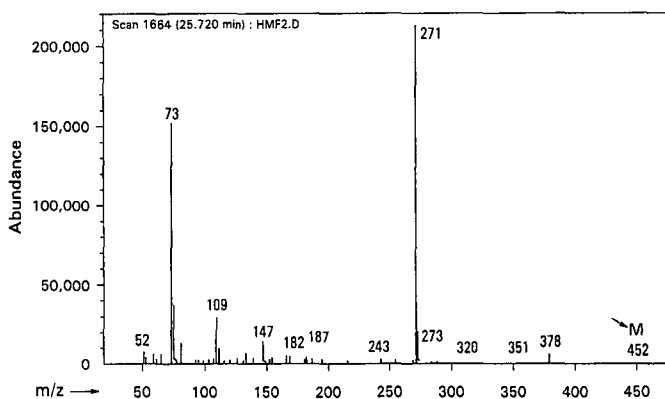
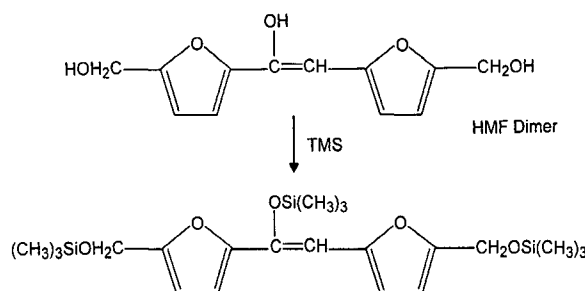


FIGURE 3: Top: Proposed structure of HMF dimer, and its TMS derivative

Bottom: Mass spectrum of the TMS derivative of HMF dimer

Saccharinic acids and saccharinic acid lactones. Saccharinic acids are formed by internal oxidation and reduction of aldomonosaccharides and are commonly formed in alkaline solution (Gortner, 1938; Sowden, 1957). Three forms are recognised, having the trivial names saccharinic acid, isosaccharinic acid and metasaccharinic acid; they are isomeric with their starting sugars, and have a strong tendency to convert to the more stable lactone.

Saccharinic acids have been described in beet sugar processing (deBruijn *et al.*, 1987; Reinefeld *et al.*, 1979).

In the present study, several peaks in the raw sugar extracts appeared to be saccharinic acid lactones, based on their spectral matches with the commercial library. These included four lactones, tentatively identified as the following:

- 11,71 min: 2-deoxy-erythropentono-1,4-lactone
- 14,14 min: xylonic acid gamma lactone
- 14,20 min: 2-C-methyl ribonic acid gamma lactone
- 17,09 min: 3-deoxy-arabino-hexonic acid gamma lactone.

The lactones increased significantly in the heated Fiji raw compared with the unheated sample. Two other tentatively identified isomeric lactones were noted in the heated Fiji raw sugar. These are characterised by an ion at m/z 348 (Ponder and Richards, 1993), and increased about five-fold in concentration when the sugar was heated. The ion chromatograms for these compounds are compared in the heated and unheated sugar in Figure 4. The two peaks are tentatively identified as isomers of 3-deoxy-2-C-hydroxymethyl-D-erythro-pentonic acid gamma lactone.

Many compounds remain to be identified in the raw sugar extracts. Many of the unknown peaks have mass ions characteristic of hexoses and hexose degradation products (m/z 147, 191, 204, 217), indicating that they may represent other closely related glucose and fructose dehydration, rearrangement and degradation products.

