

## Use of Isotopic Analyses To Determine the Authenticity of Brazilian Orange Juice (*Citrus sinensis*)

A. M. Pupin,<sup>\*,†</sup> M. J. Dennis,<sup>‡</sup> I. Parker,<sup>‡</sup> S. Kelly,<sup>‡</sup> T. Bigwood,<sup>‡</sup> and M. C. F. Toledo<sup>§</sup>

Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas (CPQBA), Universidade Estadual de Campinas, C.P. 6171, Campinas, SP CEP 13081-970, Brazil, CSL Food Science Laboratory, Norwich Research Park, Colney, Norwich NR4 7UQ, United Kingdom, and Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, C.P. 6121, Campinas, SP CEP 13081-970, Brazil

Isotopic analysis was used to characterize authentic samples of orange juice (*Citrus sinensis*) from Brazil. Site specific natural isotopic fractionation nuclear magnetic resonance (SNIF-NMR) was used to determine deuterium/hydrogen ratios at the methyl [(D/H)<sub>I</sub>] and methylene [(D/H)<sub>II</sub>] sites of ethanol produced by fermentation of orange juice. Stable isotope ratio mass spectrometry (SIRMS) was used to determine the ratio of carbon isotopes (<sup>13</sup>C/<sup>12</sup>C) in the same ethanol and the ratio of oxygen isotopes (<sup>18</sup>O/<sup>16</sup>O) in the citrus juice water. The mean ratios found for these parameters in authentic hand-squeezed orange juice were as follows: (D/H)<sub>I</sub>, 102.3 ppm (SD = 1.7); (D/H)<sub>II</sub>, 126.5 ppm (SD = 1.8); <sup>13</sup>C/<sup>12</sup>C,  $\delta^{13}\text{C} = -26.6\text{‰}$  PDB (SD = 0.9); and <sup>18</sup>O/<sup>16</sup>O,  $\delta^{18}\text{O} = +2.27\text{‰}$  SMOW (SD = 2.48). Retail samples taken from the Brazilian market place were evaluated by comparison against these data. No evidence was found for the addition of sugar to orange juice or for the dilution with tap water of samples labeled as freshly squeezed.

**Keywords:** Authenticity; orange juice; SNIF-NMR; SIRMS; isotopic analysis; D/H; <sup>13</sup>C/<sup>12</sup>C; <sup>18</sup>O/<sup>16</sup>O

### INTRODUCTION

The internal retail market for orange juice in Brazil is mainly based on freshly squeezed orange juice (FSOJ). Because certain sweeteners such as cane, corn, and beet sugars cost less than FSOJ, they could be added to the juice to illegally extend it in conjunction with the addition of groundwater. Juice that has been adulterated with these sweeteners and sold as 100% pure without the declared addition of these compounds on the label may result in prosecution by regulatory agencies.

The isotopic composition of sugars and water depends on both plant variety and geographic origin. Thus, addition of these materials can often be detected by measuring isotope ratios of the fruit juice sugars and water. The <sup>13</sup>C/<sup>12</sup>C ratio of orange juice sugars differs from that derived from cane and corn sugars. The difference originates from the way the sugars are synthesized by the plant. Sugar cane and corn belong to a group of plants that use a C4 Hatch–Slack pathway to synthesize sugars. Beet sugar and citrus plants use the C3 Calvin cycle. As a result, different amounts of <sup>13</sup>C are found in products derived from C3 and C4 plants. Typical values of  $\delta^{13}\text{C}$  for orange juice and sugar cane are  $-26.4$ , and  $-11.4\text{‰}$  PDB, respectively (Bricout and Koziet, 1985). A review of these cycles can be found in work published by O'Leary (1988).

