

Characterization of Commercial Slovenian and Cypriot Fruit Juices Using Stable Isotopes

NIVES OGRINC,^{*,†} KARMEN BAT,[†] IZTOK JOŽE KOŠIR,[‡] TEREZIJA GOLOB,[§] AND
REBECCA KOKKINOFTA[#]

[†]Department of Environmental Sciences, Jožef Stefan Institute, Jamova 39, SI-1000 Ljubljana, Slovenia,

[‡]Slovenian Institute of Hop Research and Brewing, Žalskega tabora 2, SI-3310 Žalec, Slovenia,

[§]Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia, and [#]State General Laboratory, Ministry of Health, 44 Kimonos Street, Acropolis 1451–Nicosia, Cyprus

The compositions of commercially available fruit juices on the Slovenian and Cypriot markets have been compared and checked against the corresponding declarations on the packaging. Values of $\delta^{13}\text{C}$ in the pulp, sugars, and ethanol (produced by fermentation and distillation) have been obtained by isotope ratio mass spectrometry and $(\text{D}/\text{H})_{\text{I}}$ and $(\text{D}/\text{H})_{\text{II}}$ ratios determined by Site-specific Natural Isotope Fractionation NMR and compared with literature data. These data show that some juices on the Slovenian and Cypriot markets were adulterated. Measurements of $\delta^{18}\text{O}$ in the water and of $(\text{D}/\text{H})_{\text{II}}$ in the ethanol indicated a significant difference in commercially available juices due to differences of the “tap” water in Slovenia and Cyprus, as most of the juices were diluted from concentrate. Using Principal Component Analysis, very clear differentiation can be made between juices from the two countries, arising from their distinct aquatic environments and climates. The identification of botanical origin of fruit juices was possible only in the case of Slovenian orange and apple fruit juices.

KEYWORDS: Commercial; fruit juices; IRMS; SNIF-NMR; PCA; Slovenia; Cyprus

INTRODUCTION

The authenticity of food products is important from the point of producers, consumers, and control bodies. In food production technology a variety of different additives are used to improve particular characteristics of products, such as taste, odor, and color. In some cases, the reason for addition is simply to increase production and thereby profit. In the end such practices can reflect on the quality of the product. The addition of the majority of additives is allowed by law, but the addition must be properly declared and the information about the addition must be clear to the potential consumer and control body. Identifying the fraudulent addition of additives (adulteration) to fruit juices is of great economic importance. The quality and authenticity of orange juice are of particular importance because of the large quantities consumed. Different types of adulteration exist, such as dilution with water, addition of sugar and citric acid to the pure juice, the addition of colorants, and the addition of cheaper juices from other fruits. For example, the presence of tartaric acids in fruit juices indicates the addition of grape juice.

The use of stable isotope analysis is usually the most effective way to detect adulteration. These methods are based on measurements of stable isotope contents of a product or specific component in the product. In the case of carbon, the carbon isotope ratio in foodstuffs is directly related to their botanical origin. Most plants, including beet sugar, employ C_3 photosynthesis, resulting

in $\delta^{13}\text{C}$ values between -23 and -32‰ (relative to V-PDB). A smaller but significant group of plants (e.g., sugar cane, corn) employ C_4 photosynthesis, resulting in $\delta^{13}\text{C}$ values between -10 and -16‰ (1, 2). Finally, CAM-plants use crassulacean acid metabolism and have intermediate stable isotopic composition with values varying between -10 and -20‰ . The application to fruit juices has been studied collaboratively for both the Committee of European Normalization (CEN) and the AOAC (3–5). Site-specific Natural Isotope Fractionation determined by Nuclear Magnetic Resonance (SNIF-NMR) and Isotope Ratio Mass Spectrometry (IRMS) can be used to distinguish between fruit sugar and various exogenous sugars, such as cane, beet sucrose, high-fructose corn syrup, and beet inverted syrup. For the widespread application of stable isotope techniques for beverage authentication, it is necessary to compare sample data with a database of authentic values. One of the problems is the wide range of values for authentic samples from different parts of the world. To overcome this problem stable isotope content determination of a second component of the sample can be measured as an internal reference, which reduces the variability of the authentic database. This approach is called the Specific Natural Isotopic Profile measured by Isotope Ratio Mass Spectrometry (SNIP-IRMS) and involves measurements of the ^{13}C contents of pure fractions of sugars and citric and malic acids. Such ^{13}C profiles have been determined in different fruit juices, including apple (6), pineapple (7), orange and tangerine (8), lemon (9), and also in wine (10). This sensitive method is mostly used to improve the detection limit of added sugars and organic acids. Interpretation