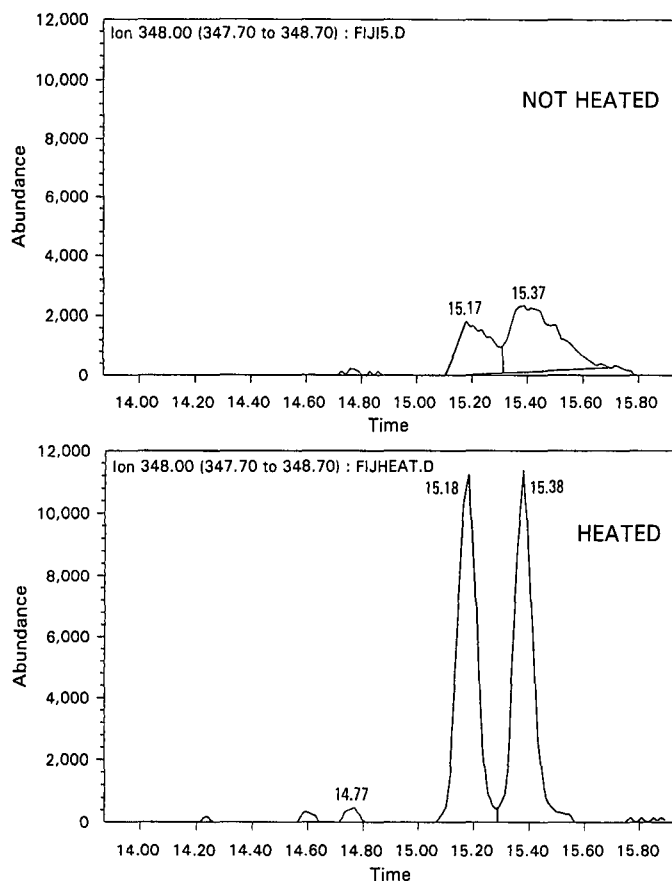


FIGURE 4: Chromatograms of mass ion m/z 348, showing the increase in two tentatively identified lactones in heated Fiji raw (bottom) compared to unheated sugar (top). The lactones are tentatively identified as isomers of 3-deoxy-2-C-hydroxymethyl-D-erythro-pentonic acid gamma lactone.

Phenolic compounds. Phenolics are ubiquitous constituents of cane plants and play a number of important roles in secondary metabolism. They are implicated in enzymatic formation of polymeric colourant and many are found in raw sugar.

Chlorogenic acid and other quinic acid esters. Chlorogenic acid was first reported in sugarcane in 1971 (Farber and Carpenter, 1971; Gross and Coombs, 1971). Chlorogenic acid is the trivial name of 5'-caffeoylquinic acid. At least seven quinic acid esters are found in plants (Trugo and Macrae, 1984), including esters of caffeic acid, ferulic acid, dicaffeic acid and *p*-coumaric acid. Paton (1992a) identified 3'-caffe-

oylquinic acid, 3'-*p*-coumaroylquinic acid and 4'-caffeoylquinic acid in cane juice in Australia, along with chlorogenic acid. Chlorogenic acid and related compounds are important colour precursors in cane sugar processing.

Both chlorogenic acid and quinic acid were identified in all of the raw sugars examined (cf, Table B). The SAX extraction procedure showed that a large amount of quinic acid was present inside the crystal in at least two sugars (53 ppm in Mozambique and 23 ppm in Fiji, cf, Table 1), and it was present as the free acid at about 75-80 ppm on solids in two varieties of cane juice from Louisiana (Godshall, 1996).

Quinic acid is characterised by a major ion at m/z 345 (46%); this ion is also present in a relative abundance of 27% in chlorogenic acid. In this study, the Fiji, Guatemala, DR and Louisiana raws exhibited six other peaks containing m/z 345 ranging in abundance from 12 to 28%, with retention times from 34 to 43 min. These peaks may be other quinic acid esters.

Traces of six of these peaks were present in the high colour Brazil and Mozambique raws, but their very low levels are in marked contrast to the other (low colour) sugars. It is possible that these components have degraded to form colour in the Brazil and Mozambique sugars. Figure 5 shows the seven m/z 345 peaks found in the Fiji raw sugar and contrasts that to the Brazil raw.

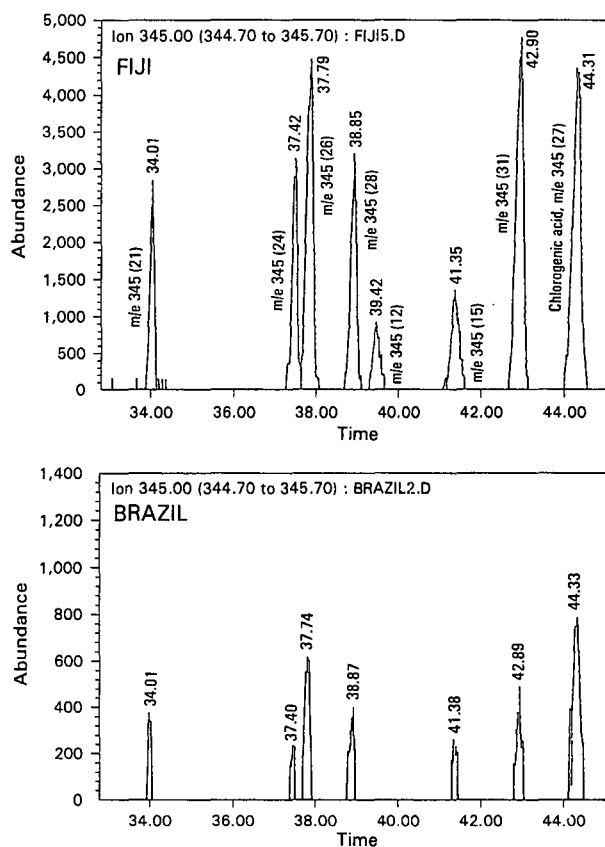


FIGURE 5: Chromatograms of mass ion m/z 345, characteristic of quinic acid and its esters. Top: Fiji raw. Bottom: Brazil raw. Figures in parentheses represent relative abundance of m/z 345.

