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Stable carbon isotope ratio analysis of Australian orange juices

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Abstract

Data are presented for stable carbon isotope ratio analysis on Australian orange juices (273 fresh and 80 concentrated samples) representing production over a five year period, with a view to establishing a database of authentic values to be used for adulteration testing. Peel extracts of Australian origin (40 samples), Brazilian pulpwashes (38 samples) and Brazilian concentrates (42 samples) were also tested. The internal correlation between carbon isotopes in sugars and acids has been determined for selected samples of fresh juice. Differences between these components ranged from 0.7 to 1.5‰. The data for fresh juices show regional differences in isotopic abundances, which are related to rootstock.

Keywords: Stable carbon isotope ratio analysis (SCIRA); Orange juice; Orange peel extract

1. Introduction

Stable carbon isotope ratio analysis (SCIRA) is widely applied to authenticity testing of fruit juices such as orange and apple juice. There is a marked difference between the carbon-13 to carbon-12 ($^{13}C/^{12}C$) ratios of juice carbohydrates and those in common adulterants such as cane sugar and corn syrup. Photosynthesis in the orange follows the Calvin-Benson (C_3) metabolic pathway whilst cane and corn utilise the Hatch-Slack (C_4) metabolism. A number of reviews of SCIRA appear in the literature (see for example Krueger, 1988, 1995).

Variations in the range of $^{13}C/^{12}C$ ratios in plant carbohydrates are quite large even within a botanical species. Consequently, in the majority of cases where juices are tested for authenticity, small amounts of sugaring are unlikely to affect the $^{13}C/^{12}C$ ratio sufficiently to take it outside the range of genuine products. This has detracted from the effectiveness of the SCIRA technique when it is applied to the total carbohydrate content of juices. Until recently, laboratories have had to rely on a probability statement based on the number of standard deviations that the $^{13}C/^{12}C$ content of a sample under investigation lies from the population mean for authentic juice of the same type (Doner, 1988). However, there is little probative value in this approach unless the same brand consistently returns an unlikely delta value.

Variations in $^{13}C/^{12}C$ ratios for different plant sugars are due to a combination of environmental and biochemical fractionations which occur in the plant leaf during photosynthesis (O'Leary, 1993). First, the diffusion of carbon dioxide to the carboxylation site is affected by the extent that plants vary the aperture of their leaf stomata to control water loss. Essentially, isotopic discrimination against ^{13}C is positively related to water availability, so that high water availability leads to proportionately less ^{13}C. Secondly, the carboxylation reaction itself contributes to isotopic differences, because different enzymes (ribulose-1,5-biphosphate carboxylase/oxygenase and phosphoenolpyruvate carboxylase) are used by orange and cane or corn, respectively, to fix carbon dioxide (Farquhar & Lloyd, 1993).

The discovery of a correlation between $^{13}C/^{12}C$ content of the sugars and other sub-fractions of juice has led to a significant improvement in SCIRA's diagnostic power. The difference between $^{13}C/^{12}C$ values of total sugars and either pulp or organic acids is fairly consistent, with the sugars containing slightly less of the

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heavier isotope than other sub-fractions (Association Interprogressionelle des Jus et Nectars de la CEE [AIJN], 1997; Jamin et al., 1998; Rossmann, Reith & Schmidt, 1995). The addition of cane or corn sugar will affect this difference, making the test for internal correlation useful in countries where pulp or citric acid is not a permitted additive. More recently, it has been reported (Hammond, 1998) that the internal ratio of natural sugars (sucrose, glucose and fructose) in juice is affected by cane sugar addition. This has further strengthened the effectiveness of SCIRA, albeit at an economic cost, because sugars must be separated by preparative liquid chromatography and analysed individually.

This paper reports on an investigation into $^{13}C/^{12}C$ ratios in fresh and concentrated Australian orange juices, Australian peel extract and imported pulpwash and orange juice. The work was carried out to establish a local orange juice database to be used in authenticity testing. Since the unlabelled addition of food acids, such as citric acid, to orange juice is not permitted in Australia, the relationship between $^{13}C/^{12}C$ of sugars and organic acids can be used to detect additions of cane sugar. To establish data which could be useful for this purpose, we decided to test a limited number of juices having unusually high or low $^{13}C/^{12}C$ values.

