

## SUGARS AND SUGAR PRODUCTS

# $\delta^{18}\text{O}$ Measurements in Water for Detection of Sugar Beet-Derived Syrups in Frozen Concentrated Orange Juice: Collaborative Study

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Stable isotope ratio mass spectrometry has shown that pure frozen concentrated orange juices (FCOJ) of 63–67° brix possess a mean  $\delta^{18}\text{O}$  value of +14.28‰, with a standard deviation of 1.80. Beet invert syrups, which are produced using ground water, possess negative values. As a result,  $\delta^{18}\text{O}$  values decrease on addition of such syrups to FCOJ. Samples with values less than +8.9‰ (3 standard deviations from the mean for pure FCOJs) can confidently be considered as adulterated. A collaborative study was conducted in which a pure FCOJ and 4 samples adulterated to various levels with medium beet invert syrup were sent to each of 6 collaborators. In all but 2 instances, juices containing more than 10% beet syrup would have been classified as adulterated by the collaborators; none would have classified pure juice as adulterated. The plot of mean  $\delta^{18}\text{O}$  values for all collaborators at each adulteration level has a correlation coefficient >0.999. The method has been adopted first action by AOAC International.

Sophisticated approaches have been required to detect some of the concoctions elaborated in recent years for use as fruit juice adulterants. Orange juice has been a popular target for adulteration, especially since 1977, when the first in a series of severe Florida freezes caused the demand for orange juice to exceed its domestic supply. Methods are now available for monitoring the addition to juices of major adulterants, such as sugar syrups derived from corn and sugar cane (1, 2) and orange pulpwash solids (3).

Mixtures of orange juice and the adulterants high fructose corn syrup (HFCS) or cane invert syrups are detected by mass spectrometry using stable carbon isotope ratio analysis (SCIRA). The basis of the test is that most elements have light and heavy stable isotopes, and there are small but measurable source-dependent differences in their ratios. The isotopes  $^{13}\text{C}$  and  $^{12}\text{C}$  in atmospheric carbon dioxide are fractionated to different extents during photosynthesis by the  $\text{C}_3$  (Calvin) and  $\text{C}_4$  (Hatch-Slack) pathways. As a result, products from  $\text{C}_3$  plants, including orange juice, have lower  $\delta^{13}\text{C}$  values than do products from  $\text{C}_4$  plants, such as HFCS and cane syrups. Adulterated mixtures possess intermediate ratios. A volume (4) on juice adulteration includes 2 chapters on the application of stable isotope ratio variations (5, 6).

Inexpensive syrups prepared by hydrolysis of sugar beet sucrose cannot be detected in orange juice from  $\delta^{13}\text{C}$  values because sugar beets are  $\text{C}_3$  plants. As a result, such syrups have been added by some processors to circumvent their detection by SCIRA. An isotopic approach was suggested from studies of the deuterium content of beet and wine sugars (7). D/H ratios of carbon-bound atoms in beet sucrose were shown to be consistently lower than the ratio in sugar from wines. It was then found that the difference in  $\delta\text{D}$  values (8, 9) between beet and orange juice sugars is even greater.  $\delta^{18}\text{O}$  values were also different, and a discriminatory formula using ratios of both elements was proposed that would describe with 99.99% confidence the province of pure juices (9). Sample preparation and mass spectrometric measurements are not as straightforward as in SCIRA. Also,  $\delta\text{D}$  determination requires nitration of a dry

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The recommendation was approved by the General Referee and the Committee on Foods II and was adopted by the Official Methods Board of the Association. See "Changes in Official Methods of Analysis" (1993) *J. AOAC Int.*, January issue.

Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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sugar fraction prepared from the juice, so that all exchangeable oxygen-bound D and H atoms are replaced. A suggestion (10) worth evaluating to enhance sensitivity of that approach involves determining  $\delta D$ ,  $\delta^{13}C$ , and  $\delta^{18}O$  values in both the pulp (essentially carbohydrate) and soluble sugar fractions of juice. The ratios in pulp serve as markers for the pure juice, and those in the soluble sugars reflect contributions from both juice and adulterant. Differences in ratios between the 2 fractions greater than those found in pure juices would indicate adulteration.