Phenolic glycosides. Palla (1982, 1983) reported the isolation and identification of several phenyl glycerols (phenyl propanetriols) in cane molasses. We used his reported mass spectra as guides to search for the presence of similar compounds in our extracts. Three sets of compounds were tentatively identified.

(1) **Mass 297 compounds.** The TMS derivative of 3-methoxy-4-hydroxy-phenyl-glycerol (*threo*-form) shows the major mass fragment to be m/z 297 (Palla, 1983). A search for this ion in the MeOH/EtAc extract of the Fiji raw sugar yielded three peaks in which m/z 297 was a major ion. The first peak had a retention time of 20,81 min (m/z 297 = 75%), and was present at a concentration of 0,36 ppm in the Fiji raw sugar, shown in Figure 6. It was also present in trace quantities in the Guatemala, Brazil, Mozambique and DR raws. The Louisiana raw had a concentration of 0,91 ppm. The mass spectrum showed ions typical of glycerol (m/z 103, 117, 133). Its retention time suggests that this is the aglycone, with a TMS/MW of 502. The other two peaks had retention times of 35,73 min (m/z 297 = 100%) and 38,03 min (m/z 297 = 94%). The retention times, as well as the presence of mass fragments characteristic of glucose (m/z 191, 204, 217) in both, suggest that these are mono-glucosides.

(2) **Mass 269 compound.** The TMS derivative of 3-methoxy-4-hydroxy-phenyl-glycerol (*erythro*-form) shows a major mass fragment at m/z 269 (77%) (Palla, 1983). A search for this ion in the MeOH/EtAc extract of the Fiji raw sugar yielded one peak with a retention time of 21,42 min in which m/z 269 represented 44%. This is tentatively identified as the named compound. It was present in low quantities in all of the sugars, with the Louisiana raw having the highest amount at 0,70 ppm.

There were no compounds with higher retention times with a prominent m/z 269 fragment that could be interpreted as glycosides of this compound.

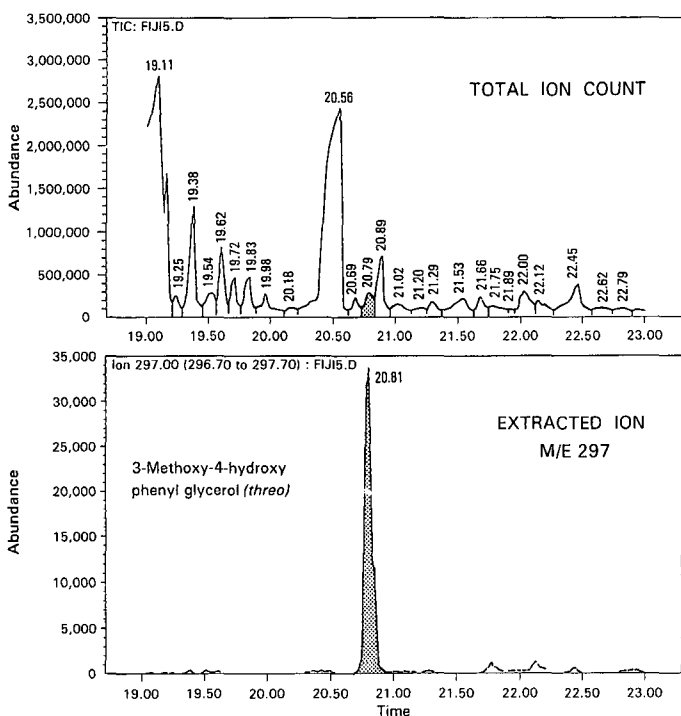


FIGURE 6: Top: Total ion count chromatogram of Fiji raw sugar, showing 3-methoxy-4-hydroxy-phenyl glycerol (*threo*) peak. Bottom: Extracted ion chromatogram for m/z 297, characteristic of the named compound. Use of an extracted ion helps to pinpoint the peak in the chromatogram.