At the beginning of the 1980s Martin et al. (1982, 1983) proposed a new method to distinguish between

certain types of sugars. The analysis measures ethanol obtained from fermentation of sugars and starches. The method is based on the measurement of the ratio of deuterium/hydrogen (D/H) at the methyl position [defined as (D/H)<sub>I</sub>] and methylene position [defined as (D/H)<sub>II</sub>] of the ethanol. The measurements are performed by <sup>2</sup>H nuclear magnetic resonance spectroscopy (NMR). It has been established that the (D/H)<sub>I</sub> ratios of ethanol are related to the sugars used for fermentation and the water in which this occurs. They can thus be used to determine the plant from which the sugars were obtained. For example, ethanol from apple has a typical (D/H)<sub>I</sub> of 100.9 ppm, and ethanol from sugar beet has a (D/H)<sub>I</sub> of 94.1 ppm (Martin et al., 1982). Therefore, if sugar beet has been added to apple juice, the <sup>2</sup>H NMR analysis will detect this adulteration. This method is called site specific natural isotopic fractionation nuclear magnetic resonance (SNIF-NMR) and has been adopted by the AOAC as the standard method for detecting beet sugar addition to fruit juice (Martin et al., 1996a).

Water from citrus juice also has particular isotopic properties, and stable isotope analysis has been used since the early 1970s to detect adulteration in fruit juice. The heavier isotope of oxygen (<sup>18</sup>O), which accounts for 0.2% of all oxygen in the air, becomes slightly more concentrated in the water of growing plants due to the phenomena of evapotranspiration. As a consequence, it is possible to distinguish between groundwater and fruit juice water. Bricout (1971, 1973) and Bricout et al. (1972) proposed measuring the oxygen isotopic ratio (<sup>18</sup>O/<sup>16</sup>O) to distinguish natural orange juice from juices reconstituted from groundwater and concentrate. Bricout (1973) found that water of orange juice from Brazil has a value for  $\delta^{18}\text{O}$  of  $+5\text{‰}$  and groundwater has a value for  $\delta^{18}\text{O}$  of  $-8\text{‰}$ .

\* Author to whom correspondence should be addressed.

† Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas.

‡ CSL Food Science Laboratory.

§ Faculdade de Engenharia de Alimentos.

Data for the analysis of  $^{13}\text{C}/^{12}\text{C}$  and D/H in orange juice are available in several works published in the literature (Bricout, 1971, 1973; Bricout et al., 1972; Nissenbaum et al., 1974; Donner and Bills, 1981, 1982; Brause et al., 1984; Bricout and Koziat, 1985, 1987; Doner et al., 1987; Tateo and Martin, 1992; Martin et al., 1996a,b). Nevertheless just a few have used the technique of SNIF-NMR to verify the authenticity of orange juice (Tateo and Martin, 1992; Martin et al., 1996a,b). The majority of the works cited involved either the analysis of frozen concentrated orange juice (FCOJ) or, in a small number of cases, freshly squeezed orange juices (FSOJ) from different countries including Brazil. However, the varieties tested were not specified.  $^{18}\text{O}/^{16}\text{O}$  ratios of FSOJ from Brazil were reported by Bricout et al. (1972) and Bricout (1973). According to Steger (1990), Pera and Natal varieties constitute 71% of the total orange production in Brazil. At present there are no data available for the isotopic ratios of the most important citrus varieties used for producing FSOJ or the resulting retail samples on the Brazilian market. Without monitoring the retail market using  $^{18}\text{O}/^{16}\text{O}$ ,  $^{13}\text{C}/^{12}\text{C}$ , D/H analyses, it is possible that the addition of groundwater, cane, corn, and beet sugars is going undetected.

Both the stable isotope ratio mass spectrometry (SIRMS) and SNIF-NMR methods rely on comparison with authentic samples. Therefore, a database of isotopic measurements from authentic juices is required.

The main objective of this work was to determine the isotopic ratios of  $^{13}\text{C}/^{12}\text{C}$ , (D/H)<sub>I</sub>, (D/H)<sub>II</sub>, and  $^{18}\text{O}/^{16}\text{O}$  in authentic samples of orange juice from Brazil. These authentic sample data were then used to assess the authenticity of samples of orange juice from the Brazilian retail market.