Isotopic analyses of water have been useful for testing orange juice authenticity.  $\delta D$  and  $\delta^{18}O$  values in ground (tap) water are a function of environmental variables such as temperature and altitude, but they are invariably negative and less than those in plants growing in this water. This provided the basis for tests to distinguish natural fruit juice from those reconstituted from concentrate by addition of ground water (11). Isotope effects associated mainly with evapotranspiration cause most enrichment in the heavy isotopes to be in plant leaf water, but enrichment also occurs in all fruits and vegetables surveyed (12). These studies suggest that ground water associated with the manufacture of products such as beet syrups and orange pulp wash could be detected in a variety of fruit and vegetable juices and used as a marker for adulteration.

### Collaborative Study

The pure frozen juice concentrate (62.6° brix) component of the collaborative samples was prepared after mixing equal proportions of hamlin, valencia, and pineapple orange juices. Medium beet invert syrup (Great Western Sugar Co.) was diluted with water to 62.6° brix and then mixed thoroughly with the orange juice. Mixtures containing 45, 60, 75, and 90% orange

juice were prepared. Two ounces of the mixtures and 2 oz pure orange juice were added to fill nalgene bottles, and the bottles were then sealed with tape and frozen. They were sent via express mail under cold packs to the collaborators.

Sophisticated expertise was required of the collaborators, who possess dedicated sample preparation systems and specialized isotope ratio mass spectrometers. Each of the stable isotope ratio analysis (SIRA) laboratories has established what it considers to be optimal experimental protocol, based on extensive experience. These protocols vary in detail among laboratories. The procedures described in the present method have been demonstrated to be very effective for sample preparation (13) and for determining  $^{18}O/^{16}O$  values.

## 992.09 Sugar Beet-Derived Syrups in Frozen Concentrated Orange Juice— $\delta^{18}O$ Measurements in Water/Stable Isotope Ratio Mass Spectrometric Method

### First Action 1992

(Applicable to classification of frozen concentrated orange juice with  $\delta^{18}O$  value  $< +8.9\%$  as diluted with ground water-prepared product.)

#### Method Performance:

100 and 90% orange juice samples, mean  $\delta^{18}O$  value = 12.24‰

$s_r = 0.88$ ;  $s_R = 1.81$ ;  $RSD_r = 7.2\%$ ;  $RSD_R = 14.8\%$

75 and 60% orange juice samples, mean  $\delta^{18}O$  value = 6.73‰

$s_r = 0.30$ ;  $s_R = 1.76$ ;  $RSD_r = 4.5\%$ ;  $RSD_R = 26.1\%$

#### A. Principle

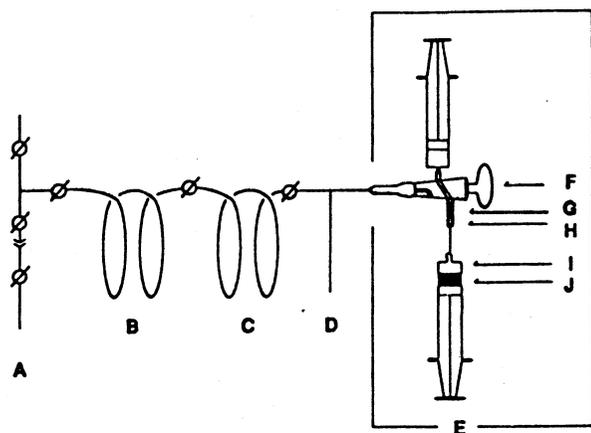
Sample is equilibrated with  $CO_2$  to achieve isotopic equilibrium between oxygen in sample water and oxygen in  $CO_2$ . After equilibration,  $CO_2$  is removed and purified, and  $^{18}O/^{16}O$  is measured by isotope ratio mass spectrometer. Differences in  $\delta^{18}O$  values for pure orange juice concentrate (63–67° brix; mean  $\delta^{18}O_{SMOW} = +14.28\%$ ; SMOW is standard mean ocean water) and ground water used in preparation of sugar beet syrups (usual range of  $\delta^{18}O_{SMOW} = -5$  to  $-10\%$ ) provide a measure of beet syrup in orange juice concentrate.