(3) **Mass 327 compounds.** The TMS derivative of 3,5-dimethoxy-4-hydroxy-phenyl-glycerol shows the major

mass fragment to be m/z 327 (100%) (Palla, 1983). A search for this ion in the MeOH/EtAc extract of the Fiji raw sugar yielded three peaks in which m/z 327 was the major ion. The first peak had a retention time of 22,12 min. The mass fragmentation pattern shows ions typical of glycerol (m/z 103, 117, 133). Its retention time suggests that this is the aglycone, with a TMS/MW of 532.

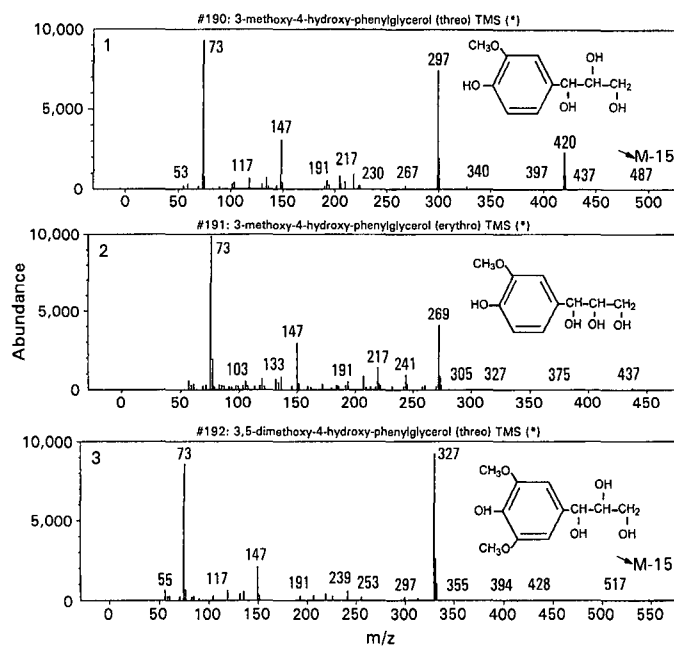


FIGURE 7: Structures and mass spectra of 3 tentatively identified phenyl glycerols.

- (1) 3-methoxy-4-hydroxy phenyl glycerol (*threo*)
- (2) 3-methoxy-4-hydroxy phenyl glycerol (*erythro*)
- (3) 3,5-dimethoxy-4-hydroxy phenyl glycerol (*threo*)

A second peak with retention time of 22,47 had a prominent m/z 327 (32%) ion, but also prominent fragments at m/z 253 (33), 355 (39) and 370 (23), indicating that it is a phenolic compound, but its structure has not been determined.

The other two peaks had retention times of 40,98 and 41,22 (m/z 327 = 100% in both). The retention times, as well as the presence of ions characteristic of glucose (m/z 191, 204, 217) in both, suggest that these are mono-glucosides.

Figure 7 shows the structures of the three phenyl glycerol aglycones discussed above.

Summary

This study has examined the constituents in six raw sugars of various origins, finding that each sugar has well over 100 peaks, representing a wide range of chemical types, including sugar degradation products, plant pigments, colourant precursors and potential catalysts of further sugar breakdown (ie, organic acids). Many of the compounds that remain unidentified may be carbohydrate degradation products, based on the nature of their mass spectra. The sugars show quantitative differences in the amounts of constituents present.

Several extraction methods were examined, and each method, except for the C18 extraction cartridges, was able to provide useful additional information. The SAX cartridges are especially convenient and easy to use, and provide a better quantitative picture of the acidic compounds in the whole crystal than any other method. The methanol/ethyl acetate

method provides information about constituents on the surface of the crystal. Liquid/liquid extraction is still a recommended procedure for the quantitative examination of the general range of sugar constituents. We hope to examine extraction cartridges filled with a form of styrene divinylbenzene, similar to the XAD-2, -4, and -16 resins that have been useful in the past for extracting phenolics and colour. These have only recently become available, and may prove to be the method needed to supplement the SAX information, thereby obviating the need to perform liquid/liquid extractions in the future.