## MATERIALS AND METHODS

**Samples.** Authentic samples of different varieties of oranges as well as commercial concentrated orange juice and water-extracted pulp (also called pulp wash) were collected from orange-processing plants in the state of São Paulo (Brazil). Retail samples (FCOJ and FSOJ) were purchased from supermarkets in the metropolitan area of Campinas (state of São Paulo, Brazil), during the years of 1995–1996.

**Sample Preparation.** The citrus fruits were hand-squeezed and the juices filtered through a stainless steel sieve (1.25 mm). All juices were stored at  $-20\text{ }^{\circ}\text{C}$  until required for analysis. Before analysis, FCOJ and frozen concentrated orange pulp wash (FCOPW) were diluted to 12 °Brix with Norwich tap water, which has a known D/H value of 148.8 ppm.

**SNIF-NMR.** *Fermentation, Distillation, and Sample Preparation.* The analysis of samples was conducted according to the method described by Martin et al. (1996a).

**Equipment.** A Bruker ARX 500 NMR (Spectra Spin, Switzerland) fitted with a specific 10 mm "deuterium" probe was used. The  $^2\text{H}$  spectra were recorded at 76.77 MHz with proton decoupling and a fluorine ( $^{19}\text{F}$ ) lock. A  $90^{\circ}$  pulse was used and an acquisition time of at least  $5T_1$ .

**Calculations.** The isotopic ratios were determined from the methyl (D/H)<sub>I</sub> and methylene (D/H)<sub>II</sub> sites of the ethanol molecule using deuterium NMR. The quantitative data were obtained by direct comparison of the sample peak with the internal standard, tetramethylurea (TMU), of known D/H value. The isotopic ratios at the two ethanol sites were determined according to eqs 1 and 2 [Commission Regulation (EEC) No. 2676/90]

$$\left(\frac{\text{D}}{\text{H}}\right)_{\text{I}} = 1.5866 T_{\text{I}} \frac{m_{\text{st}} (\text{D/H})_{\text{st}}}{m_{\text{A}} \rho_{\text{m}}} \quad (1)$$

$$\left(\frac{\text{D}}{\text{H}}\right)_{\text{II}} = 2.3799 T_{\text{II}} \frac{m_{\text{st}} (\text{D/H})_{\text{st}}}{m_{\text{A}} \rho_{\text{m}}} \quad (2)$$

where  $T_{\text{I}}$  = [height of signal I ( $\text{CH}_2\text{DCH}_2\text{OH}$ )/height of TMU signal],  $T_{\text{II}}$  = [height of signal II ( $\text{CH}_3\text{CHDOH}$ )/height of TMU signal],  $(\text{D/H})_{\text{st}}$  = isotope ratio of TMU provided by the supplier (e.g. 132.83 ppm),  $m_{\text{st}}$  = mass of TMU,  $m_{\text{A}}$  = mass of sample, and  $\rho_{\text{m}}$  = alcoholic strength by mass of the distillate from automated distillation control system (ADCS) (% m/m).

**SIRMS  $^{13}\text{C}/^{12}\text{C}$ .** The carbon isotope measurements were performed by combustion of the ethanol obtained from the distillation process. Data were compared with pulses of carbon dioxide reference gas of accurately known carbon isotope value.

**Equipment.** An elemental analyzer EA 1108 (Carlo Erba Instruments, Milan, Italy) was coupled to a Delta-S isotope ratio mass spectrometer (IRMS) (Finnigan MAT, Germany) via a ConFlo II open split interface. The quartz reactor tube was filled with tungstic oxide [tungsten(VI) oxide,  $\text{WO}_3$ ] and pure reduced copper wires and was maintained at  $1020\text{ }^{\circ}\text{C}$ .

