

Stable Carbon Isotope Ratios in Orange Juice

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The ratios $^{13}\text{C}/^{12}\text{C}$ in 42 pure orange juice samples have been determined by isotope ratio mass spectrometry. Thirty-one domestic blends, seven samples of varietal purity, and four foreign samples were tested to establish base-line values for a method to detect the undeclared addition of corn or cane sugars to orange juice. The mean $\delta^{13}\text{C}$ (ppt) value for all samples is -24.5 (ppt), and the coefficient of variation just 2.41%. This is the smallest variation encountered to this time for any orange juice component or physical property, and this uniformity will enhance the sensitivity with which adulteration can be detected.

The ratio of the stable isotopes ^{13}C and ^{12}C in a food is very close to that of its source. The act of processing sources into foods results in minimal further fractionation of these isotopes, much less than would be required to mask isotope ratio difference between the sources. While variations in $^{13}\text{C}/^{12}\text{C}$ among reservoirs of the carbon cycle were first described in 1939 (Nier and Gulbranson, 1939), only recently have applications of these variations been extended to foods. A comprehensive review recently summarized these applications (Winkler and Schmidt, 1980).

Stable carbon isotope ratio mass spectrometry is used to determine $^{13}\text{C}/^{12}\text{C}$ ratios with overall accuracy of 0.3 ppt (parts per thousand) or better. This method is applicable to foods because the $^{13}\text{C}/^{12}\text{C}$ ratio of a plant-derived material reflects the pathway of photosynthesis in the source plant. Most plant families use the Calvin (C_3) photosynthetic cycle and possess $\delta^{13}\text{C}$ values of -22 to -33 ppt; plants using the Hatch-Slack (C_4) cycle have $\delta^{13}\text{C}$ values ranging from -10 to -20 ppt (Bender, 1971; Smith and Epstein, 1971). Crassulacean acid metabolism plants, such as pineapple and cactus, have the capability for both types of carbon dioxide fixation and have intermediate $\delta^{13}\text{C}$ values.

The most important food applications of $^{13}\text{C}/^{12}\text{C}$ analysis have been in distinguishing between and detecting mixtures of C_3 and C_4 plant-derived materials. For example, Smith and Epstein (1971) showed that pure beet sugar (C_3 or "light") can easily be distinguished from pure cane sugar (C_4 or "heavy") by $^{13}\text{C}/^{12}\text{C}$ measurement. Chemically these compounds are identical. Problems in agriculture and commerce which have been solved include the fraudulent addition of inexpensive C_4 -derived materials to C_3 -derived materials and subsequent mislabeling. Examples are the additions of high-fructose corn syrup (HFCS) to honey (White and Doner, 1978a), cane syrup to maple syrup (Carro et al., 1980), and HFCS to apple juice (Doner and Phillips, 1981). These adulterations had been widespread, impacting producers, processors, and consumers of these products. Existing methods were inadequate since it was possible to manipulate the mixtures to simulate the composition of the pure product with regard to composition and physical properties. The $^{13}\text{C}/^{12}\text{C}$ ratio method is noncircumventible, as it is not feasible to manipulate this ratio.

This report establishes the uniformity of $\delta^{13}\text{C}$ values in orange juices, preliminary to using this ratio to detect the undeclared addition of HFCS to orange juice. Previous

methods for detecting citrus adulteration have been reviewed (Vandercook, 1977), and Nissenbaum et al. (1974) evaluated $\delta^{13}\text{C}$ values of Israeli and French orange juices, finding them to average -24.3 and -25.0 ppt, respectively. Unfortunately, beet sugar ($\delta^{13}\text{C} = -24.3$ ppt) is used almost exclusively in Israel, so no practical application of the method was realized. They did suggest the potential of its application elsewhere, for adulterants other than beet sugar and beet sugar invert syrups.

EXPERIMENTAL SECTION

The samples of pure orange juice chosen for $\delta^{13}\text{C}$ measurement represent prominent areas of both U.S. and foreign production, including blends, samples of varietal purity, and early-, mid-, and late-season oranges.

Florida samples (no. 1-20) were concentrated blends received from S. V. Ting, Florida Department of Citrus, Lake Alfred, FL. These samples had been collected from 14 processing plants in Florida during the months of Dec 1979 and Jan, Feb, April, and May 1980. From one to three samples from each plant were tested and from two to five samples from each month.