2. Materials and methods

2.1. Nature of the orange juice samples

The Australian fresh orange juice survey covered two programs during the years 1992–1997. Samples were either of Navel or Valencia type. Sampling was conducted by Department of Agriculture field inspectors who collected batches of whole fruit weighing approximately 5 kg. A total of 273 samples were taken between 1992 and 1997 from all of the Australian growing regions except the Northern Territory. Sampling locations included New South Wales Riverina (MIA: Stanbridge, Hillston, Lake Wyangang, Tharbogang, Leeton, Griffith, Beelbangera, Yanco and Hanwood), Victorian Sunraysia region (SUN: Cùrlwaa, Dareton, Mildura, Redcliffe, Irymple and Monak), South Australian Riverland (SA: Cooltong, Paringa, Renmark, Berri, Loxton, Waikerie, Ramco and Cadell), Western Australia (WA: Gin Gin and Chittering) and Queensland Burnett region (QLD: Mundubberah, Gayndah). Samples from New South Wales Coastal regions (COAST: Gosford, Macleay Valley and Clarence River area) were gathered by the principal author. Fig. 1 shows the location of these production areas.

Fruit was taken from trees which had been vegetatively propagated by grafting or budding Navel and Valencia varieties onto rootstocks such as Trifoliata [*Poncirus trifo1iata* (L.) Raf.], Troyer and Carizzo

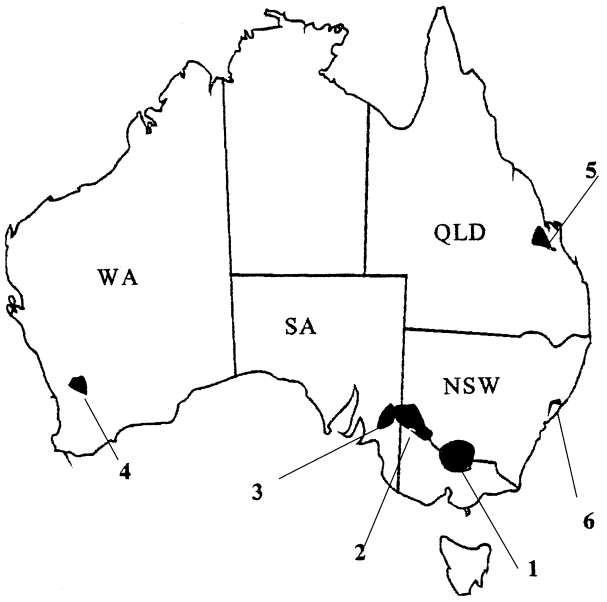

Fig. 1. Principal orange growing areas in Australia: 1 = Riverina-Murrumbidgee Irrigation Area (MIA); 2 = Sunraysia (SUN); 3 = Riverland (RIV); 4 = Chittering River (WA); 5 = Burnett District (QLD); 6 = NSW Coastal (COAST).

citrange [*C.sinensis x P.trifoliata*], sweet orange [*Citrus sinensis* (L.) Osbeck], Cleopatra mandarin [*Citrus reticulata* Blanco], citrumelo [*C. paradisi x P. trifoliata*] and rough lemon. The main rootstocks for the RIV region were sweet orange and rough lemon, because of their tolerance to chloride, whilst the trifoliata and citrange varieties predominated in MIA and WA because of their tolerance to heavier soils and resistance to root rot. Rootstock details were recorded on samples surveyed after 1994.

The fresh orange samples were shipped to the laboratory and squeezed within four days of picking. Juice was extracted with a domestic reamer (either hand or electrical), strained to remove seeds and stored in labelled 2 l polyethylene bottles in a freezer at −20°C until analysis. The Australian concentrates (prime orange juice concentrates and water-soluble peel extracts) were sampled during the early part of the program (1992–1994) from regional processing plants. Concentrated juice samples were produced on falling film evaporators such as APV, Wigand or Alfa-Laval types. Generally, Navel concentrates were produced at around 57° Brix, whilst Valencia concentrates were produced at around 63° Brix, because of their lower pectin contents. Peel extracts were produced from pectinase-degraded orange peels which had been centrifuged following enzyme treatment to remove solid material, followed by resin-adsorption to remove bitter ingredients, and then evaporatively concentrated in a similar manner to the juices. Deacidified juices were prepared by using ion-exchange to remove citric acid. The Brazilian pulpwash samples (water-extracted soluble orange solids) were supplied as

concentrates by Schutzgemeinschaft fur der Fruchtsaft-Industrie (SGF). The concentrated juices and peel extracts and pulpwashes were all supplied as finished products and stored in a frozen state at −20°C after receipt until analysis.