*Corresponding author (telephone +386 1 588 5387; fax +386 1 588 5346; e-mail nives.ogrinc@ijs.si).

of the results is usually based on the correlation between $\delta^{13}\text{C}$ values of sugars, malic acid, and citric acid, with one of the acids being taken as internal reference. In addition, isotopic analysis of the fruit juice pulp as a common internal reference could offer an additional opportunity to detect simultaneous adulteration of sugars and organic acids (11). There are some reasons, however, to exclude pulp as a suitable internal reference. The complex composition of the pulp fraction can be altered by some physical or chemical processes, or some clarified juices or concentrates may not contain any pulp fraction. There are also a few experimental difficulties during purification of the pulp (12). Recently, fruit proteins were reported to be a more convenient internal standard of fruit juices and concentrates (13).

The determination of the $\delta^{18}\text{O}$ in water is a reliable procedure for differentiating between a natural single-strength juice and a juice reconstituted from concentrate, particularly in the case of citrus, apple, and grape juices (14, 15). The main factor that influences the $\delta^{18}\text{O}$ value of plant water is the source of plant-available water, usually groundwater (resulting from precipitation). During transportation from the source (oceans, seas) over continents air masses cool and lose water vapor along the way as precipitation. Isotopically enriched rain falls, and the vapor becomes progressively depleted in its ^{18}O content. The combination of climatic, geographic, and topographic conditions results in a worldwide distinct pattern of meteoric waters (16). Furthermore, the climatic conditions of a location additionally modify the transpiration of leaves and fruit, which always causes an enrichment of the heavy oxygen isotopic content of plant water as compared with groundwater. This enrichment is additionally related to the climate, being higher when the climate is dry and warm. Therefore, isotopic "fingerprinting" not only makes it possible to estimate precisely the degree of dilution with water but, using all of the isotope data together with the appropriate statistical tool, also enables the determination of the source and geographical origin of the product.

The aim of the present study is to characterize, using the stable isotope approach, commercially available fruit juices made in Slovenia and Cyprus.

MATERIALS AND METHODS

Thirty-six commercially available fruit juices made in Slovenia ($n = 11$) and Cyprus ($n = 25$) from 2007 formed the basis of the study. Samples included orange and apple juices from both countries, and pineapple, grapefruit, peach, sour cherry, and grape juices from Cyprus. The stable carbon isotope ratio ($^{13}\text{C}/^{12}\text{C}$) of pulp and sugars and the stable oxygen isotope ratio ($^{18}\text{O}/^{16}\text{O}$) in waters were determined. $^{13}\text{C}/^{12}\text{C}$ was also measured in ethanol. In this case fruit juice sugars were converted to ethanol by fermentation. SNIF-NMR measurements were also made on ethanol to determine site-specific (D/H) ratios. IRMS and SNIF-NMR measurements of ethanol produced by fermentation of the juice were also performed on authentic Cyprus orange, grapefruit, and mandarin juices prepared from fresh fruits ($n = 11$) squeezed in the laboratory with known origin and variety.

Sample Preparation. Sugars were separated and purified from fruit juice according to the method of Koziet et al. (17). The insoluble constituents (pulp) of 50 mL of juice were separated by centrifugation (10 min at 4000 rpm). The pulp was stored for further purification and isotope determination. The supernatant was decanted, and 2 g of calcium oxide was added to precipitate organic and amino acids. The supernatant was heated and stirred on a water bath at 90 °C for 3 min and then centrifuged (10 min at 4000 rpm), and the clear supernatant was decanted and acidified with 1 M sulfuric acid to pH ≈ 5 , when the color of the solution changed. This solution contained mainly sugars, calcium sulfate, and some colorants as minor ingredients. The solution was refrigerated overnight at 4 °C to allow the calcium sulfate to precipitate. The final solution, containing mainly sugars (fructose, glucose, sucrose), was ready

for measurements and were introduced into the elemental analyzer as liquid. Residual water was removed after combustion (7).

The pulp was resuspended in water, mixed, and centrifuged (10 min at 4000 rpm) and the supernatant discarded. The residue was additionally washed with water, twice with acetone, and allowed to dry. The pulp was homogenized by mixing with a spatula and used for measurements (12).