REFERENCES

- Clarke, MA, Blanco, RS and Godshall, MA (1984). Colour tests and other indicators of raw sugar refining characteristics. *Proc Sug Processing Res Conf*, pp 284-302.
- deBuijn, JM, van der Poel, PW, Kieboom, APG, and van Bekkum, H (1987). Reactions of monosaccharides in aqueous alkaline solutions. *CITS*, Vol. 28, pp. 1-25.
- Farber, L and Carpenter, FG (1971). Chlorogenic and caffeic acids identified as colourants in cane sugar. *int Sug J* 73: 99.
- Giraldo, JJ (1995). Compuestos colorados en azucar crudo y en jugos de caña de azucar. Thesis, Universidad del Valle, Facultad de Ciencias, 84 pp.
- Godshall, MA (1996). Recent progress in sugar colourants. *Sug Refining Res Conf* (in press).
- Godshall, MA and Grimm, CC (1994). Leucoanthocyanins, aconitic acid and turbidity in sugarcane. An update on cane colourants. *Proc Sug Processing Res Conf* pp 334-357.
- Godshall, MA (1975). Gas-liquid chromatography of minor constituents in sugars. *Proc 1972 Technical Session Cane Sug Refining Res* pp 93-100.
- Gortner, RA (1938). *Outlines of Biochemistry*. John Wiley & Sons, Inc., London.
- Gross, D (1967). The fractionation and characterization of sugar colour by modern separation methods. *int Sug J* 69: 360-365.
- Gross, D and Coombs, J (1971). Chlorogenic and caffeic acids in sugar cane. *int Sug J* 73: 100.
- Gross, D and Coombs, J (1976a). Enzymic colour formation in beet and cane juices. Part I. *int Sug J* 78: 69-73.
- Gross, D and Coombs, J (1976b). Enzymic colour formation in beet and cane juices. Part II. *int Sug J* 78: 106-109.
- Hilton, HW (1963). Di-D-fructose dianhydrides. In: *Methods in Carbohydrate Chemistry*, Vol. 2, Chapter 50. RL Whistler, ML Wolfrom and JN BeMiller (Eds). Academic Press, New York, pp 199-203.
- Hodge, JE (1953). Chemistry of browning reactions in model systems. *J Agric Food Chem* Vol. 1, pp 928-943.
- Isbell, HS, Frush, HL, Wade, CWR and Hunter, CE (1969). Transformations of sugars in alkaline solutions. *Carbohydr Res* Vol. 9, pp 163-175.
- Larrahondo, JE, Giraldo, JJ, Godshall, MA and Clarke, MA (1995). Separation and GC/MS identification of Colombian raw sugar constituents. *Proc int Soc Sug Cane Technol* (in press).
- Larrahondo, JE, Godshall, MA and Clarke, MA (1996). Separation and identification of constituents of Colombian raw sugar. *Proc Sug Processing Res Conf* (in press).
- McWeeny, DJ (1973). Carbohydrates in nonenzymic browning. In: *Molecular Structure and Function of Food Carbohydrates*. GG Birch and LF Green (Eds). John Wiley & Sons, New York, pp 21-31.
- Nef, JU. Dissociationsvorgänge in der Zuckergruppe. *Ann*, 357: 214-312 (1907) and *Ann*, 403: 204-383 (1914).
- Newth, FH (1951). The formation of furan compounds from hexoses. *Adv Carb Chem* Vol. 6, pp 83-106.
- Palla, G (1982). Isolation and identification of phenolic glucosides in liquid sugars from cane molasses. *J Agric Food Chem* 30: 764-766.
- Palla, G (1983). Characterization of the main secondary components of the liquid sugars from cane molasses. *J Agric Food Chem* 31: 545-548.
- Paton, NH (1992a). Sugar cane phenolics and first expressed juice colour. Part I. Determination of chlorogenic acid and other phenolics in sugar cane by HPLC. *int Sug J* 94: 99-108.
- Paton, NH (1992b). The origin of colour in raw sugar. *Proc Aus Soc Sug Cane Technol* 14: 8-17.
- Ponder, GR and Richards, GN (1993). Pyrolysis of inulin, glucose, and fructose. *Carb Res* 244: 341-359.
- Reinefeld, E, Bleisener, KM, Kowitz-Treynhagen, P and Brandes, E (1979). Studies on acid formation and elimination in technical sugar juices. I. Formation and detection of saccharinic acids. *Zuckerind* 104: 504-510.
- Roberts, EJ (1980). internal SPRI report.
- Shallenberger, RS, and Birch, GG (1975). *Sugar chemistry*. pp 169-193, Van Nostrand Reinhold/AVI, New York.
- Shallenberger, RS and Mattick, LR (1983). Relative stability of glucose and fructose at acid pH. *Food Chem* 12: 159-166.
- Smith, P and Gregory, PE (1971). Analytical techniques for colour studies. *Proc int Soc Sug Cane Technol* 14: 1415-1425.