Prior to analysis, samples were brought to room temperature and thoroughly mixed to eliminate any isotopic fractionation that may have occurred during thawing.

2.2. Carbon $^{13}C/^{12}C$ ratios of total carbohydrates

Samples were centrifuged to precipitate the pulp, and clear serum (20 μl) was then combusted following well-established methods (Association of Official Analytical Chemists [AOAC], 1994). Carbon dioxide analysis was carried out on a Finnigan-MAT 252 Isotope Ratio Mass Spectrometer. Results were expressed according to the familiar delta notation:

$$\delta\,{}^{13}C/{}^{12}C(\text{permil}) = ({}^{13}C/{}^{12}C_{\text{sample}}/{}^{13}C/{}^{12}C_{\text{standard}} - 1) \times 1000$$

using a limestone standard referenced to PDB (Craig, 1957).

2.3. Carbon $^{13}C/^{12}C$ ratios of sugar and acid subfractions

Carbon $^{13}C/^{12}C$ ratios of sugar and acid subfractions of fresh juice were determined after precipitation of acids as calcium salts. Centrifuged juice (50 ml) was added to 50% calcium chloride solution (20 ml) and the pH was adjusted to 8.5–9.0 by addition of 25% calcium hydroxide solution. The mixture was heated to 98°C on a boiling water bath for 10 min. The precipitated calcium salts of the organic acids were separated from the sugars by centrifugation at 3000 *g* for 5 min, carefully washed with acetone and dried overnight at 80°C. A subsample of the well-mixed acid salt (4 mg) was analysed by the same method as whole juice. A 20 μl portion of the supernatant liquid from the last centrifugation was used for analysis of sugars.

2.4. Quality assurance

A "repeat" sample of beet sugar was reanalysed with each batch of orange juices. Data on 25 redeterminations established that repeatability was within ±0.2‰. Inter-laboratory comparisons with a specialist overseas laboratory and participation in a number of proficiency trials including CEN/TC 174 interlaboratory study on sugars and pulp (Rossman, Koziet, Martin & Dennis, 1997), were also conducted to ensure the quality of results.

3. Results and discussion

3.1. Carbon $^{13}C/^{12}C$ ratios of total carbohydrates in juices

The $^{13}C/^{12}C$ ratios of total carbohydrates in the fresh juices, reconstituted concentrates, peel extracts and pulpwashes are summarised in Table 1. The data show the following.

(a) Average values for fresh and concentrated Australian juices were consistent with those found by other investigators on overseas products. However, the range for fresh juices was found to be much wider than expected, and indeed the maximum value at −22.5‰ is less negative than we have seen published elsewhere. The range for fresh samples was wider than for concentrates. We note that small sample sizes were taken for fresh fruit (~5 kg), and additionally they were taken from specific areas. This would certainly lead to much greater variance in results for fresh fruit than would be expected for concentrated samples drawn from a commercial extraction plant, where the juice is taken from a much larger blend. A similar broad distribution for small samples has been reported for laboratory-prepared wines (Rossmann, Schmidt, Reniero, Versini, Moussa & Merle, 1996). The distribution for fresh juice appears in Fig. 2.

(b) The mean $\delta\,{}^{13}C/{}^{12}C$ values of the sub-groups (fresh, concentrated, peel, pulpwash and Brazilian products) were not statistically different. This was determined by conducting a two-sample test of means for large samples at the 0.05 significance level (Mansfield, 1983) on the data for each type. The distributions of $\delta\,{}^{13}C/{}^{12}C$ values for Australian concentrates and Australian peel extracts appear in Figs. 3 and 4 respectively.

(c) Navel and Valencia types were not statistically different.

3.2. Internal correlation

A total of 23 samples were tested for internal correlation between sugars and acids. These were chosen to include samples having very high and very low carbon delta values. Results are illustrated in Fig. 5. The difference between sub-components was consistently within the range of 0.7–1.5‰. This accorded well with AIJN guidelines which state that the difference between the $\delta\,{}^{13}C$ content of acids (precipitated as calcium salts) and the $\delta\,{}^{13}C$ content of sugars from the same juice should be between +1 and +2‰, and also with published data (Rossmann et al., 1995) which reported that citrate $\delta\,{}^{13}C$ was enriched by about 1.5‰ compared to sugar for a wide range of samples.