The orange juice concentrates 21-29 were received from C. E. Vandercook, U.S. Department of Agriculture, Fruit and Vegetable Chemistry Laboratory, Pasadena, CA. Samples 21-23 were California juices of the Navel variety, 24 and 25, Arizona blends, 26 and 27, Mexican samples, and samples 28 and 29 originated in Spain and South Africa, respectively.

Samples 30-42 represented Texas juices and were received from R. F. Albach, U.S. Department of Agriculture, Food Crops Utilization Research, Weslaco, TX. Numbers 30, 31, and 32 were single strength juices processed in his laboratory from fruit of varieties Marrs, Hamlin, and Valencia, respectively. Samples 33-39 were single strength juice blends from a processing plant in Weslaco, TX, representing two seasons: 1977-1978 and 1978-1979. Samples 40-42 were orange juice concentrates from a processing plant at Mission, TX, sample 42 being of the Valencia variety.

Stable Carbon Isotope Ratio Analysis (SCIRA). $^{13}\text{C}/^{12}\text{C}$ ratios in the samples were determined by Coastal Science Laboratories, Inc., Port Arkansas, TX, on a Micromass 602D mass spectrometer with a dual-capillary inlet. The samples were thoroughly mixed and an aliquot (1 drop) was taken for analysis. Both single-strength and concentrated orange juices were combusted directly, and the details of $^{13}\text{C}/^{12}\text{C}$ ratio determination were described previously (Doner et al., 1980). The $^{13}\text{C}/^{12}\text{C}$ ratios are expressed as $\delta^{13}\text{C}$ values, where

$$\delta^{13}\text{C} \text{ (ppt)} = \left[\frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} - 1 \right] \times 10^3$$

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Table I. $\delta^{13}\text{C}$ (ppt) of Orange Juices

| provenience | $\delta^{13}\text{C}$, ppt | provenience | $\delta^{13}\text{C}$, ppt |
|-------------|-----------------------------|--------------|-----------------------------|
| Florida | 1 -24.4 | California | 21 -24.1 |
| | 2 -23.8 | | 22 -23.7 |
| | 3 -24.4 | | 23 -24.2 |
| | 4 -24.4 | Arizona | 24 -25.0 |
| | 5 -24.0 | | 25 -23.6 |
| | 6 -24.6 | Mexico | 26 -23.4 |
| | 7 -24.6 | | 27 -25.2 |
| | 8 -25.1 | Spain | 28 -23.9 |
| | 9 -25.1 | South Africa | 29 -24.8 |
| | 10 -24.0 | Texas | 30 -24.7 |
| | 11 -25.2 | | 31 -24.6 |
| | 12 -24.9 | | 32 -24.3 |
| | 13 -24.5 | | 33 -24.1 |
| | 14 -23.8 | | 34 -24.2 |
| | 15 -25.5 | | 35 -23.7 |
| | 16 -25.2 | | 36 -24.6 |
| | 17 -25.6 | | 37 -23.8 |
| | 18 -25.5 | | 38 -23.9 |
| | 19 -25.0 | | 39 -24.7 |
| | 20 -25.3 | | 40 -23.8 |
| | | | 41 -24.1 |
| | | | 42 -24.0 |

Table II. Statistical Evaluation of Data in Table I

| provenience | no. of samples | $\delta^{13}\text{C}$, ppt | | | CV, % |
|--------------|----------------|-----------------------------|-------|-------|-------|
| | | range | mean | SD | |
| Florida | 20 | -23.8 to -25.6 | -24.7 | 0.575 | 2.32 |
| Texas | 13 | -23.8 to -24.7 | -24.2 | 0.359 | 1.48 |
| California | 3 | -23.7 to -24.2 | -24.0 | | |
| Arizona | 2 | -23.6 to -25.0 | -24.3 | | |
| Mexico | 2 | -23.4 to -25.2 | -24.3 | | |
| Spain | 1 | | -23.9 | | |
| South Africa | 1 | | -24.8 | | |
| all samples | 42 | -23.4 to -25.6 | -24.5 | 0.591 | 2.41 |

