

## SUGARS AND SUGAR PRODUCTS

### Mass Spectrometric $^{13}\text{C}/^{12}\text{C}$ Determinations to Detect High Fructose Corn Syrup in Orange Juice: Collaborative Study

LANDIS W. DONER and DONALD D. BILLS

*U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Philadelphia, PA 19118*

Collaborators: O. Carro; R. Drimmie; P. Fritz; J. N. Gearing; C. Hillaire-Marcel; P. L. Parker; R. M. Reeseaman; B. N. Smith; H. Ziegler

The  $^{13}\text{C}/^{12}\text{C}$  ratios in orange juice are sufficiently uniform and different from those in high fructose corn syrup (HFCS) so that the addition of HFCS to orange juice can be detected. HFCS averages  $-9.7\text{‰}$  (parts per thousand)  $\delta^{13}\text{C}$ , orange juice averages  $-24.5\text{‰}$ , and mixtures of HFCS and orange juice possess intermediate values. One pure orange juice and 4 orange juice-HFCS mixtures containing from 25 to 70% orange juice were properly classified by 7 collaborators. Samples with  $\delta^{13}\text{C}$  values less negative than  $-22.1\text{‰}$ , 4 standard deviations from the mean of pure juices, can, with a high degree of confidence, be classified as adulterated. Samples with values more negative than  $-22.1\text{‰}$  must be considered unadulterated with HFCS, because pure orange juices possess a range of  $\delta^{13}\text{C}$  values. The  $^{13}\text{C}/^{12}\text{C}$  mass spectrometric method was adopted official first action for detecting HFCS in orange juice.

The economic incentives to adulterate orange juice with less expensive solutions of sugars are significant, and previous methods developed to indicate such adulteration have been reviewed (1-3). Orange juice is also adulterated by addition of orange pulp wash (water-extracted soluble orange solids from orange pulp), and recently an effective spectrophotometric method was developed to detect such adulteration (4). A microbiological assay procedure has been developed (5) which indicates the content of orange juice in a product; the versatility of this latter method has been demonstrated by extension to other food adulteration problems, such as detecting grape juice added to apple juice (6).

The present report demonstrates the application of stable carbon isotope ratio analysis (SCIRA) for detecting illegal addition of high fructose corn syrup (HFCS) to orange juice. Sugar analysis is not suitable for this purpose, because HFCSs are available which, when added to orange juice, will result in glucose and fructose levels within the range found for authentic juices. We previously applied SCIRA to the detection of HFCS in honey (7) and apple juice (8), and the AOAC official first action methods are widely used. The SCIRA approach takes advantage of the fact that organic materials derived from  $\text{C}_4$  plants such as corn have elevated  $^{13}\text{C}/^{12}\text{C}$  ratios compared with those derived from  $\text{C}_3$  plants such as apple trees and all honey floral sources. We recently reported (9) that pure orange juices (42 samples) from several locations are extremely uniform in  $\delta^{13}\text{C}$ , averaging  $-24.5\text{‰}$  with a coefficient of variation of 2.41%. The orange juice samples included domestic blends, samples of varietal purity, and foreign samples. The results agreed with earlier analyses by Nissenbaum et al. of Israeli and French juices (10). Being derived from a  $\text{C}_4$  plant, HFCS is richer in  $^{13}\text{C}$ , with  $\delta^{13}\text{C}$  values averaging  $-9.7$  (7).

#### Experimental

##### Materials

Pure orange juice was squeezed from oranges of the Navel variety and determined to contain 11.60% solids. Navel orange juice was chosen because its  $\delta^{13}\text{C}$  values have been shown (9) to fall in the range of values for pure juices. HFCS was obtained from Corn Sweeteners Co. (Cedar Rapids, IA), and diluted to 11.60% solids with water. Orange juice and HFCS were then combined to give adulterated mixtures containing the following proportions of orange juice: A, 70.0%; B, 25.0%; C, 100.0%; D, 40.0%; and E, 55.0%. These 5 samples were freeze-dried, and

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The recommendation of the Associate Referee was approved by the General Referee and Committee D and was adopted by the Association. See the report of the committee, March 1982 issue, for detailed recommendations.

**Table 1. Instruments and conditions used in collaborative study**

Coll.	Mass spectrometer	Combustion <sup>a</sup>		Coll. study working std
		Temp., °C	Recirc. time, min	
1	Micromass 602 D	600	sealed tube with CuO	AER vacuum pump oil
2	Micromass 602 D	850	10	charcoal ( $\delta^{13}\text{C} = -23.3\%$ vs PDB) and marble ( $\delta^{13}\text{C} = 0.0\%$ vs PDB) calibrated against NBS-20 solenhofen limestone ( $\delta^{13}\text{C} = -29.5\%$ vs PDB)
3	Micromass 602 C	1200	—	UQ2 marble vs NBS-22 and PDB
4	Nier type, 6 in. 60° sector, dual collecting	810	20	BYU carbonate calibrated against NBS-22, NBS-oxalate, and NBS-21
5	Micromass 602	>1500	—	bender limestone
6	Mat 250, Varian	750	—	flask CO <sub>2</sub> vs PDB
7a	Micromass 903	—	—	tank CO <sub>2</sub>

<sup>a</sup> Sample combustion in Parr bomb, electrically ignited after charging with 12 atmospheres O<sub>2</sub>.

portions were added to screw-top vials labeled A through E and sent to collaborators.