Table 1
$\delta^{13}C$ (‰) values for database (all samples)

Type	Number	Mean	Standard deviation	Minimum	Maximum
Fresh (all samples)	273	−24.77	0.83	−27.3	−22.5
Fresh Valencia	185	−24.73	0.86	−27.3	−22.5
Fresh Navel	88	−24.87	0.73	−26.6	−22.8
Deacidified	16	−25.06	0.12	−25.3	−24.9
Concentrated Valencia	57	−25.25	0.33	−26.2	−24.4
Concentrated Navel	23	−25.08	0.33	−25.9	−24.2
Peel Extract	40	−25.37	0.39	−25.8	−23.9
Pulpwash	38	−25.6	0.28	−26.4	−25
Brazilian concentrates	30	−25.35	0.28	−26.1	−24.9
Brazilian fresh	12	−25.39	0.33	−26	−24.7

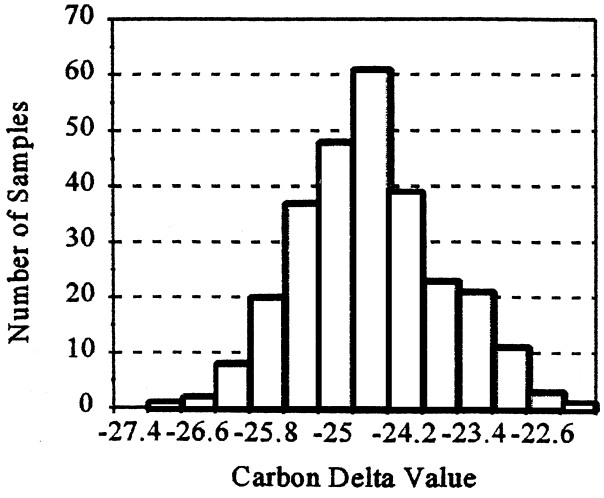

Fig. 2. Distribution of $\delta^{13}C$ in fresh Australian orange juices.

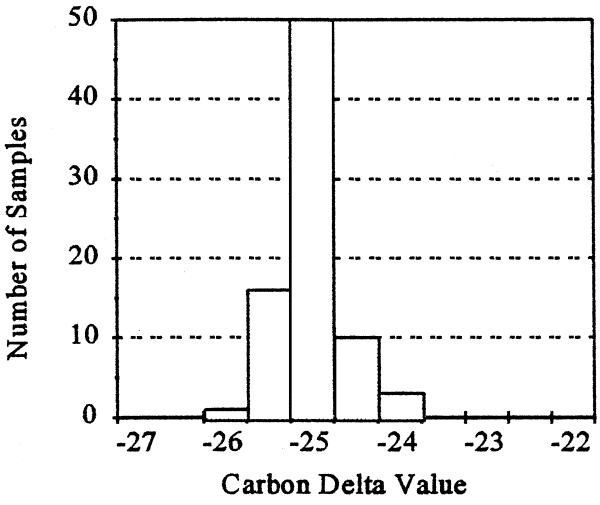

Fig. 3. Distribution of $\delta^{13}C$ in Australian orange concentrates.

3.3. Regional differences

We observed that small regional differences occurred in $\delta^{13}C$ values over the duration of the survey. These data are presented in Table 2. They appear to have little intrinsic value in determining the region from which the fruit originated, and were certainly not of the same magnitude as that reported for some overseas juices. For example, a mean difference of 1.7‰ between Floridian and Honduran samples was observed, with the Honduran samples being more negative (Krueger, 1995). Moreover, assuming that leaf $\delta^{13}C$ values translate to the fruit, these differences did not appear to conform to the Farquhar model referenced below, which predicts that RIV samples should be less negative than MIA because, although latitudinally similar, weather bureau records show that RIV has higher average temperatures and lower humidity. We acknowledge that there is considerable complexity in developing a model to explain regional differences in carbon isotope levels. A theoretical interpretation of photosynthetic processes (Farquhar, Hubrick, Condon & Richards, 1988) has been developed, predicting a linear

