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Control of the Authenticity of Orange Juice by Isotopic Analysis

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Control of the authenticity of orange juices involves the detection of undeclared addition of sugar. The $^{13}\text{C}/^{12}\text{C}$ ratio of sugar from orange juice is lower than the $^{13}\text{C}/^{12}\text{C}$ ratio in cane or corn sweetener but similar to the $^{13}\text{C}/^{12}\text{C}$ ratio in beet sugar. For this reason, we have determined the $^2\text{H}/^1\text{H}$ ratio of nonexchangeable carbon-bound hydrogen in sugar from orange and beet. The difference in $\delta(^2\text{H})$ of these two sources of sugars is quite large and reflects probably the difference of climate in which these two plants grow.

In Western Europe, orange juices are generally prepared by redilution of concentrates imported from different countries of tropical or subtropical areas. It is necessary to have the ability to verify the purity of orange concentrate and to detect adulteration. Many analytical and statistical methods have been proposed for this purpose (Bielig et al., 1984; Richard and Coursin, 1980; Vandercook et al., 1983); generally, the concentrations of different elements are measured and those results compared to a data base, originally established by analyzing pure juices of different origins. This comparison can be made by different statistical tests (Brown and Cohen, 1983). However, the analytical parameters of oranges are generally not unique but are substances that occur in many types of fruits and vegetables, and are available as cheap chemicals.

In recent years, it was suggested that the isotopic composition of different constituents of our food can be used for the determination of their origin (Bricout, 1982). For example, the isotopic ratio $^{13}\text{C}/^{12}\text{C}$ can be used to detect the addition of synthetic vanillin to vanilla extract (Bricout et al., 1974) or of high-fructose corn syrup or cane sugar to honey (White and Doner, 1978), apple juice (Doner et al., 1980), and orange juice (Doner and Bills, 1980).

The $^{18}\text{O}/^{16}\text{O}$ and $^2\text{H}/^1\text{H}$ ratios of water allow a confident distinction between natural fruit juices (particularly orange) and reconstituted juices (Bricout, 1973). The en-

richment in ^2H and ^{18}O of plant water as compared to rain and ground water is due to isotopic fractionation by evapotranspiration. It was shown that this enrichment is higher when the climate is dry and warm (Lesaint et al., 1974), a climatic situation that occurs in some orange-producing areas. A further extension of this observation was the detection of sugar syrup or pulp wash to orange concentrate by the determination of the $^{18}\text{O}/^{16}\text{O}$ ratio in the water of orange concentrate (Brause et al. 1984) as sugar syrup and pulp wash as manufactured with ground water.

We can anticipate that the $^2\text{H}/^1\text{H}$ and the $^{18}\text{O}/^{16}\text{O}$ ratios of plant water can influence the isotopic composition of plant organic matter. $^2\text{H}/^1\text{H}$ ratios were used to distinguish natural aromatic substances from their synthetic equivalents (Bricout and Koziat, 1978). It was shown that orange sugars have higher $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$ ratios than beet sugar (Bricout, 1978). However, some experimental difficulties were encountered during these first analyses. The $^{18}\text{O}/^{16}\text{O}$ analysis of sugar is difficult and the $^2\text{H}/^1\text{H}$ ratio of sugar is not constant due to an isotopic exchange between water and hydrogen bound to oxygen. This difficulty can be overcome by nitration, which removes all the exchangeable hydroxyl hydrogen atoms. This method was first developed for cellulose analysis (Epstein et al., 1976) and was extended for grape sugar (Dunbar et al., 1983). The results obtained on plant cellulose have shown a low sensitivity of $^{18}\text{O}/^{16}\text{O}$ ratio of cellulose to source water (Epstein et al., 1977).

For these reasons, we have examined the $^2\text{H}/^1\text{H}$ ratio in nitrated sugars from oranges of known origin and in

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Table I. Isotopic Composition of Sugars of Orange Juice