Fruit juices were fermented with yeast. Different types of yeast were evaluated, along with the time of fermentation, to determine the optimal fermentation conditions. *Zymasil Bayesianus* (Pascal Biotech, Paris, France) was chosen as the best yeast for fermentation. Samples of juice were weighted in Erlenmeyer flasks corked with plastic caps with the cone filled with cotton to get anaerobic conditions. Samples were then fermented in a closed incubator "SELECTA" with temperature controller (20 ± 2 °C) to avoid ethanol evaporation. Clinintests (Bayer Diagnostics Mfg. Ltd, U.K.) were used to monitor the fermentation of sugar into ethanol. Fermentation was complete when the amount of sugar was ≤ 2.5 g/L (which can take about a week for commercial juices and 3–4 days for fresh juice). The samples were then distilled to obtain the ethanol using the ADCS (Eurofins, Nantes Cedex, France) system. The water content in the distillate was measured by Karl Fischer 787 (Methrom) apparatus.

IRMS Measurements. Stable carbon isotope ratios were determined in sugar samples, pulp, and ethanol using the isotope ratio mass spectrometer (IRMS) Europa Scientific 20-20 (Crewe, U.K.) with ANCA-SL preparation module for solid and liquid samples. The stable oxygen isotope ratio ($^{18}\text{O}/^{16}\text{O}$) was determined in fruit juice water after equilibration with reference CO_2 at 25 °C for 24 h (18) using a Varian MAT 250 mass spectrometer. The $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ ratios are expressed in the delta notation, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, as deviation in per mil (‰) from the V-PDB standard for carbon and V-SMOW standard for oxygen. The precisions of measurements made on two replicates were ± 0.2 and $\pm 0.1\%$ for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, respectively. Analyses were calibrated against international standards: IAEA-NBS22 (oil), IAEA-CH-7, and IAEA-CH-6 with $\delta^{13}\text{C}$ values of -29.7 ± 0.2 , -31.8 ± 0.2 , and $-10.4 \pm 0.2\%$, respectively. To ensure the accuracy of IRMS measurements in ethanol certified reference material from the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) CRM/BCR 656 with a $\delta^{13}\text{C}$ value of $-26.91 \pm 0.07\%$ was used. Analyses for $\delta^{18}\text{O}$ measurements were calibrated against laboratory-used standard seawater with the average value of $\delta^{18}\text{O} = -0.25 \pm 0.08\%$ and tap water with the average value of $-9.45 \pm 0.06\%$. Data quality control charts were also systematically recorded throughout the study period. In addition, to ensure the validity and comparability of stable isotope results, interlaboratory proficiency testing organized by Eurofins (Nantes Cedex, France) was performed.

NMR Measurements. Deuterium isotope ratios (D/H)_I and (D/H)_{II} were determined in the distillates of the samples by following the standard method described in European Regulation 2676/90 for wines (4). The alcohol grade was measured with a DMA-5000 (Anton Paar) density meter after the distillation to calculate the yield obtained, which was $\geq 90\%$. ^2H NMR spectra were recorded (10 times) on a Bruker 400 MHz NMR spectrometer using X-WIN NMR 3.1 software for processing the spectra and EUROSPEC (Eurofins, Nantes Cedex, France) software for the calculations. The ratios (D/H)_I and (D/H)_{II} are expressed in parts per million. The precision of measurements was $< \pm 0.5\%$ for both (D/H)_I and (D/H)_{II}. All of the samples were measured under the same laboratory conditions. For deuterium NMR analysis of ethanol, *N,N*-tetramethylurea (TMU-Institute for Reference Materials) was used as an internal standard with a D/H ratio determined on the V-SMOW/SLAP scale. As a secondary internal standard a commercial sample of orange juice was taken in large amount, homogenized in the laboratory, and analyzed 10 times to create the control charts of (D/H)_I and (D/H)_{II}. Different Certified Reference Materials were used to control the measurements with the density meter (ERM 5, 10, 15% vol), the Karl-Fischer (BCR 656) and the NMR (CRM 123: VB27, BB13, CB13).

Statistical Analysis. The stable isotope data were evaluated statistically using principal component analysis (PCA). Statistical analysis was performed using the SCANWIN software (Minitab Inc., State College, PA).