**SIRMS  $^{18}\text{O}/^{16}\text{O}$  Analysis.** The  $\delta^{18}\text{O}_{\text{‰}}$  values of the aqueous fraction of fruit juice samples were determined using the carbon dioxide equilibration method. The sample (5 mL) was placed in a small glass vessel and the vessel evacuated. The atmosphere was then replaced with a low-pressure headspace of carbon dioxide. The samples were shaken in a water bath thermostated to a temperature of  $20\text{ }^{\circ}\text{C}$  over an 8 h period. This established an equilibrium between the oxygen isotopes in water, carbon dioxide ( $\text{CO}_2$ ), and carbonic acid ( $\text{H}_2\text{CO}_3$ ). The  $\delta^{18}\text{O}_{\text{‰}}$  value of the sample water was then determined by measuring the oxygen isotope ratio of the carbon dioxide (46:44).

**Equipment.** The equilibration device was connected to the dual inlet system of the Finnigan MAT Delta S SIRMS via a transfer line that incorporated a cold trap ( $-70\text{ }^{\circ}\text{C}$ ), which was used to remove water vapor from the sample gas.

**Calculation.** The amount of  $^{13}\text{C}/^{12}\text{C}$  and  $^{18}\text{O}/^{16}\text{O}$  in the samples of ethanol was determined according to

$$\delta \text{ sample } \text{‰} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000 \quad (3)$$

where  $R$  is the ratio  $^{13}\text{C}/^{12}\text{C}$  or  $^{18}\text{O}/^{16}\text{O}$ .

The ratios were reported against the following standards: Pee Dee Belemnite (PDB) for  $^{13}\text{C}/^{12}\text{C}$  and standard mean ocean water (SMOW) for  $^{18}\text{O}/^{16}\text{O}$ .

**Quality Control.** For each method of analysis (SNIF-NMR, SIRMS  $^{13}\text{C}/^{12}\text{C}$ , and SIRMS  $^{18}\text{O}/^{16}\text{O}$ ) one type of in-house reference material (IHRM) was used to ascertain that the method was "in control" and to determine the repeatability of the method. For SNIF-NMR a sample of orange juice with a nominal (D/H)<sub>I</sub> value of 103.0 ppm (SD = 0.51) was analyzed with every batch of samples. To make sure that the absolute values obtained by the NMR were correct, three BCR-certified reference ethanols from different sources (beet, cane, and grape) of known D/H content were acquired before each batch analysis. For SIRMS of  $^{13}\text{C}$  a sample of ethanol with a nominal  $\delta^{13}\text{C}_{\text{‰}}$  value of  $-25.1\text{‰}$  (SD = 0.5) was analyzed with every batch of samples. The analytical precision (absolute difference between two measurements on one sample) was  $\pm 0.5\text{‰}$  or better. For SIRMS of  $^{18}\text{O}$  a sample of orange juice with a nominal  $\delta^{18}\text{O}_{\text{‰}}$  value of  $+2.21$  was analyzed with every batch of samples. If the values obtained for the IHRM fell outside the average  $\pm 2$  SD, the batch was rejected.

## RESULTS AND DISCUSSION

**Carbon-13.** *Quality Control.* The mean value determined for the IHRM was  $\delta^{13}\text{C} = -25.4\text{‰}$  PDB (SD =  $0.2\text{‰}$ ;  $n = 11$ ). The data demonstrate the good repeatability of the method.