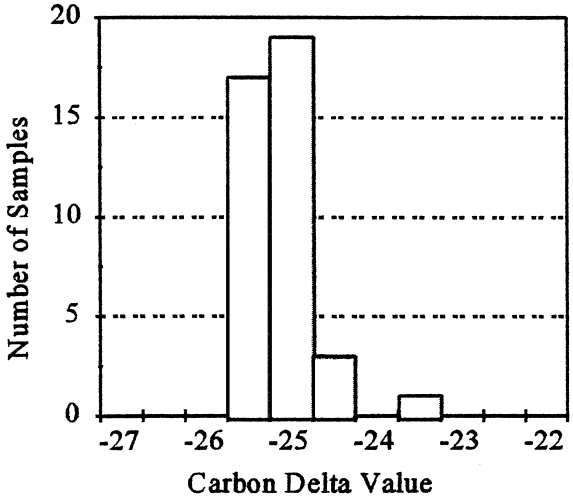

Fig. 4. Distribution of $\delta^{13}C$ in Australian orange peel extracts.

relationship between $\delta^{13}C$ and the partial pressure of carbon dioxide within the stomata. The carbon isotope discrimination in fruit sugars represents a long-term integration of these partial pressures but, within any growing region, there are a multiplicity of other factors

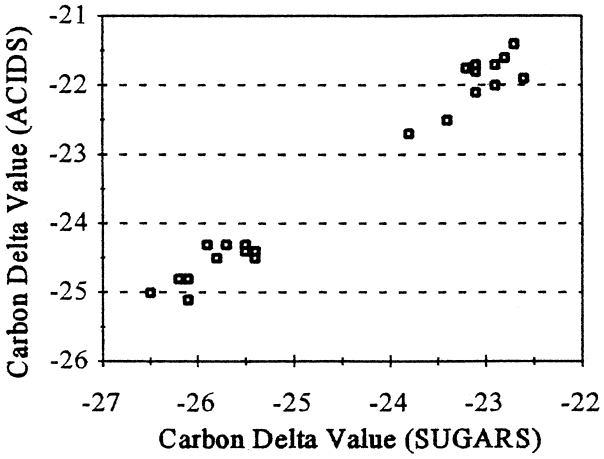

Fig. 5. $\delta^{13}C$ values for sugars and acids from same juices.

Table 2
Regional distribution of $\delta^{13}C$ (‰) values

Region	Number	Mean	Minimum	Maximum
MIA	108	−24.4	−25.7	−22.5
SUN	42	−25.4	−26.5	−23.4
RIV	64	−24.9	−27.3	−23.0
WA	32	−24.2	−25.2	−23.1
QLD	19	−25.5	−26.6	−24.6
Coast	9	−25.0	−26.6	−24.6

which affect isotopic ratios. These include variable photosynthetic capacity due to light limitations, plant age, the input of respiratory carbon dioxide from the soil and decaying detritus, altitude and water availability at critical stages of growth. Of these, water availability can be linked to the extent to which orchards are irrigated, and also how well soils retain irrigation water. More critically, water use efficiency may be a function of the plant's root system, and therefore linked to rootstock variety. Rootstock is mostly selected for its physical performance and resistance to biological attack, with some varieties preferred in particular regions because of their compatibility with soil structure and climate. Thus Trifoliata and Citrange rootstocks are used fairly ubiquitously in most regions, except RIV where sweet orange and rough lemon predominate. These reportedly have a more vigorous growth than other types.

Our results show a significant difference in $\delta^{13}C$ values between different rootstocks. Fruit from species grafted onto Trifoliata and Citrange had less negative values than those from sweet orange and rough lemon. This can be seen from data in Table 3. We suggest that these differences are due to the varying efficiency in water uptake of the different rootstocks. This leads to greater water availability in the leaves at the point of photosynthesis.

Table 3
$\delta^{13}C$ (‰) values for different rootstocks

Type	Number	Mean	Minimum	Maximum
Trifoliata	69	−24.3	−26.2	−22.5
Citrange	46	−24.4	−26.5	−22.9
Sweet orange	28	−24.8	−26.5	−23.2
Rough lemon	27	−25.1	−27.3	−23.6
Cleopatra mandarin	4	−25.1	−26.6	−24.2

4. Conclusions

This investigation into stable carbon isotope ratios of orange juices and concentrates available in Australia provides a statistical database of $\delta^{13}C$ values, which will be useful in determining authenticity of juices being sold commercially. Juices having a $\delta^{13}C$ higher than 3σ from the database mean should be suspected of sucrose addition, following well established practice. The values for fresh juices were observed to cover a wide range, making detection of even substantial amounts of sucrose difficult. We found a 0.7–1.5‰ difference between total carbohydrates and acids from the same juice, and recommend that this difference should be measured when cane sugar addition to juices is suspected. Our data also indicates that the variety of rootstock contributes to differences in $\delta^{13}C$.

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