RESULTS AND DISCUSSION

Carbon Isotope Ratios of Sugars, Pulp, and Ethanol. Results obtained for all parameters determined in commercial Slovenian

Table 1. Type, Botanical Origin, Sample Declaration, SNIF-NMR Parameters [(D/H)_I, (D/H)_{II}, R], $\delta^{13}\text{C}$ Values for Ethanol, Pulp, and Sugar, and $\delta^{18}\text{O}$ of Water Determined in Slovenian (SLO) and Cypriot (CYP) Commercially Available Fruit Juices^a

no.	country	type	botanical origin	sample declaration	(D/H) _I (ppm)	(D/H) _{II} (ppm)	R	$\delta^{13}\text{C}$ (‰ ethanol)	$\delta^{13}\text{C}$ (‰ pulp)	$\delta^{13}\text{C}$ (‰ sugar)	$\delta^{18}\text{O}$ (‰ water)	Δ (‰)	AD-I
1*	CYP	juice	orange	natural juice without added sugars	104.3	127.6	2.445	-23.7	-25.9	-16.5	-0.1	9.5	IRMS, NMR
2	CYP	juice	orange	natural juice without added sugars	104.0	127.4	2.449	-27.2	-25.9	-25.7	-3.1	0.3	
3*	CYP	juice	orange	natural juice without added sugars	97.9	125.5	2.564	-26.6	-26.6	-25.3	-4.4	1.2	NMR
4	CYP	juice	orange	natural juice without added sugars	99.7	123.3	2.427	-27.5	-26.1	-23.2	-2.8	3.0	IRMS, NMR
5	CYP	natural	orange	natural juice without added sugars	104.6	125.5	2.398	-26.1	-25.9	-25.7	-3.9	0.2	
6	CYP	natural	orange	natural juice without added sugars	102.2	122.1	2.390	-24.1	-25.2	-23.0	-4.2	2.2	IRMS, NMR
7	CYP	natural	orange	natural juice without added sugars	103.5	125.8	2.431	-24.0	-25.8	-23.5	2.0	2.3	IRMS, NMR
8	SLO	natural	orange	natural juice without added sugars	101.0	118.2	2.341	-23.8	-26.0	-23.2	-8.4	2.8	IRMS
9	SLO	natural	orange	natural juice without added sugars	102.4	123.0	2.404	-26.1	-25.8	-25.5	-7.5	0.3	
10	CYP	juice	apple	natural juice without added sugars	99.4	125.5	2.526	-27.2	n/a	-25.5	-3.6		
11	CYP	juice	apple	natural juice without added sugars	97.0	127.0	2.619	n/a	n/a	-25.1	-2.4		
12	CYP	natural	apple	natural juice without added sugars	98.5	123.9	2.516	-23.0	n/a	-21.2	-4.3		IRMS
13	CYP	natural	apple	natural juice without added sugars	97.9	124.2	2.537	-27.5	n/a	-26.5	-4.8		
14	SLO	juice	apple	juice containing added sugars	93.5	121.3	2.593	-28.1	n/a	-26.4	-9.3		
15	SLO	juice	apple	natural juice without added sugars	96.2	120.7	2.509	-28.2	n/a	-24.3	-10.0		
16	SLO	juice	apple	juice containing added sugars	91.4	118.2	2.610	-26.9	n/a	-25.8	-10.2		
17	SLO	juice	apple	juice containing added sugars	102.6	119.5	2.330	-18.3	n/a	-18.1	-10.4		
18*	SLO	natural	apple	natural juice without added sugars	96.6	125.4	2.605	n/a	n/a	-26.8	-5.3		
19	SLO	natural	apple	natural juice without added sugars	97.7	121.7	2.493	-27.6	n/a	-26.6	-8.7		
20	SLO	natural	apple	juice containing added sugars	101.9	119.2	2.340	-18.6	n/a	-18.0	-10.4		
21	SLO	natural	apple	juice containing added sugars	92.5	119.5	2.583	-27.8	n/a	-26.0	-10.7		
22	SLO	natural	apple	juice containing added sugars	91.6	120.3	2.626	-27.3	n/a	-25.6	-9.7		
23	CYP	natural	grapefruit	natural juice without added sugars	102.1	123.9	2.427	-26.6	-25.5	-26.1	-4.6	-0.6	
24	CYP	juice	grapefruit	juice containing added sugars	107.8	126.9	2.354	-16.5	-25.3	-16.0	0.7	9.3	
25	CYP	juice	grapefruit	natural juice without added sugars	99.2	126.1	2.542	-25.2	-25.2	-24.5	-3.5	0.7	NMR
26	CYP	juice	grapefruit	natural juice without sugars	95.3	125.5	2.632	-27.0	-24.7	-25.3	-4.0	-0.6	NMR
27	CYP	juice	grapefruit	natural juice without added sugars	102.0	125.9	2.470	-27.4	-25.5	-26.0	-3.7	-0.5	
28	CYP	natural	pineapple	natural juice without added sugars	105.0	124.6	2.374	-14.9	-12.7	-16.5	-4.5	6.1	IRMS
29	CYP	juice	pineapple	natural juice without added sugars	96.6	117.8	2.513	-15.4	n/a	-16.6	-4.4	-3.8	IRMS
30	CYP	juice	pineapple	natural juice without added sugars	106.6	127.0	2.384	-14.0	-14.0	-12.4	-1.1		
31	CYP	juice	peach	juice containing added sugars	94.0	126.7	2.762	-27.6	-26.7	-26.0	-3.9	1.6	
32	CYP	juice	peach	juice containing added sugars	106.8	130.0	2.435	-16.3	-23.4	-16.7	-1.0	0.6	
33	CYP	juice	peach	juice containing added sugars	93.6	126.9	2.713	-27.2	-26.6	-25.9	-3.5	6.7	
34	CYP	juice	sour cherry	juice containing added sugar	91.9	122.6	2.668	-27.2	n/a	-26.5	-6.1	0.7	
35	CYP	juice	grape	natural juice without added sugars	102.7	126.8	2.470	-25.6	n/a	-24.5	-3.8		
36	CYP	juice	grape	natural juice without added sugars	102.5	127.9	2.521	-24.6	n/a	-23.4	-4.1		