#### B. Apparatus

(a) *Equilibration system.*—60 mL plastic syringes, 2.5 cm 22 gauge needles, 1.8 cm diam. steel balls, 40 cm diam. wheel to which 12 syringes can be attached and rotated at 10–12 rpm by electric motor.

(b) *Purification system.*—Vacuum-tight glass manifold including liquid nitrogen traps, sample collection bottle, and high vacuum source (Figure 992.09).

(c) *Mass spectrometer.*—Instrument designed or modified for isotope ratio measurement and capable of accuracy of 0.01% of abundance at mass 46 (VG Micromass 602, 903, PRISM VG Instruments Inc., 32 Commerce Centre, Cherry Hill Dr, Danvers, MA 01923, or equivalent).



**Figure 992.09.** Schematic diagram of vacuum line for collection and purification of  $CO_2$ : (A) sample bottle for collection of  $CO_2$ ; (B) multitrap 2; (C) multitrap 1; (D) cold finger; (E) sample inlet, illustrated in detail; (F) 3-way stopcock; (G) capillary glass tubing; (H) silicone rubber septum; (I) equilibrated  $CO_2$  in gaseous phase; (J) water sample.

Table 1.  $\delta^{18}\text{O}$  values (‰) for 63–67° brix orange juices from different growing areas<sup>a</sup>

| Source      | No. of samples | Mean, ‰ | Range, ‰ |       | SD   | CV, % |
|-------------|----------------|---------|----------|-------|------|-------|
|             |                |         | Low      | High  |      |       |
| Florida     | 15             | +14.06  | +12.3    | +16.2 | 1.12 | 8.0   |
| California  | 6              | +14.57  | +12.0    | +17.6 | 2.67 | 18.3  |
| Brazil      | 9              | +15.10  | +12.8    | +18.2 | 1.98 | 13.1  |
| Others      | 4              | +12.87  | +11.5    | +15.0 | 1.43 | 11.1  |
| All samples | 34             | +14.28  | +11.5    | +18.2 | 1.80 | 12.6  |

<sup>a</sup> From reference 14.

### C. Reagents

(a) Pressurized carbon dioxide.—100%.

(b) Reference water standard.—Standard mean ocean water (SMOW).

(c) Carbonate standards.—(1) NIST SRM 20 Solenhofen limestone ( $\delta^{13}\text{C}_{\text{PDB}} = -1.06\text{‰}$ ;  $\delta^{18}\text{O}_{\text{PDB}} = -4.14\text{‰}$ ). (2) NIST SRM 19 TS limestone ( $\delta^{13}\text{C}_{\text{PDB}} = 1.92\text{‰}$ ;  $\delta^{18}\text{O}_{\text{PDB}} = -2.19\text{‰}$ ), or equivalent. (PDB is Pee Dee belemnite limestone standard.)

(d) Phosphoric acid.—100%

### D. Preparation of Sample

Place steel ball in barrel of syringe, and pour 30 mL sample into barrel. Insert plunger and expel as much air as possible from syringe. Attach 2.5 cm 22 gauge needle. Use rubber septum attached to pressurized reservoir of pure  $\text{CO}_2$  to add 20 cc of  $\text{CO}_2$ . Remove syringe from septum, and seal needle by piercing a #000 rubber stopper. Attach syringe to wheel, and rotate in vertical plane at 10–12 rpm for  $\geq 2$  h. Maintain air (sample) temperature at  $25 \pm 0.5^\circ$ . Insert needle into septum on preparation line, Figure 992.09. Transfer  $\text{CO}_2$  from syringe into preparation line; move  $\text{CO}_2$  into evacuated portion of preparation line up to first stopcock, and cool finger with liquid nitrogen. Pump away any air through multitrap 1, also cooled in liquid nitrogen. Water vapor is retained in multitrap 1 and 2, which are cooled with dry ice–acetone slurry. For each sample set, reference water standard (SMOW) should be analyzed 2 $\times$ . Analyze a standard before and after samples.