country	variety	date of processing	$\delta(^{13}\text{C}), \text{‰ PDB}$		$\delta(^2\text{H}), \text{‰ SMOW}$ of nitrate esters
			sugars	pulp	
Israel	Shamouti	12-17-84	-25.1	-25.1	-24
		12-23-84	-25.0	-25.4	-27
		12-27-84	-24.8	-25.4	-23
		1-01-85	-24.8	-25.7	-21
		1-09-85	-25.1	-25.7	-30
		1-10-85	-25.0	-25.8	-27
		1-14-85	-24.9	-24.9	-25
		1-21-85	-24.9	-25.5	-25
		1-23-85	-24.9	-25.5	-25
		1-23-85	-24.6	-24.9	-18
		1-28-85	-24.7	-25.3	-20
		2-07-85	-24.4	-25.5	-18
		2-24-85	-24.5	-24.1	-15
		Feb 1985	-25.4	-25.8	-44
		Feb 1985	-24.9	-25.5	-27
		3-06-85	-24.9	-24.9	-14
		3-12-85	-24.9	-25.4	-10
	3-21-85	-24.7	-25.2	-2	
	3-26-85	-24.9	-25.5	-15	
	3-28-85	-25.1	-25.3	-12	
	Valencia	4-15-85	-24.7	-25.3	-25
		4-17-85	-24.7	-25.4	-20
		4-21-85	-24.6	-25.2	-10
		4-22-85	-24.7	-25.3	-14
		4-28-85	-24.9	-25.5	-13
		5-03-85	-25.9	-25.6	-12
5-06-85		-24.9	-25.7	-8	
5-12-85		-23.8	-25.0	-14	
5-23-85		-24.3	-24.8	-21	
U.S. (FL)	Hamlin	10-24-84	-26.2	-26.4	-31
	Hamlin	1-31-85	-24.8	-25.5	-30
	Parson Brown	10-25-84	-26.0	-26.9	-17
	Parson Brown	1-31-85	-25.6	-26.5	-17
	Valencia	2-14-85		-26.9	-32
	Valencia	4-25-85	-23.5	-24.9	-20
	Temple	2-14-85	-26.5	-26.9	-35
Brazil	Dancy Tangerine	11-15-84	-26.5	-26.5	-40
		4-12-85	-28.1	-28.1	-26
		9-04-81	-26.9	-26.9	-32
			-26.3	-26.6	-49
mean			-25.1	-25.6	-22.1
std dev			0.9	0.8	10

industrial sugars. The $^{13}\text{C}/^{12}\text{C}$ ratios were also measured.

EXPERIMENTAL SECTION

Pure single-strength orange juices were obtained from the Ministry of Industry in Israel, along with industrial sugar available in Israel. Orange concentrate from Florida and Brazil and industrial sugar from the United States and Canada were obtained from the Florida Citrus Commission.

^{13}C Analysis. Juice or concentrate rediluted to 11.5° Brix was centrifuged. The pulp was washed with acetone and dried under vacuum. The supernatant fluid was clarified by adding Carrez I and II reagent and centrifuged. The clarified juice was freeze-dried. The freeze-dried material (10–20 mg) was combusted in pure oxygen and the CO_2 analyzed with a mass spectrometer VG Micromass 602C for the determination of the $^{13}\text{C}/^{12}\text{C}$ ratio. Similarly, the dried pulp was combusted and analyzed. The results are expressed as $\delta(^{13}\text{C})$ defined as parts per thousand difference in the $^{13}\text{C}/^{12}\text{C}$ ratio of a sample from that in Standard Pee Dee Belemite (Craig, 1957):

$$\delta(^{13}\text{C}) = [(^{13}\text{C}/^{12}\text{C})_{\text{sample}} / (^{13}\text{C}/^{12}\text{C})_{\text{std}} - 1] \times 1000$$

The industrial sugars in either dry or syrup form were directly analyzed.

^2H Analysis. Of the freeze-dried solids obtained after clarification of the juice with Carrez reagent 1 g was ni-

trated with nitric acid in the presence of acetic anhydride and acetic acid according to Dunbar (1983). The precipitate of nitrate esters of sugars was washed with water, dried under vacuum, and combusted in an atmosphere of pure oxygen to CO_2 , H_2O , and nitrogen oxides. Water purified by cryodistillation was allowed to pass over hot uranium, and the liberated H_2 was collected and analyzed for the $^2\text{H}/^1\text{H}$ ratio on a VG Micromass 602C mass spectrometer. Industrial sugars were processed after vacuum drying. The $^2\text{H}/^1\text{H}$ ratio is expressed relative to that of VSMOW [Vienna Standard Mean Ocean Water (Craig, 1961)] according to

$$\delta(^2\text{H}) = \left[\frac{(^2\text{H}/^1\text{H})_{\text{sample}}}{(^2\text{H}/^1\text{H})_{\text{std}}} - 1 \right] \times 1000$$

RESULTS AND DISCUSSION

The results of the isotopic analysis of orange juices are presented in Table I and the results of sugars in Table II. $\delta(^{13}\text{C})$ values of sugars of orange juice are in the range expected for C3 plants from $\delta(^{13}\text{C}) = -28.0$ to $\delta(^{13}\text{C}) = -23.5\text{‰ PDB}$. These results confirm the earlier studies (Doner and Bills, 1981). The pulp, cautiously washed with acetone to eliminate lipids, has a slightly more negative value than sugar. The difference is generally less than 1.5‰, and thus the detection of the illicit addition of cane

