Detection of Adulteration in Australian Orange Juices by Stable Carbon Isotope Ratio Analysis (SCIRA)

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Stable carbon isotope ratio analysis (SCIRA) was used to determine the authenticity of commercial Australian orange juices. Thirty-five samples of Valencia ($\delta^{13}C$ values from −23.8 to −24.7 ppt) and eight samples of Navel juices ($\delta^{13}C$ values from −24.1 to −24.5 ppt) of known origin were used to establish a decision level before analysis. No significant seasonal variations in $^{13}C/^{12}C$ ratio were observed. Variations in combustion temperature in the method were also found to be insignificant.

Keywords: *Authenticity; adulteration; SCIRA; stable carbon isotope ratio; orange juice; Valencia; Navel*

INTRODUCTION

Adulteration of orange juice (*1*) by the addition of high-fructose corn syrup (HFCS) or beet invert syrup plus water is common as sugar represents a major ingredient of juice. High-precision isotope measurement was developed as a method to detect this form of juice adulteration. Stable carbon isotope ratio analysis (SCIRA) measures small variations in the ^{13}C content of the carbon in different plants. Methods based on measurement of the $^{13}C/^{12}C$ ratio are used for the detection of C_4 sugars. This technique is based on the constancy of the $^{13}C/^{12}C$ ratio in plants using a common photosynthetic pathway for carbon dioxide fixation.

Natural variations in this ratio have been immensely useful in detecting adulterated foods. The measurement requires high precision because ^{13}C represents only 1% of the total carbon present, and the maximum relative variation in the $^{13}C/^{12}C$ is ∼5% (*2*). The application of $^{13}C/^{12}C$ ratio analysis to the detection of food adulteration began in the early 1970s when the relatively inexpensive high-fructose corn syrup (HFCS) was being widely added to honey. Thus, SCIRA was used to determine sugar addition to Israeli orange juices (*3*) and adulteration of honey with cane sugar or corn syrup (*4*). Since then, the technique has been successfully used in assessing the authenticity of apple juice (*5*), maple products (*6*), orange juice (*7*, *8*), grape juice (*9*), cranberry juice (*10*), and various fruits (*11*) and juices (*12*). Other kinds of additives such as organic acids, essence, and flavor constituents can also be detected by SCIRA. Byrne et al. (*13*) reported the use of $^{13}C/^{12}C$ ratio analysis to distinguish the ethyl butyrate which was naturally present in orange juice from that produced by fermentation.

There is a natural variation in $\delta^{13}C$ values in orange juice. In a comprehensive study of orange juice samples from four countries (United States, Mexico, South Africa, and Spain) Doner and Bills (*7*) reported a mean $\delta^{13}C$ of −24.5 ppt (range = −23.3 to −25.6 ppt) of all samples from the different growing areas, whereas in a more restricted study (*3*) of 42 Israeli and French orange juices the mean $\delta^{13}C$ values were −24.3 and −25.0 ppt, respectively. The mean $\delta^{13}C$ values (*14*) for orange juice sugars and orange pulp derived from Israel, United States, and Brazil were −25.1 and −25.6 ppt, respectively. The most negative values have been reported (*15*) for orange juices from Central America, averaging ∼−27 ppt. Thus, analysis of orange juices from different locations suggests that $\delta^{13}C$ values for oranges are relatively uniform. This facilitates the detection of C_4 adulterants (HFCS and cane invert syrup with $\delta^{13}C$ values near −10 ppt) in orange juice. In 1982, Doner and Bills (*16*) conducted an interlaboratory study of $\delta^{13}C$ values in orange juices and HFCS mixtures. The agreement among different laboratories was excellent. Nonetheless, Doner (*17*) reported that detection of orange juice adulteration using $^{13}C/^{12}C$ ratio measurement is qualitative rather than quantitative because the $\delta^{13}C$ value of a given pure juice sample before it is adulterated cannot be determined. Using a conservative approach, they concluded that any orange juice having a $\delta^{13}C$ value more negative than −22.1 ppt (4 standard deviations from the mean) was pure and unadulterated.

An interlaboratory trial (*18*) using an internal reference method (*19*), in which the $^{13}C/^{12}C$ ratio of free sugar was compared to the $^{13}C/^{12}C$ ratio of the pulp in each sample, found that smaller quantities of adulterants could be detected. Good correlation between laboratories was found, but the method is more complicated, and great care is needed to prepare the pulp samples to prevent interference from lipids.