### E. Determination

Calibrate reference gas of mass spectrometer using at least 2 carbonate standards. React carbonate standards with 100%  $\text{H}_3\text{PO}_4$  at  $25 \pm 0.1^\circ$ . Make any necessary corrections due to instrumental error, such as zero enrichment, peak tailing, or gas mixing. Correct for  $^{17}\text{O}$  contribution to mass 45.

### F. Calculations

Calculate  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  from as follows:

$$\delta^{\text{E}} (\text{‰}) = \left[ \frac{(\text{H}^{\text{E}}/\text{L}^{\text{E}})_{\text{Sample}}}{(\text{H}^{\text{E}}/\text{L}^{\text{E}})_{\text{Std}}} - 1 \right] \times 1000$$

where  $\text{H}^{\text{E}}$  and  $\text{L}^{\text{E}}$  are heavy and light isotopic species of element E, respectively. A frozen orange juice concentrate with  $\delta^{18}\text{O}$  value of  $+15\text{‰}$  would have an  $^{18}\text{O}/^{16}\text{O}$  ratio 15‰ greater

than  $^{18}\text{O}/^{16}\text{O}$  ratio in SMOW and would be “heavy” in  $^{18}\text{O}$  relative to the standard.

Convert analytical data obtained relative to reference gas to PDB scale using the following equation:

$$\delta_{(x-\text{PDB})} = \delta_{(x-\text{B})} + \delta_{(\text{B}-\text{PDB})} + \left\{ (\delta_{(x-\text{B})}) (\delta_{(\text{B}-\text{PDB})}) \right\} 10^{-3}$$

where  $(x - \text{B})$  and  $(x - \text{PDB})$  refer to analysis of sample (x) relative to standard (B) and to PDB, and  $(\text{B} - \text{PDB})$  is analysis of standard (B) relative to PDB; all  $\delta$  values expressed in ‰ (standard B defined as carbon dioxide source used, derived from standard limestone, graphite, or crude oil).

Convert  $\delta^{18}\text{O}_{\text{PDB}}$  values to  $\delta^{18}\text{O}_{\text{SMOW}}$  by adding  $-0.26\text{‰}$ .

Correct sample values by average deviation from expected value found for water standards by adding or subtracting appropriate ‰ value. A  $\delta^{18}\text{O}$  value  $< +8.9\text{‰}$  for sample of frozen concentrated orange juice indicates the presence of ground water-prepared product (typically sugar beet-derived syrups).

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## Results and Discussion

Orange juice is normally stored and transported as 63–67° brix concentrate, and is retailed either as a concentrate at about 42° brix or as single strength juice at about 12° brix. Conversion of single strength juices to 63–67° brix concentrates results in the preferential evaporation of “light water” and causes  $\delta^{18}\text{O}$  values to increase by about 10‰ units. Ground water is almost invariably negative in  $\delta^{18}\text{O}$ , and it is used in the manufacture of beet invert syrups. Adding beet invert syrups to 63–67° brix concentrates causes  $\delta^{18}\text{O}$  values to decrease. The more beet invert syrup added, the lower the resulting  $\delta^{18}\text{O}$  value in the adulterated frozen orange juice concentrate.

Earlier we surveyed  $\delta^{18}\text{O}$  values of water in orange juices (14), and the results for the 63–67° brix orange juice concentrates are listed in Table 1. The mean  $\delta^{18}\text{O}$  value for 34 samples was  $+14.28\text{‰}$ , with a standard deviation of 1.80. We recommend a cutoff of  $+8.9\text{‰}$  for orange juice concentrates over 62° brix. This is 3 standard deviations from the mean, so it is extremely probable that a pure concentrate will have  $\delta^{18}\text{O}$  value greater than  $+8.9\text{‰}$ .

