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Detecting Sugar Beet Syrups in Orange Juice by D/H and $^{18}O/^{16}O$ Analysis of Sucrose

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Sucrose from pure orange juices and beet sugar syrups was isolated by preparative HPLC. A portion was converted to sucrose octanitrate, to remove the readily exchangeable hydroxyl hydrogens. D/H ratios were then determined on the carbon-bound hydrogen atoms by mass spectrometric stable-isotope ratio analysis. The mean D/H ratios in orange sucrose were significantly elevated compared to those in beet sucrose, the δ(D) values being −27 and −143/mil respectively. $^{18}O/^{16}O$ ratios were determined for underivatized sucrose from both soures. With a single exception, $\delta(^{18}O)$ values were higher in orange sucrose than in beet sucrose. From these data, a discriminatory formula has been developed describing a 99.99% confidence ellipse about pure orange juices. The availability of this method to identify orange juices as pure or adulterated with beet sugar will provide a disincentive for the economic adulteration of orange juice.

Variations in the natural abundance of stable isotopes in plant components provide the means whereby adulteration of food products can be identified. Carbon isotopes have been especially useful in this regard. Most plants that are cultivated for food utilize the C_3 photosynthetic mode, with two major exceptions being the plants sugar cane and corn, which use the C_4 mode. C_4 plants and their metabolites are substantially enriched in ^{13}C compared to C_3 plants. Analysis of $^{13}C/^{12}C$ ratios thus readily identifies food products derived from C_3 plants that have been adulterated by the undeclared addition of low-cost sugar cane and corn-derived syrups. These adulterants bring no unique components to the mixture, and their sugar profiles are within the range found in most juices. So conventional nonisotopic analytical approaches cannot discriminate between pure and adulterated samples. Application of carbon isotope analysis by enforcement agencies has largely discouraged the addition of C_4 plant derived cane or high-fructose corn syrups to maple syrup (Hillaire-Marcel et al., 1977), apple juice (Doner et al., 1980), orange juice (Nissenbaum et al., 1974; Doner and Bills, 1982), honey (Doner and White, 1977), and fermented beverages (Bricout, 1982).

Carbon isotope analysis is not useful for identifying C_3 plant food products adulterated with inexpensive invert syrups prepared from the C_3 plant sugar beet, and no other useful procedures are available. This is now the major form of orange juice adulteration in the United States. The market value of orange juice is about $2 billion in the United States alone, so the economic impact of such adulteration is significant. Water in orange juice has higher deuterium and oxygen-18 concentrations than sugar beet invert syrup watter. This observation has permitted development of an isotopic method for detecting beet invert syrups added to orange juice (Brause et al., 1984). However, the availability of fourth-stage condensate water from orange juice concentrate production, enriched in oxygen-18 and deuterium, that can be added to orange juice along with sugar beet invert syrup has rendered this method of detecting adulteration ineffective.

We describe here a method that permits identification of orange juice samples to which have been added sugar beet invert syrups. The method is based on D/H and $^{18}O/^{16}O$ ratios in sucrose.

EXPERIMENTAL SECTION

Preparative HPLC of Sucrose from Orange Juice and Beet Invert Syrups. Prior to HPLC of orange juice, 25 mL of single-strength juice or 5 g of concentrate diluted to 10 mL with water was deionized by passing through a column of Amberlite MB-3 resin (50 mL). The column was rinsed with 50 mL of water, and the column eluant was lyophilized and dissolved in water to give 500 mg/mL solutions, which consisted primarily of the orange juice sugars fructose, glucose, and sucrose. The deionization step was unnecessary for the beet invert syrup samples (about 70° Brix). For these, 1.2 g of syrup was diluted with 1.8 mL of water. Of the diluted beet syrups or deionized orange juice samples 500 μL was injected onto an Aminex Q-15S (Ca^{2+} form) column (2.2 × 30 cm), which was then eluted at 2.0 mL/min with water at 85°C. Eluted peaks were monitored by refractive index detection, and the first peak (sucrose, retention time 15 min) was collected and