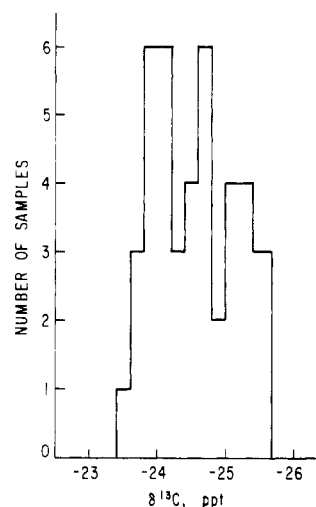
and the reference standard is CO_2 produced from Peedee belemnite (PDB) calcium carbonate. Reproducibility for the $\delta^{13}\text{C}$ determinations is 0.2 ppt or better.

RESULTS AND DISCUSSION

In Table I are listed the $\delta^{13}\text{C}$ values for all 42 samples included in this study, as well as the provenience of each sample. The major areas of U.S. production, California, Florida, Arizona, and Texas, are represented, as well as the countries of Mexico, Spain, and South Africa. The distribution of $\delta^{13}\text{C}$ values is presented in Figure 1, and a statistical analysis of the data in Table II.

There is no apparent correlation between $\delta^{13}\text{C}$ value and provenience or variety of the orange juices analyzed, which suggests that a method can be developed to detect the presence of C_4 plant derived materials, such as HFCS, in orange juices (C_3 plant derived). A collaborative study is presently being conducted to establish the validity of this method.

The uniformity of the data is remarkable, with a coefficient of variation for all samples of just 2.41%. This variance is even less than that seen with honey and apple juice and, as with those materials, may result because a high percentage of the carbon in orange juice resides in the carbohydrates. The ranges of $\delta^{13}\text{C}$ values for classes of compounds from single plants (Whelan et al., 1970) are

Figure 1. Distribution of $\delta^{13}\text{C}$ (ppt) values among orange juices.

wider than the range found for all orange juice samples in this present study.

The mean value for all samples, -24.5 ppt, establishes that the carbon in orange juice is somewhat heavier than that in either honey (White and Doner, 1978a) or apple juice (Doner et al., 1980). Honey (119 samples) and apple juice (40 samples) both averaged -25.4 ppt in $\delta^{13}\text{C}$. It is interesting that the three citrus honeys which had been analyzed (White and Doner, 1978b) for $\delta^{13}\text{C}$ were significantly richer in ^{13}C than the mean value for all the honeys, with $\delta^{13}\text{C}$ values of -23.0 , -23.5 , and -23.8 ppt. It is possible that plants of the family Rutaceae discriminate against atmospheric $^{13}\text{CO}_2$ during photosynthesis to a lesser extent than other C_3 plants.

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LITERATURE CITED

- Bender, M. M. *Phytochemistry* 1971, 10, 1239.
 Carro, O.; Hillaire-Marcel, D.; Gagnon, M. *J. Assoc. Off. Anal. Chem.* 1980, 63, 840.
 Doner, L. W.; Krueger, H. W.; Reesman, R. H. *J. Agric. Food Chem.* 1980, 28, 362.
 Doner, L. W.; Phillips, J. G. *J. Assoc. Off. Anal. Chem.* 1981, 64, 85.
 Nier, A. O. C.; Gulbranson, E. A. *J. Am. Chem. Soc.* 1939, 61, 697.
 Nissenbaum, A.; Lifshitz, A.; Stepak, Y. *Lebensm.-Wiss. Technol.* 1974, 7, 152.
 Smith, B. N.; Epstein, S. *Plant Physiol.* 1971, 47, 380.
 Vandercook, C. E. *Food Chem.* 1977, 2, 219.
 Whelan, T.; Sackett, W. M.; Benedict, C. E. *Biochem. Biophys. Res. Commun.* 1970, 41, 1205.
 White, J. W., Jr.; Doner, L. W. *J. Assoc. Off. Anal. Chem.* 1978a, 61, 746.
 White, J. W., Jr.; Doner, L. W. *J. Apic. Res.* 1978b, 17, 94.
 Winkler, F. J.; Schmidt, H. L. *Z. Lebensm.-Unters.-Forsch.* 1980, 171, 85.

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