- Sowden, JC (1957). The saccharinic acids. *Adv Carb Chem* 12: 35-79.
- Trugo, LC and Macrae, R (1984). A study of the effect of roasting on the chlorogenic acid composition of coffee using HPLC. *Food Chem* 15: 219-227.
- Zerban, FW (1947). The colour problem in sucrose manufacturing. Technological Report Series No. 2, Sugar Research Foundation, Inc., New York.

APPENDIX: Table A

Comparison of extraction procedures on amount of components extracted from a Fijian raw sugar (reported as ppm on sugar).

R.t. (min)	Compound	MeOH/EtAc not acidified	MeOH/EtAc acidified	EtAc/H ⁺ liq/liq	SAX cartridge	C18 cartridge
3.50	Lactic acid	1,98	18,02	22,29	55,45	7,36
3.85	Unk (m/z 73,147,205)	0,51	2,92	1,19	10,50	0,59
4.04	2-furancarboxylic acid	0	0,13	0,06	0	0
4.22	3-OH-propanoic acid	0,15	0,70	0,55	1,87	0
5.38	Malonic acid	0,02	2,18	0,66	0,50	0,064
5.96	3-or-4-Me-2-OH-pentanoic acid	0,006	0,08	0,14	0	0,04
6.02	3-or-4-Me-2-OH-pentanoic acid	0,004	0,13	0,14	0	0,04
6.08	Benzoic acid	0,009	0,12	0,12	trace	0,04
6.85	Glycerol	0,92	1,01	0,37	1,28	0
6.90	Phosphoric acid	trace	0,10	trace	1,73	1,96
7.35	Unknown (m/z 145)	2,61	3,17	6,19	0	0,11
7.63	Succinic acid	0,14	2,00	1,26	1,38	0,08
8.11	Glyceric acid	0,05	0,71	0,37	2,63	0,07
8.22	Fumaric acid	trace	0,35	0,41	trace	trace
8.36	Citraconic acid	0	0,13	0,32	0	0
9.34	Resorcinol	0,10	0,09	0,30	0	0
10.38	Fructose degrd. product (m/z 273) (FDP-1)	0,64	0,68	1,53	0	trace
11.10	Fructose degrd. product (m/z 271) (FDP-2)	0	0,03	0	0	0
11.50	Malic acid	0,13	5,07	4,77	8,69	0,07
11.52	Lactic acid lactate	0,14	0,17	0,71	trace	0
11.71	Lactone (m/z 147, 189, 261)	0,60	0,63	0,65	0	0
11.91	5-oxo-Proline	0,02	2,23	1,24	2,52	0,04
11.94	Aspartic acid	0	0	0	2,71	0
12.49	5-OHMe-2-furan-carboxylic acid	0	0,25	0,85	0	0
12.60	Erythronic acid	0,007	0,58	0,03	2,28	0,04
12.91	C4 acid	0	0,13	0	0,27	0
13.80	p-OH-Benzoic acid	0,58	0,87	1,31	0	0
14.03	3-OH-Benzeneacetic	0	0	0	0	0
14.14	Xyloic acid lactone	0	0,25	0	0,67	0
14.20	2C-Mc-Ribonic acid lactone	0,58	1,40	0	1,51	0
16.38	Vanillic acid	0,61	*	*	0	0,09
16.90	Aconitic acid	0,88	45,9	56,76	5,77	0,25
17.46	3,4-di-OH-benzoic	trace	trace	trace	0	0
18.04	Citric acid	0	**	**	11,43	0
18.50	Quinic acid	1,06	1,67	0,24	23,40	0,19
18.72	Gluconolactone	0	0	0	2,91	0
18.73	Syringic acid	1,50	3,18	0,68	0	0,66
19.27	p-OH-Cinnamic acid	1,08	1,32	1,37	0	0,46
19.48	Cetyl alcohol	0,23	0,41	0,59	0	0,11
20.71	Gluconic acid	0	0	0	6,87	0
20.83	Unknown (m/z 297) ⁽¹⁾	0,34	0,36	0	0	trace
20.87	Palmitic acid	0,96	0,76	0,86	1,28	0,88
21.41	Unknown (m/z 269) ⁽²⁾	0,22	0,13	0	0	trace
21.64	Ferulic acid	0,39	0,26	0,45	0	0,13
22.12	Unknown (m/z 327) ⁽³⁾	0,30	0,16	0,34	trace	0,25
22.38	Caffeic acid	0	trace	0	0	0
22.46	Unk (m/z 253,327,355,370)	0,77	0,90	0,79	0	0,13
23.30	9,12-octadecadienoic	0,29	0,31	0,48	0,12	0,13
23.37	Oleic acid	0,58	0,60	0,40	0,16	0,15
23.72	Stearic acid	1,12	0,47	0,38	2,92	1,53
24.85	Unknown (m/z 267) ⁽⁴⁾	0,02	0,34	0,71	0	0
25.18	Unknown (m/z 267) ⁽⁴⁾	trace	0,47	0,65	0	trace
25.94	Hexanedioic acid ester	0	0	0	0	trace
25.95	Phosphoric acid triphenyl ester (m/z 326)	0,56	0,74	0	0	0
27.78	Phthalate	2,70	2,08	1,41	9,03	1,61
35.73	Glucoside of (1) (m/z 297)	0,16	0,02	0,21	0	trace
38.03	Glucoside of (1) (m/z 297)	0,08	0,23	0,33	0	trace
40.98	Glucoside of (3) (m/z 327)	0,08	0,32	0,40	trace	trace
41.22	Glucoside of (3) (m/z 327)	trace	0,11	0,10	trace	trace
42.62	Unknown (m/z 467)	trace	0,20	0	0	0
42.86	Chlorogenic isomer	trace	0,27	0,06	0	0,23
44.29	Chlorogenic acid	trace	0,27	0,06	0	trace