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Table I. $\delta(^{18}O)$ and $\delta(D)$ Values (Given per Mil) of Sucrose and Sucrose Octanitrate Prepared from Beet Sugar Products and Orange Juice

sample			description	$\delta(^{18}O)$	$\delta(D)$
beet	A	Alberta, Canada	50% invert syrup	+25.0	−172
	B	Alberta, Canada	50% invert syrup	+26.5	−178
	C	MN	95% invert syrup	+24.4	−144
	D	MN	crystalline sucrose	+26.6	−157
	E	MN	crystalline sucrose	+26.9	−142
	F	MN	50% invert syrup	+26.8	−140
	G	MN	liquid sucrose syrup	+27.3	−134
	H	MN	50% invert syrup	+28.7	−150
	I	MI	50% invert syrup	+26.7	−134
	J	NE, CO, WY	crystalline sucrose	+26.7	−149
	K	NE, CO, WY	50% invert syrup	+29.6	−130
	L	TX	50% invert syrup	+30.8	−108
	M	CA	50% invert syrup	+35.2	−126
orange	N	FL	varietal blend, 45° Brix	+31.7	−23
	O	FL	varietal blend, 45° Brix	+32.1	−24
	P	FL	varietal blend, 45° Brix	+33.2	−31
	Q	FL	varietal blend, 45° Brix	+33.5	−31
	R	FL	varietal blend, 45° Brix	+34.0	−24
	S	FL	varietal blend, 45° Brix	+36.2	−43
	T	TX	varietal blend, 65° Brix	+34.8	−29
	U	TX	Mars, single strength	+35.2	−13
	V	TX	Valencia, single strength	+35.6	−15
	W	TX	Hamlin, single strength	+36.4	−16
	X	CA	Navel, 60° Brix	+33.6	−42
	Y	CA	Valencia, single strength	+37.1	−28
	X	CA	Valencia, single strength	+37.6	−31
	Z′	CA	Valencia, single strength	+38.3	−24

		mean	range	SD
beet	$\delta(^{18}O)$	+27.8	+24.4 to +35.2	2.8
	$\delta(D)$	−143	−178 to −108	19
orange	$\delta(^{18}O)$	+34.9	+31.7 to +38.3	2.0
	$\delta(D)$	−27	−43 to −13	9.0

lyophilized. Orange juice and beet invert syrup samples typically yielded 50–100 mg of sucrose/injection, which crystallized upon evaporation. The entire sucrose peak was collected in order to avoid isotope fractionation during chromatography, as confirmed by analysis of pure sucrose before and after chromatography and of sucrose nitrate prepared from these two sucrose samples. No exchange of deuterium and oxygen between sucrose and the water eluant occurred during chromatography, as identical $\delta(^{18}O)$ and $\delta(D)$ values were obtained when samples were prepared with water that differed by over +70/mil in $\delta(^{18}O)$ values and +200/mil in $\delta(D)$ values.

Mass Spectrometric Determination of D/H and $^{18}O/^{16}O$ Ratios. $^{18}O/^{16}O$ ratios in sucrose were determined after pyrolysis to carbon dioxide by the method of Rittenberg and Ponticorvo (1956) as modified by Burk (1979). For D/H ratio analysis, exchangeable hydroxyl hydrogens in sucrose were removed by nitration with nitric acid in the presence of acetic acid and acetic anhydride (Dunbar and Schmidt, 1984). Element ratios (C/N, H/N, C/H) in sucrose octanitrate were close to that expected for $C_{12}H_{14}N_8O_{27}$, and the natural-abundance nuclear magnetic resonance spectrum showed the chemical shifts and carbon resonance ratios consistent with the octanitrate structure. D/H ratios of the sucrose octanitrate were determined as described previously for cellulose nitrate (Northfelt et al., 1981), after combustion water was reduced to hydrogen. Isotope ratios are expressed as δ values

$$\delta = [(R_{sample}/R_{std}) - 1] \times 1000 \text{ per mil}$$

where R represents $^{18}O/^{16}O$ for $\delta(^{18}O)$ values and D/H for $\delta(D)$ values. The standard for hydrogen and oxygen is the standard mean ocean water (SMOW).

Replicate analyses of a beet sucrose sample gave $\delta(^{18}O)$ values of +27.1, +26.8, +26.8, +27.0, and +27.7/mil; for sucrose octanitrate, $\delta(D)$ values of −141, −141, −140, −137, and −143/mil were obtained.