MATERIALS AND METHODS

Reagents. Milli-Q water was used for carbon isotope ratio analysis. Standards for carbon isotope ratio analysis included CSR white cane sugar (CSR Ltd. Australia) and ANUC4 sugar (C_4 sugar standard supplied by Alan Chivers, Australian National University, Canberra, Australia).

Citrus Juice Samples. Orange juice samples were supplied by Leeton Citrus Juice Limited, Leeton, NSW, Australia. Sample collection was performed under the supervision of the authors. These juice samples were made from two cultivars (Valencia and Navel oranges), harvested at different times from sampling locations in New South Wales Riverina and Victorian Sunraysia regions. Navel oranges were sampled 8

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times from August 7, 1992, to October 16, 1992, and Valencia oranges were sampled 35 times between August 12, 1992, and June 1, 1993. The concentrated juices were produced on a falling film evaporator and supplied as finished products. The juice samples were stored in polyethylene bottles in a freezer at −5 °C until required for sample preparation.

Commercial ready-to-consume orange juice samples were purchased from local supermarkets. The brands of these samples were labeled A−J. All products were labeled "100% pure orange juice" with the exception of sample J, which was a fruit juice drink. Sample K, a 100% grapefruit juice, was also purchased from the supermarket. In addition, two fresh grapefruit juice samples were extracted from white (sample L) and ruby (sample M) grapefruits with a domestic hand reamer.

Standard Preparation for SCIRA. Aqueous solutions (50 mg/mL) of CSR white cane sugar and ANUC4 sugar solution were prepared. An aliquot (10 μL) of the solution was transferred into a series of tin capsules and treated as described for the samples. In the SCIRA, CSR white sugar was chosen as a routine standard, with ANUC4 sugar used as reference to CSR white sugar.

Sample Preparation for SCIRA. Tin capsules were cleaned by immersion and agitation in chloroform for a few minutes and dried in an oven at 100 °C. The dried capsules were then stored in a clean container for later use.

Concentrated orange juice samples were defrosted at room temperature, thoroughly mixed to eliminate any isotopic fractionation that occurred during freezing and thawing, and then diluted to single-strength drinking concentration by addition of an appropriate amount of Milli-Q water (typically seven parts by mass) to orange concentrate (one part by mass). Pulp was removed by centrifugation, and a sample (10 μL) of the clear diluted orange juice was transferred to a tin capsule held in a microtiter. Five replicates were performed on each sample. The microtiter was placed in a freeze-dryer (Pirani II, Edwards, −1.0 bar) for ∼24 h at room temperature. Following drying the tin capsules were sealed by crushing tightly with a pair of forceps on the surface of a clean mirror. The crushed capsules were shaped into balls and transferred into an autosampler for the δ^{13}C measurement.

Isotopic Ratio Analysis of Total Carbohydrates. SCIRA was performed on a Europa Scientific isotope ratio mass spectrometer (IRMS) consisting of a Europa Scientific Roboprep-CN biological sample converter and a Europa Scientific Tracemass stable isotope analyzer (Europa Scientific Ltd., Crewe, U.K.).

The MS system comprised three stages: automated Dumas combustion, chemical and gas chromatographic purification of sample-derived CO_2, and on-line isotope analysis by mass spectrometry. Fruit juice was first combusted with the aid of a Cr_2O_3 catalyst and a software-selected pulse of O_2. This converted carbon in the sample to CO_2, which was separated from excess O_2 and other combustion products. Finally, the CO_2 pulse was swept by helium carrier gas directly to the ion source of the mass spectrometer for isotopic analysis.

Three blank tin capsules were run before the sample measurement for blank calibration. Sealed capsules containing samples were placed in the 66-position autosampler of the combustion unit (Roboprep-CN Europa Scientific Ltd.). References of CSR white sugar (δ^{13}C = −11.88 ppt) were positioned after every five juice samples. Carrier flow rate was 60 mL min^{-1}, and a 15 mL pulse of high-purity oxygen (99.999%, v/v) was injected into the oxidation tube at 1000 °C. The reduction stage was maintained at 600 °C and the GC column at 150 °C. Carbon isotope ratios were determined on an 11-cm-radius triple collector, 90° magnetic sector IRMS. Ion currents at *m*/*z* 44, 45, and 46 were simultaneously integrated and corrected for background, ^{17}O contribution at *m*/*z* 45, and any drift between references.

RESULTS AND DISCUSSION

A series of juice samples were measured by isotope ratio mass spectrometry to establish decision levels for a method to detect the undeclared addition of corn or cane sugars to orange juice. The samples included 8 concentrated Navel and 35 concentrated Valencia orange juices, which were produced in early, middle, and late seasons (from August 7, 1992, to June 1, 1993). Ten commercial orange juices and three grapefruit juices and single-strength pure orange juices were also measured and compared with the above values to evaluate their sugar content.