* Interference by aconitic acid

** Interference by fructose

C18 also extracted the steroids, stigmasterol and sitosterol.

⁽¹⁾ Tentatively identified as 3-methoxy-4-hydroxy-phenyl-glycerol aglycone (*threo*-form) (Palla, 1983)

⁽²⁾ Tentatively identified as 3-methoxy-4-hydroxy-phenyl-glycerol aglycone (*erythro*-form) (Palla, 1983)

⁽³⁾ Tentatively identified as 3,5-dimethoxy-4-hydroxy-phenyl-glycerol aglycone (Palla, 1983)

⁽⁴⁾ Structurally similar to 4-hydroxy-phenyl-(alpha-hydroxy) acetic acid

APPENDIX: Table B

Components in raw sugars obtained by methanol/ethyl acetate extraction (reported as ppm on sugar)

R.t. (min)	Compound	Guate	Brazil	Fiji	Mozamb	D.R.	La.
3.50	Lactic acid	7,44	16,78	18,02	21,64	9,98	8,69
3.85	Unk (m/z 73, 147, 205)	2,97	8,27	2,92	7,44	4,46	2,78
4.04	2-furancarboxylic acid	0,23	0,30	0,13	0,15	0,15	0,37
4.22	3-OH-propanoic acid	1,23	4,23	0,70	2,27	1,78	0,89
5.38	Malonic acid	1,65	0,15	2,18	3,14	2,20	2,49
5.96	3-or-4-Me-2-OH-pentanoic acid	0,14	0	0,08	0,08	0,14	0,40
6.02	3-or-4-Me-2-OH-pentanoic acid	0,19	0,69	0,13	0,26	0,15	0,51
6.08	Benzoic acid	0,15	0	0,12	0,24	Trace	0,46
6.85	Glycerol	1,32	2,49	1,01	1,61	1,12	0,87
6.90	Phosphoric acid	0,13	Trace	0,10	0,16	0,11	0,09
7.35	Unknown (m/z 145)	2,39	2,93	3,17	1,01	3,04	2,96
7.63	Succinic acid	3,07	4,67	2,00	5,29	2,68	4,14
8.11	Glyceric acid	1,28	2,98	0,71	2,35	1,28	0,55
8.22	Fumaric acid	0,55	0,18	0,35	0,18	0,30	1,92
8.36	Citraconic acid	0,29	0,004	0,13	0,09	0,09	0,98
9.34	Resorcinol	0,17	0,33	0,09	0,53	0,16	0,21
10.38	Fructose dehydr. prod. (m/z 273) (FDP-1)	0,38	0,33	0,68	0	0,66	1,47
11.10	Fructose dehydr. prod. (m/z 271) (FDP-2)	trace	0,09	0,03	0	trace	0
11.50	Malic acid	6,11	4,77	5,07	6,33	6,17	9,22
11.52	Lactic acid lactate	0,30	0,56	0,17	1,40	0,33	0
11.71	Lactone (m/z 147, 189, 261)	1,41	3,29	0,63	1,80	2,16	0
11.91	5-oxo-Proline	1,62	1,34	2,23	1,40	1,14	4,01
12.49	5-OHMe-2-furan-carboxylic acid	0,45	2,51	0,25	0,48	0,51	0,26
12.60	Erythronic acid	0,34	0,72	0,58	0,16	0,23	trace
12.91	C4 acid	0,19	0,21	0,13	trace	0,04	trace
13.80	p-OH-Benzoic acid	0,56	0,84	0,87	1,17	1,00	1,58
14.03	3-OH-Benzeneacetic	0,08	0,11	0	0,17	0,10	0
14.14	Xylopic acid lactone	0	0,86	0,20	0,37	0,42	0
14.20	2-C-Me-Ribonic acid lactone	0	0	1,40	0	0	0
16.38	Vanillic acid	*	0,90	*	*	*	*