**Table 1. Levels of  $^{13}\text{C}/^{12}\text{C}$ , (D/H)<sub>I</sub>, (D/H)<sub>II</sub>, and  $^{18}\text{O}/^{16}\text{O}$  in Authentic Samples of Orange Juice, Authentic FCOJ, FCOPW, Retail FCOJ, and Retail FSOJ**

type	date of collection	$^{13}\text{C}/^{12}\text{C}$ ( $\delta\text{‰}$ PDB) <sup>a</sup>	(D/H) <sub>I</sub> (ppm)	(D/H) <sub>II</sub> (ppm)	$^{18}\text{O}/^{16}\text{O}$ (VSMOW)
Authentic Samples (Laboratory-Squeezed Orange Juice)					
Pera	May 1995	-26.1	100.6	123.4	-1.25
	Sept 1995	-26.3	104.5	127.7	3.73
		-26.6	103.3	127.2	3.24
		-26.1	103.7	128.0	3.32
		-26.1	na <sup>b</sup>	na	2.91
		-26.3	104.2	128.3	6.76
		-26.7	103.6	126.1	-0.54
Valencia	Sept 1995	-26.6	101.0	126.4	3.54
		-28.0	103.0	127.1	4.13
		-26.9	103.3	126.7	0.62
Hamlin	Sept 1995	-25.8	100.9	128.8	4.13
		-26.4	100.9	128.7	3.57
		-26.1	101.5	129.5	na
		-25.8	103.7	126.2	na
Natal	Sept 1995	-26.6	101.7	126.9	4.66
	Jan 1996	-25.5	103.8	125.4	na
		-26.0	104.8	125.7	na
		-26.5	103.1	126.3	-0.42
Lima	May 1995	-26.9	99.8	122.7	-0.90
Cravo	May 1995	-29.2	99.9	124.3	na
Baía	May 1995	-27.9	99.2	124.7	-1.12
Samples from Factory					
FCOJ	Sept 1995	-26.5	101.3	121.9	na
		-26.8	101.1	121.5	na
		-27.0	101.1	122.8	na
FCOPW	Sept 1995	-26.9	101.6	122.8	na
Retail Samples					
FCOJ	Sept 1995	-26.5	103.1	121.9	na
	Jan 1996	-26.3	102.8	120.1	na
		-26.3	104.3	121.5	na
FSOJ ( <i>n</i> = 20)	Sept 1995–Sept 1996	-29.0 to -25.7	102.8–105.9	121.1–127.9	-1.44 to 6.67 <sup>c</sup>

<sup>a</sup> In ethanol. <sup>b</sup> na, not available. <sup>c</sup> *n* = 18.**Table 2. Mean Isotopic Values for Brazilian Samples of Orange Juice**

type of juice	year of harvest	no. of samples	$^{13}\text{C}/^{12}\text{C}$ <sup>a</sup> ( $\delta\text{‰}$ PDB)	(D/H) <sub>I</sub> (ppm)	(D/H) <sub>II</sub> (ppm)	$^{18}\text{O}/^{16}\text{O}$ (VSMOW)
authentic freshly	1995	17	-26.6	102.0 <sup>b</sup>	126.7 <sup>b</sup>	2.82
			SD = 0.9	SD = 1.7	SD = 2.0	SD = 2.4 ( <i>n</i> = 13)
squeezed	1996	4	-26.1	103.8	125.9	-0.11
			SD = 0.5	SD = 0.7	SD = 0.4	SD = 2.0 ( <i>n</i> = 3)
authentic FCOJ	1995	3	-26.8	101.3	121.8	na <sup>c</sup>
			SD = 0.3	SD = 0.1	SD = 0.6	
retail FSOJ	1995	2	-26.8	103.1	125.8	6.67
			SD = 0.6	SD = 0.4	SD = 1.6	( <i>n</i> = 1)
	1996	18	-26.7	104.5	125.8	3.26
			SD = 0.9	SD = 0.8	SD = 1.6	SD = 2.5
retail FCOJ	1995	1	-26.5	103.1	121.9	na
	1996	2	-26.3	103.6	120.8	na
			SD = 0	SD = 1.1	SD = 1.0	

<sup>a</sup> In ethanol. <sup>b</sup> Average of 16 samples. <sup>c</sup> na, not applicable.