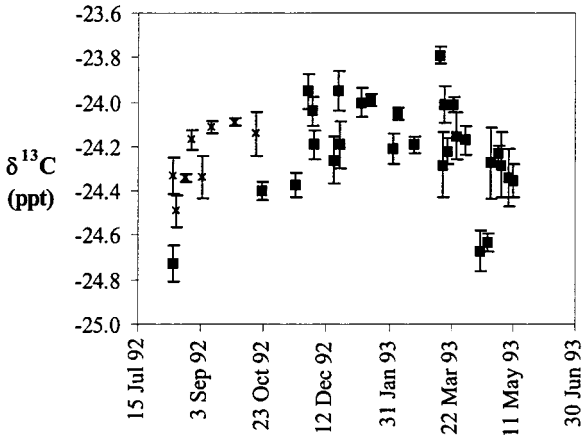

Figure 1. SCIRA values for orange juices: (■) Valencia; (×) Navel.

Results are presented in Figure 1 as the δ^{13}C value, which is the relative difference per thousand between the ^{13}C and ^{12}C ratios of a sample in relation to the international standard, Pee Dee Belemnite (PDB) from South Carolina in the United States. This is a fossil calcium carbonate with an isotopic ratio $(^{13}C/^{12}C)_{PDB}$ of 0.0112372 for emitted CO_2. This value is the reference point of the common international PDB scale for δ^{13}C (*20*):

$$\delta^{13}C_{PDB} = 1000\{(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard} - 1\}‰$$

Most carbon in nature has a lower $^{13}C/^{12}C$ ratio than that of the standard and thus a negative δ^{13}C value.

The δ^{13}C values for eight concentrated Navel orange juices ranged from −24.1 to −24.5 ppt, with a mean of −24.3 ppt (SD = 0.15), whereas the δ^{13}C values for 35 concentrated Valencia orange juices ranged from −23.8 to −24.7 ppt, with a mean of −24.2 ppt (SD = 0.23). When the two groups of concentrated orange juice samples were combined, the δ^{13}C values were in the range expected for C_3 plants from −23.8 to −24.7 ppt, with a mean of −24.2 ppt (SD = 0.21). These results are similar to the results of previous work on Australian (*20*) and Israeli (*14*) orange juices and are typical of the type of results that have been observed for the Mediterranean region generally. They are considerably more positive than the results reported for other citrus areas such as Florida and Brazil except for the work reported by Doner and Bills (*7*).

Statistical analysis of the data shows that there is no significant difference between the δ^{13}C values of both cultivars at different harvesting times. Environmental factors such as temperature, rainfall, and harvest time have minimal effect on δ^{13}C values. Thus, the δ^{13}C values of plant-derived materials are determined largely by the photosynthetic pathway in the plant, and typical values in C_3 plants are ∼−25 ppt as further illustrated by the mean value for grapefruit of −25.4 ppt. Nevertheless, minor differences in δ^{13}C such as are observed

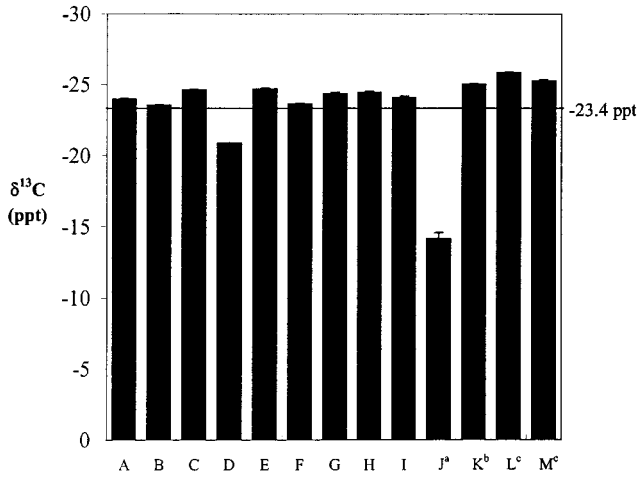

Figure 2. SCIRA values for commercial citrus juices (A−I), an orange fruit drink (J), a commercial grapefruit juice (K), and grapefruit juices squeezed from fresh grapefruit (L, M).

within a given cultivar (Figure 1) do occur. Variables such as the local mean temperature, the lighting period and the plant, which itself is influenced by the vegetative cover (*6*), account for the slight spread of the isotopic composition.