16.90	Aconitic acid	33,04	2,54	45,9	19,07	8,30	86,78
17.46	3,4-di-OH-benzoic	1,35	2,58	trace	1,90	trace	trace
18.04	Citric acid	2,90	trace	**	**	**	3,81
18.50	Quinic acid	1,12	1,63	1,67	1,46	1,65	3,20
18.73	Syringic acid	1,19	1,56	3,18	1,81	2,34	2,61
19.27	p-OH-Cinnamic acid	0,49	0,15	1,32	0,42	0,97	2,82
19.48	Cetyl alcohol	0,34	trace	0,41	trace	0,17	0,42
19.77	3,4,5-tri-OH-Benzoic	0,05	0,09	0	0	0,10	0,28
20.57	Unknown (m/z 287)	0,25	0	0	0	0,24	0,19
20.71	Gluconic acid	0,13	0,09	0	0,22	trace	trace
20.83	Unknown (m/z 297) ⁽¹⁾	trace	trace	0,36	trace	trace	0,91
20.87	Palmitic acid	1,95	2,54	0,76	2,50	1,35	1,64
21.41	Unknown (m/z 269) ⁽²⁾	0,14	0,14	0,13	trace	0,21	0,70
21.64	Ferulic acid	0,08	0,08	0,26	0,15	0,35	0,88
22.12	Unknown (m/z 327) ⁽³⁾	0,05	0,24	0,16	trace	0,23	0,70
22.38	Caffeic acid	0,03	trace	trace	trace	0,23	0,90
22.46	Unknown (m/z 253, 327, 355, 370)	0,28	0,46	0,90	0,76	0,65	0,98
23.30	9,12-octadecadienoic	1,63	1,46	0,31	0,81	0,63	0,87
23.37	Oleic acid	0,83	0,70	0,30	0,63	0,39	0,64
23.72	Stearic acid	0,60	1,26	0,60	1,59	0,61	0,76
24.85	Unknown (m/z 267) ⁽⁴⁾	0,11	trace	0,34	trace	0,29	0,59
25.18	Unknown (m/z 267) ⁽⁴⁾	0,25	trace	0,47	trace	0,26	1,14
25.71	Unknown (m/z 239)	0,10	0	0	trace	0,09	0,57
25.94	Hexanedioic acid ester	4,23	0,11	0	trace	3,97	2,80
25.95	Phosphoric acid, tri-phenyl ester (m/z 326)	0	0,11	0,74	1,18	0	0
27.78	Phthalate	2,54	1,00	2,08	0,47	2,37	2,12
35.29	Unknown (m/z 439)	0,11	0,05	0	0,14	0,09	1,27
35.73	Glucoside of (1) (m/z 297)	trace	trace	0,16	trace	0,13	0,49
38.03	Glucoside of (1) (m/z 297)	trace	trace	0,23	trace	0,41	0,37
40.98	Glucoside of (3) (m/z 327)	trace	0,02	0,32	trace	0,22	0,16
41.22	Glucoside of (3) (m/z 327)	0,11	0,01	0,11	trace	0,25	0,07
42.62	Unknown (m/z 467)	0,66	0,24	0,20	0,32	0,47	2,44
42.86	Chlorogenic isomer	0,18	0,03	0,27	0,08	0,52	0,51
44.29	Chlorogenic acid	0,27	0,07	0,27	0,24	0,32	1,41

* Interference by aconitic acid

** Interference by fructose

⁽¹⁾ Tentatively identified as 3-methoxy-4-hydroxy-phenyl-glycerol aglycone (*threo*-form) (Palla, 1983)⁽²⁾ Tentatively identified as 3-methoxy-4-hydroxy-phenyl-glycerol aglycone (*erythro*-form) (Palla, 1983)⁽³⁾ Tentatively identified as 3,5-dimethoxy-4-hydroxy-phenyl-glycerol aglycone (Palla, 1983)⁽⁴⁾ Structurally similar to 4-hydroxy-phenyl-(α -hydroxy) acetic acid