RESULTS AND DISCUSSION

The method we describe is based on two observations: First, the D/H and $^{18}O/^{16}O$ ratios of ground water in the warm regions where oranges are produced are substantially higher than those in the colder areas where most sugar beets are grown. The differences in $\delta(D)$ and $\delta(^{18}O)$ values between the two types of ground waters are on the order of 80/ and 10/mil, respectively (Taylor, 1974; Craig, 1961). Second, the hydrogen and oxygen isotopic compositions of plant cellulose (and probably other carbohydrates) reflect the isotopic composition of the ground water available to the plants (Epstein et al., 1976, 1977; DeNiro and Epstein, 1979). Because the differences in $\delta(D)$ and $\delta(^{18}O)$ values of cellulose among different species of C_3 plants that grow together in the field are generally smaller than the differences in the isotopic composition of ground waters where oranges and sugar beets are grown (Sternberg and DeNiro, 1983; Sternberg et al., 1984), we expected that the isotope ratios of carbohydrate from the two sources would be substantially different.

$\delta(^{18}O)$ and $\delta(D)$ values of sucrose and sucrose octanitrate prepared from orange and sugar beet sources produced in the significant North American growing areas are given in Table I. Also included in Table I are results for various items of commerce, including single-strength and concentrated orange juices, varietally pure and mixed orange juices, and pure and inverted beet sucrose samples. Sucrose samples from sugar beets are significantly depleted in carbon-bound deuterium relative to the values obtained for orange juice sucrose, with the mean values differing by 116/mil. The $\delta(D)$ difference between beet and orange sucrose octanitrate was observed even when both were grown in climatically similar regions, as shown for samples from Texas and California. As a result, sucrose from adulterated juices will possess intermediate $\delta(D)$ values,

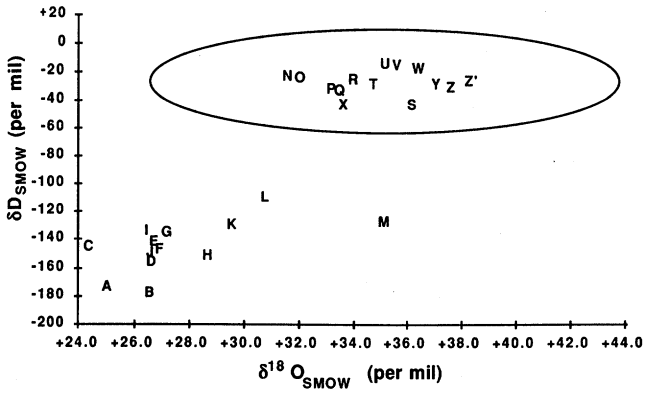

Figure 1. δ(D) values of sucrose octanitrate plotted against $\delta(^{18}O)$ values of sucrose for beet- (A–M) and orange- (N–Z′) derived samples. Data point designations correspond to sample letters in Table I. The orange juice samples are surrounded by the 99.99% confidence ellipse described by the discriminatory functions discussed in the text.

since the values in mixtures will be the weighted sums of the δ(D) values of the components. Classification of pure and adulterated juices will be enhanced by the finding that the $\delta(^{18}O)$ values for sucrose from beet sugar products were, with a single exception, less than the values for orange juice sucrose (Table I).

In Figure 1, δ(D) values are plotted against $\delta(^{18}O)$ values. A discriminatory formula

$$\left[\frac{\delta(^{18}O) - 34.9/\text{mil}}{1.96/\text{mil}}\right]^2 + \left[\frac{\delta(D) + 27/\text{mil}}{8.60/\text{mil}}\right]^2 < 18$$

has been developed that describes the 99.99% confidence ellipse about the orange sucrose data points. Pure orange juices will give a value of less than 18, with only one chance in 10000 that sucrose from a pure orange juice will generate a point falling outside of this ellipse. From Figure 1, it is apparent that sucrose from sugar beets grown in colder areas, such as Alberta and Minnesota, is depleted in both D and ^{18}O compared to sucrose from sugar beets grown in warmer areas. This trend is especially notable for δ(D) values, so sugar from beets grown in northern areas will be detected in orange juice with greater sensitivity.

The approach described in this report will provide regulatory agencies with the means to detect the adulteration of orange juices with beet invert syrups and thereby minimize the economic impact of such practices. δ(D) values of sugars isolated from German wines and then nitrated have been used to detect samples to which beet sugar had been added (Dunbar et al., 1983), and such measurements have promise for detecting beet sugar addition to other C_3 plant derived syrups. For example, an apple sucrose sample from Pennsylvania we analyzed had $\delta(^{18}O)$ and δ(D) values of +27.3 and −58/mil, suggesting that δ(D) measurements have potential for identifying adulterated apple juices.

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