Table II. Isotopic Composition of Industrial Sugars

country	origin	$\delta(^{13}\text{C})$, ‰ PDB	$\delta(^2\text{H})$, ‰ SMOW of nitrate esters
Israel			
1	beet	-26.6	-163
2	beet	-25.7	-151
3	cane	-11.3	
4	beet	-25.9	-153
5	cane	-11.6	
6	cane	-11.2	
7	cane	-11.4	
8	beet	-26.0	-141
9	beet	-26.0	-146
Canada			
Alberta	beet	-25.2	-160
U.S.			
MI	beet	-25.8	-127
IL	beet	-24.7	-122
GA	cane	-11.7	-63
CA	beet	-25	-131
TX	beet	-24.6	-109
	beet	-24.7	-163
	beet	-24.8	-144
France	beet	-26.0	-116
	corn	-13.5	-31
	cane	-11.4	-41

or corn sweetener can be enhanced, as indicated by Parker (1982). We can calculate that the addition of 15 g of cane sugar [$\delta(^{13}\text{C}) = -11\text{‰}$] to 10° Brix orange juice [$\delta(^{13}\text{C}) = -25\text{‰}$] will result in a product with $\delta(^{13}\text{C}) = -23.2\text{‰}$ PDB. This value can be acceptable for true orange juice as stated by Brause (1984), but the difference between $\delta(^{13}\text{C})$ of sugar and pulp would be certainly more than 2‰, a value that seems objectionable.

The results obtained by deuterium analysis of the nitrate esters of sugars are more interesting. If we consider the beet sugar of North America, we will observe a gradient of deuterium concentration with latitude from $\delta(^2\text{H}) = -160\text{‰}$ for Canada to $\delta(^2\text{H}) = -122$ and 127 for Illinois and Michigan and $\delta(^2\text{H}) = -109\text{‰}$ for Texas. This observation confirms that $\delta(^2\text{H})$ of the nitrate of sugars is affected by climatic conditions (Epstein et al., 1977). It is not surprising that orange sugars show a less negative $\delta(^2\text{H})$ value than beet sugars. $\delta(^2\text{H})$ values of nitrate esters of orange sugars are in the range $-50 < \delta(^2\text{H}) < 0\text{‰}$ SMOW. The results obtained on Israeli orange juice show a slight increase of $\delta(^2\text{H})$ with the season. Until end of January, $\delta(^2\text{H})$ values of nitrate esters of sugars are between -30 and -20‰, but from February until end of May, $\delta(^2\text{H})$ variation is between -20 and -10‰ with only one exception. The nitrate esters of sugars of orange juice from Brazil and Florida show generally lower values than juice from Israel, which seems to relate to the drier climate of Israel.

Cane sugar and high-fructose corn syrup show higher $\delta(^2\text{H})$ values than beet sugar (Table II) and similar values to orange juice. Two factors may contribute to this observation. Cane and corn grow generally in warmer climate than beet, and it was also observed that C4 plants contain more deuterium in their organic material than do C3 plants (Ziegler et al., 1976), even when they grow in the same climatic conditions. We have shown that, in the same greenhouse, leaf water of corn is more enriched in ^2H and ^{18}O as compared to soil water than tomato leaf (Lesaint et al., 1974), and originally all the hydrogens of plant organic material are derived from leaf water. On the other hand, the similarity in $\delta(^2\text{H})$ content of sugar from orange

and from corn or cane is not a drawback as the difference in ^{13}C content is large enough.

It is interesting to compare the $\delta(^2\text{H})$ of the nitrate esters of sugars with the values published on nitrate esters of cellulose of different plants (Epstein et al., 1977). Epstein et al. observed a range of $\delta(^2\text{H})$ for cellulose nitrate from -180‰ (Yukon territory) to -1‰ SMOW (Florida). The following values are close to our data: -160‰ for beet sugar of Canada; -17‰ for Parson Brown Orange from Florida. All those experimental data have led to the conclusion that $\delta(^2\text{H})$ of the nitrate ester of sugar or cellulose is related to the $\delta(^2\text{H})$ of leaf water and therefore to the climate, although we cannot ignore some characteristic effects of the plant itself.

In conclusion, we can assume that careful examination of the isotopic composition of orange sugars [$\delta(^{13}\text{C})$ and $\delta(^2\text{H})$ of nitrate esters] allows the detection of the addition of sugar from beet, cane, or corn, the main sources of industrial sugar.

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