**Authentic Samples.** Table 1 gives the  $^{13}\text{C}/^{12}\text{C}$ , D/H, and  $^{18}\text{O}/^{16}\text{O}$  results from all of the samples analyzed. A summary of the results is given in Table 2.

Table 1, column 3, presents the  $\delta^{13}\text{C}\text{‰}$  results. The  $\delta^{13}\text{C}$  value of ethanol fermented from laboratory-squeezed samples varied between -29.2 and -25.5‰ with a mean of -26.6‰ (SD = 0.9) (Table 2). Our results are similar to those published by Martin et al. (1996b) (mean = -26.6‰; SD = 0.45). These values are

in general slightly lower than those obtained by measuring the  $\delta^{13}\text{C}$  value directly from the sugar. According to Martin et al. (1996b) this is due to the limited fractionation that occurs during the fermentation (usually slightly higher than 1‰), although the possibility that this is due to the nonrandom distribution in hexoses should also be considered. Other authors measured juices directly (that is, containing other components such as acids, amino acids, pulp) to obtain

the  $\delta^{13}\text{C}$  value in the samples. Therefore, small variations in the results should be expected when whole sample data, sugar data, and ethanol data are compared.

From this study there is no evidence that the ratio of carbon-13 is related to variety, as only one variety of orange (Cravo) fell outside the mean  $\pm 2$  SD (97.5% confidence limit) ( $-26.6 \pm 1.8\text{‰}$ ). As only one sample of this variety was available for analysis, it is not possible to reach a firm conclusion on this issue. The small seasonal effect observed between samples from 1995 and 1996 is not statistically significant. Mean  $\delta^{13}\text{C}$  values for 1995 and 1996 season are  $-26.6$  and  $-26.1\text{‰}$ , respectively (Table 2). Martin et al. (1996b) did not find any significant seasonal effect or varietal effects on carbon isotopic ratios of samples from Florida.

**FCOJ and FCOPW.** Commercial FCOJ and FCOPW (Table 1) showed similar  $^{13}\text{C}/^{12}\text{C}$  ratios (in the ethanol produced after dilution to 12 °Brix with Norwich tap water and fermentation) when compared to the ethanol produced from fermentation of authentic samples of orange juice. The values were  $-26.9\text{‰}$  PDB for FCOPW and ranged from  $-27.0$  to  $-26.5\text{‰}$  for FCOJ. This indicates that the concentration process did not alter the  $^{13}\text{C}/^{12}\text{C}$  isotope ratio.

**Retail Samples.** (a) *FCOJ.* The values of  $\delta^{13}\text{C}$  of ethanol fermented from retail samples of FCOJ detected varied between  $-26.5$  and  $-26.3\text{‰}$  (Table 1). These were entirely in accord with those expected from pure orange juices and gave no indication of the presence of cane sugar.

(b) *FSOJ.* The mean value of  $^{13}\text{C}/^{12}\text{C}$  for all of the samples analyzed was  $-26.7\text{‰}$  PDB (SD = 0.8), similar to that for authentic samples (mean =  $-26.6\text{‰}$ , SD = 0.9) (Table 1). Only one sample had a value ( $-29\text{‰}$ ) outside the mean  $\pm 2$  SD for authentic samples (mean =  $-26.6\text{‰}$ ; SD = 1.8). This should be considered unusual but not impossible because an authentic sample had a similar value. Normally, orange juice processed in a factory results from mixing orange juices from several varieties and from different regions. Therefore, the values obtained may be expected to have a similar mean but a narrower range than those hand-squeezed fruit. This is supported by the fact that authentic and retail samples of FCOJ had  $\delta^{13}\text{C}$  values which were close to each other. In these circumstances the best approach is to obtain further analytical data. Most authentic orange juices with depleted  $\delta^{13}\text{C}$  values also have low values of (D/H)<sub>I</sub>. Thus, the elevated value of (D/H)<sub>I</sub> is a further suggestion of a potential problem with the sample. This could be confirmed or excluded on the basis of further compositional data.