Results of the collaborative study are compiled in Table 2. In all but 2 instances, juices containing more than 10% beet syrup would have been classified as adulterated by the collab-

Table 2.  $\delta^{18}\text{O}$  values (‰) for collaborative samples of beet invert syrup/orange juice mixtures

| Collaborator | Sample   |            |            |            |            |
|--------------|----------|------------|------------|------------|------------|
|              | 5 (pure) | 1 (90% OJ) | 4 (75% OJ) | 2 (60% OJ) | 3 (45% OJ) |
| 1            | +15.4    | +13.1      | +10.2      | +7.2       | +3.7       |
| 2            | +13.1    | + 8.7      | + 6.1      | +3.0       | +0.8       |
| 3            | +11.4    | + 9.6      | + 7.0      | +4.3       | +1.3       |
| 4            | +15.7    | +13.1      | +10.6      | +7.0       | +3.6       |
| 5            | +13.5    | +10.7      | + 8.2      | +5.8       | -0.4       |
| 6            | +11.6    | +11.0      | + 7.0      | +4.4       | -3.0       |

orators, and none would have been misclassified pure juice as adulterated. From these data, the limit in discriminating between false negatives is between 60 and 75% orange juice. Results from the study were analyzed by using a Youden matched pair analysis to estimate repeatability and reproducibility. Samples 1 and 5 were paired, as were samples 2 and 4. Sample 3, with less than 50% pure frozen orange juice concentrate, did not have a matching sample. For samples 5 and 1, the average and estimates of repeatability and reproducibility are as follows: average, 12.24‰;  $s_r$ , 0.88;  $s_R$ , 1.81;  $RSD_r$ , 7.2%;  $RSD_R$ , 14.8%. Values for samples 4 and 2 are as follows: average, 6.73‰;  $s_r$ , 0.30;  $s_R$ , 1.76;  $RSD_r$ , 4.5%;  $RSD_R$ , 26.1%.

Both pure orange juice concentrates (Table 1) and beet syrups possess a range of values. Therefore, from  $\delta^{18}\text{O}$  values in a suspect sample, the level of adulteration cannot be assigned but the degree of certainty as to purity can be. This is also the case for the  $\delta^{13}\text{C}$  adulteration detection methods.

The plot of the mean  $\delta^{18}\text{O}$  values from data reported by all collaborators at each adulteration level has a correlation coefficient greater than 0.999 (Figure 1). The cutoff value of +8.9‰ for pure juices is indicated on the x-axis. The range shown at each adulteration level represents twice the standard deviation at that level and indicates that all adulteration levels were sig-

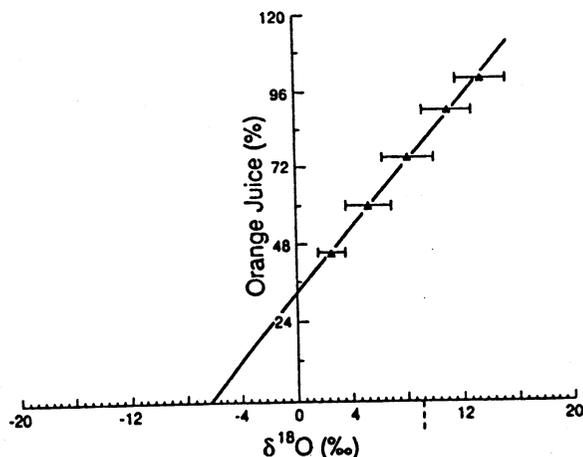


Figure 1. Plot of mean  $\delta^{18}\text{O}$  value for pure product and each level of adulteration of frozen orange juice concentrate with beet invert syrup for all collaborators. Line is described by  $Y = 5.0839X + 32.841$ ;  $r = 0.99907$ .  $\text{—|—} = 2 \times 0.05 \times (s^2/6)^{1/2}$ . Dashes indicate cutoff value at 8.9‰ for pure juices.

nificantly different from one another. The intercept at 0% orange juice is -6.2‰, a  $\delta^{18}\text{O}$  value typical for pure beet invert syrups (14).

### Recommendation

We recommend that the stable isotope ratio mass spectrometric method for detection of sugar beet-derived syrups in frozen concentrated orange juice by using  $\delta^{18}\text{O}$  measurements in water be adopted first action.

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