Doner (*21*) recommended that a statistical approach should be used to interpret the $\delta^{13}C$ results on suspect juices. Using this procedure, juices that possessed $\delta^{13}C$ values within 4 standard deviations from the mean were classified as acceptable. However, inclusion of the relatively positive Mediterranean and Australian juices plus the relatively negative Central American juices in Doner's database would substantially widen his 4σ confidence interval. The specified criterion of mean $\pm$ 4 SD [$-24.2 \pm (4 \times 0.175)$] when restricted to the current samples yields a critical $\delta^{13}C$ value of -23.5 ppt for authenticity of an Australian juice. Thus, there is a 99.996% probability that the $\delta^{13}C$ value of an authentic orange juice sample (New South Wales, Australia) is more negative than this limit value. The use of a broad range in this way ensures that it is extremely unlikely that an authentic juice will be classified as unacceptable or adulterated. This approach is useful in a regulatory or litigation environment, when the risk of false positives is much more important than the risk of false negatives. In a quality assurance environment, a narrower interval is more appropriate, more evenly balancing the risks of false positives and false negatives.

The $\delta^{13}C$ values of commercial orange juices and grapefruit juices (Figure 2) were compared with the limit value. With the exception of one juice sample, all commercial products showed $\delta^{13}C$ values more negative than -23.5 ppt, indicating that no extra C_4 sugars were added to the orange juices. One commercial orange juice (sample D, labeled "100% pure orange juice and no added sugar") showed a more positive $\delta^{13}C$ value (-20.9 ppt), indicating that it was likely that this juice sample was adulterated with ~30% C_4 sugar. Another sample (sample J, labeled a fruit juice drink) is distinguished from fruit juice because such products can contain added water, sugar, and sometimes organic acid, gum arabic, and artificial flavors and color with a fruit juice or a blend of fruit juices. This accounts for the $\delta^{13}C$ value of -14.1 ppt for this sample, which indicated a significant (>80%) C_4 sugar content in the drink.

A comparison of the MS isotope ratio data with previous work and results obtained independently (mean

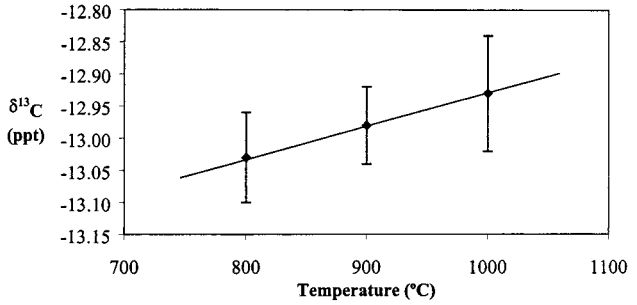

Figure 3. $\delta^{13}C$ values of cane sugar at different combustion temperatures.

$\delta^{13}C = -25.2$ ppt) (*20*) using off-line combustion at 900 °C and trapping reveals a difference of ~1 ppt. In general, samples may be prepared for ^{13}C measurement by combustion using one of three procedures. The Association of Official Analytical Chemists' procedure involves combustion in oxygen at 800 °C over copper oxide (*22*). A second method is based on combustion under vacuum at 550 °C with copper oxide alone. These two sample preparation procedures are performed off-line, the carbon dioxide being trapped and subsequently analyzed. Krueger (*23*, *24*) found that the 550 °C combustion procedure gave results that were biased ~±1 ppt relative to the AOAC procedure. The method used in the present study differed from both of the above procedures, which involved combustion of the sample in oxygen at 1000 °C over Cr_2O_3 and CuO catalysts. The CO_2 was then fed directly into the mass spectrometer.

To assess the effect of combustion temperature on the $\delta^{13}C$ value, samples of sugar were checked at different temperatures on the MS instrument. The results (Figure 3) obtained between 800 and 1000 °C showed no significant differences ($P = 0.05$). The use of beet sugar (a C_3 sugar) as a standard ($\delta^{13}C = -25.96$ ppt) was also investigated. It should be noted that the addition of a C_3 sugar such as beet to orange juices cannot be detected by the SCIRA technique. The results using the C_3 standard were shifted toward positive values by ~0.06 ppt, again indicating no significant difference ($P = 0.05$).

SCIRA is a powerful method for determining adulteration by C_4 sugars in fruit juices. Our studies indicate that seasonal changes and instrumental operating conditions have little effect on the data. There does, however, seem to be a variation due to measuring technique, and so it is important to carefully calibrate the system based on a large number of known samples to confidently analyze unknown samples.

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