**SNIF-NMR.** *Quality Control.* The mean value of (D/H)<sub>I</sub> determined for the IHRM for seven analyses was 103.2 ppm (SD = 0.18 ppm). This demonstrates that the analysis was in control and indicates the good repeatability of the method.

**Correction to VSMOW.** When sugars are fermented to ethanol, some hydrogen atoms from the fermentation medium are incorporated in the ethanol molecule. Approximately 70% of the deuterium at (D/H)<sub>I</sub> comes from the sugar molecule, whereas the proportion of solvent-derived deuterium at (D/H)<sub>II</sub> is much larger. Dilution of concentrate with laboratory water before fermentation leads to small differences in deuterium content of the solvent from that naturally present in the fruit juice and these become incorporated into the

ethanol. To facilitate comparison of juice made from concentrated with pure fruit juices, Martin et al. (1986) normalized data to a standard solvent deuterium content (VSMOW = 155.7 ppm). We prefer to report our results without correction, but a small adjustment enables our data to be readily compared.

concentrated juices diluted with Norwich tap water:

$$(\text{D}/\text{H})_{\text{VSMOW}} = (\text{D}/\text{H})_{\text{I}} + 1.3 \text{ ppm}$$

$$(\text{D}/\text{H})_{\text{IIVSMOW}} = (\text{D}/\text{H})_{\text{II}} + 5.3 \text{ ppm}$$

For 26 Brazilian authentic juices squeezed from fresh fruit, including those recorded in this paper, we have established a deuterium content of the water fraction of 157.2 ppm (SD = 2.4). Only a small correction is required to compare with data reported as VSMOW.

$$(\text{D}/\text{H})_{\text{IOJ}} = (\text{D}/\text{H})_{\text{I}} - 0.2 \text{ ppm}$$

$$(\text{D}/\text{H})_{\text{IIIOJ}} = (\text{D}/\text{H})_{\text{II}} - 0.9 \text{ ppm}$$

**Authentic Samples.** Table 1, columns 4 and 5, show the (D/H)<sub>I</sub> and (D/H)<sub>II</sub> ratios for the authentic samples of FSOJ. The values obtained varied from 99.2 to 104.8 ppm and from 122.7 to 129.5 ppm, respectively, for (D/H)<sub>I</sub> and (D/H)<sub>II</sub>. As can be observed from Table 2, there is a slight increase in the mean value of (D/H)<sub>I</sub> for samples from the year 1996 compared to 1995, although the numbers of the samples analyzed in 1996 were not large enough to determine whether this was significant. Martin et al. (1996b) concluded that the seasonal effect in Florida samples for three consecutive seasons is  $\sim 1$  ppm for (D/H)<sub>I</sub> and  $\sim 2$  ppm for (D/H)<sub>II</sub> even in extreme drought.

In general, there were no varietal trends in the data. However, single samples of Baía, Cravo, and Lima oranges showed relatively low values of (D/H)<sub>I</sub> and (D/H)<sub>II</sub> (99.2–99.9 and 122.7–124.7 ppm, respectively) when compared to the other varieties collected in the same year (100.9–104.8 and 123.4–129.5 ppm, respectively). However, the small number of samples available does not permit an evaluation of whether a seasonal or varietal effect was occurring. Martin et al. (1996b) have correlated certain varieties with specific origins, climates, or harvest times with small variations in the isotope ratios (usually  $< 1$  ppm).

The data presented in our work were similar to those found by Martin et al. (1996b) analyzing authentic FSOJ from Brazil. They report ratios (corrected to VSMOW) of (D/H)<sub>I</sub> and (D/H)<sub>II</sub> for authentic hand-squeezed orange juice in the seasons 1992 and 1993 ranged from 103.0 to 105.8 ppm and from 121.7 to 128.0 ppm, respectively.