^aThe differences (Δ) $\delta^{13}\text{C}_{\text{sugar}} - \delta^{13}\text{C}_{\text{pulp}}$ are also included together with the identification by which method adulteration was detected (AD-I). All samples in the table are juices made from concentrate except samples 1*, 3*, and 18*, which were natural single-strength juices. n/a, not applicable.

and Cyprus fruit juices samples are listed in **Table 1**. The $\delta^{13}\text{C}$ values in sugars ranged from -26.8 to -12.4 ‰. As expected, the highest values were observed in pineapple juices, because pineapple is a CAM plant with higher $\delta^{13}\text{C}$ values. The sugar values for pineapple juices ranged from -16.6 to -12.4 ‰, whereas the corresponding $\delta^{13}\text{C}$ values in ethanol (i.e., from the same juice) ranged from -15.4 to -14.0 ‰. Only one of the pineapple fruit juices (sample 30) (**Table 1**) has appropriate $\delta^{13}\text{C}$ values of sugar and fell in the $\delta^{13}\text{C}$ range between -13.7 and -10.7 ‰ for authentic pineapples collected from a large number of production areas around the world (7, 13). Two other samples (samples 28 and 29) had lower $\delta^{13}\text{C}$ values of sugars. The European guidelines concerning pineapple juices state that $\delta^{13}\text{C}$ values of sugars < -15 ‰ indicate the addition of C_3 product (19). The presence of C_3 product in the pineapple fruit juice could also be seen from the difference between $\delta^{13}\text{C}$ values of sugar and the pulp (sample 28) (**Table 1**). This fruit juice was declared to be pure pineapple juice without any sugar addition.

The results show that some fruit juices (samples 1, 12, 17, 20, 24, and 32) contain cane sugar, because $\delta^{13}\text{C}$ values of sugars ranged between -16.0 and -21.2 ‰, higher than those for authentic fruit juices. The addition of sugar was, however, declared by the producers, except in two samples (samples 1 and 12). We also observed that in two Slovenian apple nectars (samples 17 and 20)

the addition of sugar was declared, but the isotopic data show the addition of cane sugar despite the fact that the most frequently added sugar in Slovenia is beet. The addition of cane sugar has previously been detected in some commercially available orange juices in Slovenia (20). Sample 18 is declared to be a natural single-strength juice from only apples. This declaration is supported by the $\delta^{13}\text{C}$ value of -26.8 ‰ determined in sugar and by the $\delta^{18}\text{O}$ value of -5.3 ‰ determined in the fruit juice water.