**Sample Combustion and Determination of  $\delta^{13}\text{C}$**

Various isotope ratio mass spectrometers are used in laboratories which determine <sup>13</sup>C/<sup>12</sup>C ratios. Also, different procedures are used to quantitatively burn the sample to carbon dioxide and water. Overall accuracy, including combustion and <sup>13</sup>C/<sup>12</sup>C determination, is 0.3‰ or better. The instruments, combustion conditions, and standards used by the collaborators are given in Table 1.  $\delta^{13}\text{C}$  (‰) values are reported from comparisons with carbon dioxide generated from the reference standard PeeDee belemnite (PDB) and calculated from the following formula after comparison of the <sup>13</sup>C<sup>16</sup>O<sub>2</sub> and <sup>12</sup>C<sup>16</sup>O<sub>2</sub> ion beams:

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

A  $\delta^{13}\text{C}$  value of -25‰ means that the <sup>13</sup>C/<sup>12</sup>C ratio of the sample is 2.5% less than that of the

PDB standard (<sup>13</sup>C/<sup>12</sup>C PDB = 0.011237). Corrections are applied to the measured differences, including any zero enrichment in the capillary inlet system, valve mixing between sample and standard valves, and tailing of major onto minor peak signal.

**Results and Recommendations**

The results of our recent survey (9) are given in Table 2. The probability of a given orange juice sample being unadulterated may be determined from multiples of the standard deviation, as shown in Table 3. A sample with a value less negative than -22.1‰ should be classified as adulterated. Because of the range of values found for pure orange juices (-23.4‰ to -25.6‰), a statistical approach is necessary to interpret the results of the test.

The results of the collaborative study are compiled in Table 4. Regression lines of percent orange juice on  $\delta^{13}\text{C}$  (range of values for all collaborators) demonstrate the good agreement among the collaborators and are shown in Figure 1. These lines were all significant ( $P < 0.01$ ) with very high correlation coefficients, -1.00 <  $r < -0.99$ . All adulterated samples in the study

**Table 2.  $\delta^{13}\text{C}$  Values of orange juice and HFCS<sup>a</sup>**

Sample	No. of samples	Mean, ‰	Range, %		SD	CV, %
			Low	High		
Orange juice	42	-24.5	-25.6	-23.4	0.591	2.41
HFCS	4	-9.7	-9.8	-9.5	0.14	1.4

<sup>a</sup> See ref. 9 for individual values for 42 orange juice samples, including 3 domestic blends, 7 samples of varietal purity, and 4 foreign samples.

**Table 3. Probability of  $\delta^{13}\text{C}$  value of authentic orange juice sample being more negative than stated limit**

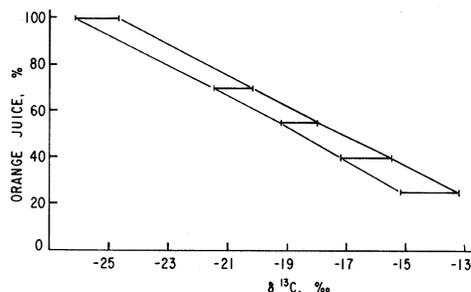
Probability, %	Limit of $\delta^{13}\text{C}$ , ‰
5 of 6 = 84.1	-23.9
43 of 44 = 97.72	-23.3
769 of 770 = 99.87	-22.7
24 999 of 25 000 = 99.997	-22.1

**Table 4.  $\delta^{13}\text{C}$  (‰ vs PDB) for collaborative samples**

Coll.	Sample				
	A	B	C	D	E
1	-20.2	-13.2	-24.9	-15.5	-18.0
2	-21.3	-14.8	-25.9	-17.2	-19.2
3	-21.3	-14.5	-26.0	-16.7	-19.2
4	-20.7	-14.2	-24.7	-16.0	-19.0
5	-21.5	-15.2	-25.3	-16.6	-18.9
6	-21.5	-14.5	-26.1	-16.7	-18.8
7	-21.2	-14.5	-26.2	-16.4	-18.8
Mean	-21.1	-14.4	-25.6	-16.4	-18.8

(A, B, D, E) were properly classified, according to the highest probability limit set in Table 3.

From these results, the Associate Referee recommends that the  $^{13}\text{C}/^{12}\text{C}$  mass spectrometric method be adopted official first action for detecting HFCS in orange juice.



**Figure 1. Graphic representation of collaborative study results, showing range in  $\delta^{13}\text{C}$  values reported by all collaborators for orange juice with various levels of HFCS added.**

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## REFERENCES

- (1) Vandercook, C. E. (1977) *Food Chem.* **2**, 219-233
- (2) Anonymous (1980) *Anal. Chem.* **52**, 1269A-1272A
- (3) Petrus, D. R., & Vandercook, C. E. (1980) in *Citrus Quality and Nutrition*, S. Nagy and J. A. Attaway (Eds), ACS Symposium Series 143, American Chemical Society, Washington, DC, pp. 395-422
- (4) Petrus, D. R., & Attaway, J. A. (1980) *J. Assoc. Off. Anal. Chem.* **63**, 1317-1331
- (5) Vandercook, C. E., Smolensky, D. C., Nakamura, L. K., & Price, R. L. (1976) *J. Food Sci.* **41**, 709-710
- (6) Smolensky, D. C., & Vandercook, C. E. (1980) *J. Food Sci.* **45**, 1773-1780
- (7) White, J. W., Jr, & Doner, L. W. (1978) *J. Assoc. Off. Anal. Chem.* **61**, 746-750
- (8) Doner, L. W., & Phillips, J. G. (1981) *J. Assoc. Off. Anal. Chem.* **64**, 85-90
- (9) Doner, L. W., & Bills, D. D. (1981) *J. Agric. Food Chem.* **29**, 803-804
- (10) Nissenbaum, A., Lifshitz, A., & Stepak, Y. (1974) *Lebensm. Wiss. Technol.* **7**, 152-154