**FCOJ and FCOPW.** Factory samples of FCOJ (Table 1) gave isotope ratios [(D/H)<sub>I</sub> = 101.1–101.3 ppm and (D/H)<sub>II</sub> = 121.5–122.8 ppm] within the range found for authentic samples of hand-squeezed orange juice [(D/H)<sub>I</sub> = 99.2–104.8 ppm and (D/H)<sub>II</sub> = 122.7–129.5 ppm]. Martin et al. (1996b) analyzed authentic samples of Brazilian FCOJ during the seasons 1992 and 1993. The ratios for (D/H)<sub>I</sub> and (D/H)<sub>II</sub> ranged from 103.2 to 105.8 ppm and from 124.4 to 128.5 ppm ( $n = 73$ ), respectively. FCOPW [(D/H)<sub>I</sub> = 101.6 ppm and (D/H)<sub>II</sub> = 122.8 ppm] (Table 1) showed no isotopic differences from FCOJ and authentic hand-squeezed orange juice.

**Retail Samples.** *FCOJ.* The ratios measured varied between 102.8 and 104.3 ppm and between 120.1 and

121.9 ppm, respectively, for (D/H)<sub>I</sub> and (D/H)<sub>II</sub> (Table 1). The ratios of (D/H)<sub>I</sub> are within the range found for authentic samples of hand-squeezed orange juice (99.2–104.8 ppm) and higher than those found for commercial samples of FCOJ (101.1–101.3 ppm). The results for the retail samples are consistent with those expected from pure orange juice and give no indication of the presence of other sugars. The (D/H)<sub>II</sub> ratios were also similar to those found for factory authentic samples of FCOJ (121.5–122.8 ppm).

**FSOJ.** The values found for (D/H)<sub>I</sub> and (D/H)<sub>II</sub> in FSOJ ranged from 102.8 to 105.9 ppm (mean = 104.4 ppm) and from 121.1 to 127.9 ppm (mean = 125.2 ppm), respectively (Table 1). The (D/H)<sub>I</sub> ratios were in general higher than those found for authentic samples. Only two samples (both with values of 105.9 ppm) fell outside the mean  $\pm$  2 SD (97.5% confidence limit) for authentic samples of hand-squeezed orange juice. The results obtained for carbon-13 analysis for these samples (Table 1, retail samples) did not give any indication of the addition of cane sugar to the orange juice.

**Oxygen-18.** *Quality Control.* The mean value determined for the IHRM was  $\delta^{18}\text{O} = +2.21\text{‰}$  SMOW (SD = 0.14 ‰;  $n = 17$ ). The data demonstrate the good repeatability of the method.

*Authentic Samples.* Table 1, column 6, presents the  $\delta^{18}\text{O}$  results. The  $\delta^{18}\text{O}$  of the laboratory-squeezed samples, collected in different regions and periods of the year in the state of São Paulo (Brazil), ranged from  $-1.25$  to  $+6.76\text{‰}$  with a mean of  $+2.27\text{‰}$  (SD = 2.48). As can be seen, a wide range of values was obtained. Compared with the literature data (Bricout et al., 1972; Bricout, 1973; Nissenbaum et al., 1974), our results showed a much wider variation, possibly reflecting climatic effects. As the results presented showed larger variations for individuals, it is difficult to make any conclusions related to varietal or seasonal effects.

*Retail Samples.* The data obtained for  $^{18}\text{O}$  in retail samples of FSOJ ( $-1.44$  to  $+6.67\text{‰}$ ) showed a range similar to that of authentic samples of FSOJ ( $-1.25$  to  $+6.76\text{‰}$ ) (Table 1). A typical sample of Brazilian groundwater gave a value of  $-5.1\text{‰}$ . If concentrated orange juice had been diluted with this water and added to samples of FSOJ, some diminution in the  $^{18}\text{O}\text{‰}$  level might have been expected; however, none was observed.

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