We used $\delta^{13}\text{C}$ values of the pulp as an additional method for authentication. It is likely that $\delta^{13}\text{C}$ values of sugar and pulp will be in a constant ratio because the respective metabolic pools are closely related due to the same environmental influences. The data proposed by Rossman et al. (12) demonstrate the value of the internal reference procedure in detecting relatively small amounts of added sugar. For orange juice the limiting value for the difference between sugar and pulp for the detection of added cane sugar is 1.25‰, whereas for grapefruit juice the value is 1.04‰ (12). It was proposed that the range of authentic $\delta^{13}\text{C}$ values of sugars in orange juice lies between -23.4 and -25.6 ‰ with an upper limit of -24.0 ‰ for citrus (19). According to the AIJN CoP limit for citrus, it is quite obvious that samples 1, 4, 6, 7, and 8 contain added C_4 or mixed C_4/C_3 sugars. In addition, it was found that besides sample 1 the difference between the $\delta^{13}\text{C}$ values of sugar and pulp was higher in four samples of orange

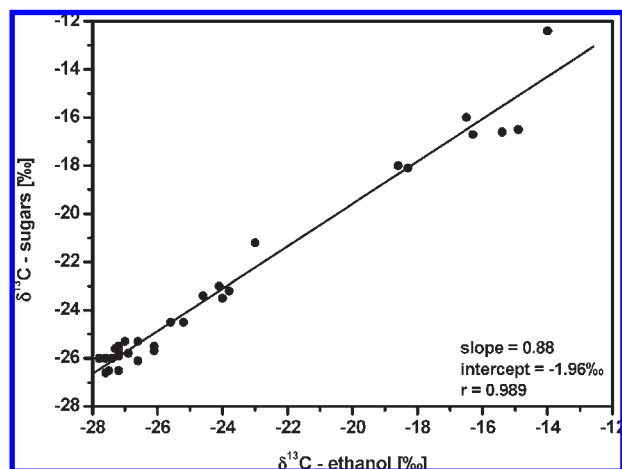


Figure 1. Correlation between $\delta^{13}\text{C}$ values determined in isolated sugars and ethanol from fruit juices.

juice (samples 4, 6, 7, and 8), ranging between 2.2 and 3.0%. These data indicate a small amount of added cane sugar, which was not declared by the producers.

$\delta^{13}\text{C}$ analyses are usually carried out on ethanol rather than on the precursor sugars. One of the main advantages of examining the alcohol is the purity of the analyte. When fermentation and distillation are performed under controlled conditions, the $\delta^{13}\text{C}$ values of sugars and ethanol correlated well, as was found in our samples (**Figure 1**). The $\delta^{13}\text{C}$ value for extracted sugar is $> 1\%$ higher than that for ethanol due to the isotopic fractionation of CO_2 production during fermentation (4). This shift is not problematic, because the same, reproducible shift occurs in all cases, and thus the results are directly comparable. $\delta^{13}\text{C}$ measurements in ethanol were also proposed as the method adopted by First Action AOAC Official Methods for the determination of the $\delta^{13}\text{C}$ deviation of ethanol derived from fruit juices and maple syrup (21).

The $\delta^{13}\text{C}$ values of sugars and ethanol in other samples from our study fall in the range of the $\delta^{13}\text{C}$ values for beet sugar ($\delta^{13}\text{C}$ value of -25.6%), and thus either it is not possible to detect this kind of adulteration or the samples are really authentic. The best way to maximize the detection level of any sugar addition is by the combined use of SNIF-NMR and IRMS fingerprinting.

IRMS and SNIF-NMR. The combined use of SNIF-NMR and IRMS data can be illustrated by an adulteration triangle constructed from the plane of $\delta^{13}\text{C}$ values versus $(\text{D}/\text{H})_I$ values from three different botanical sources (**Figure 2**). The triangle is constructed from isotopic parameters from authentic reference alcohols from beet, cane, and fruit sugars. The cut-off point is determined from authentic samples performed in the laboratory. Possible adulteration could be detected from the position of the sample. A nonadulterated sample falls in the reference zone (REF). If beet or cane sugar was added, the sample falls in zone BR or CR, respectively. The BCR zone indicates the addition of the mixture of beet or cane sugars, whereas the BC zone may indicate a high level of adulteration, for example, with a product from a source other than the given fruit (4, 21). This approach was also used in our study for orange juices and grapefruit juices from Cyprus. IRMS and SNIF-NMR parameters determined in ethanol are presented in **Table 1**, whereas **Table 2** summarizes the results of SNIF-NMR and IRMS performed on ethanol from authentic citrus juices.

So-called cut-off points or reference zones (REF) for authentic orange, grapefruit, and mandarin juices were established from authentic samples using a statistical approach. The cut-off points

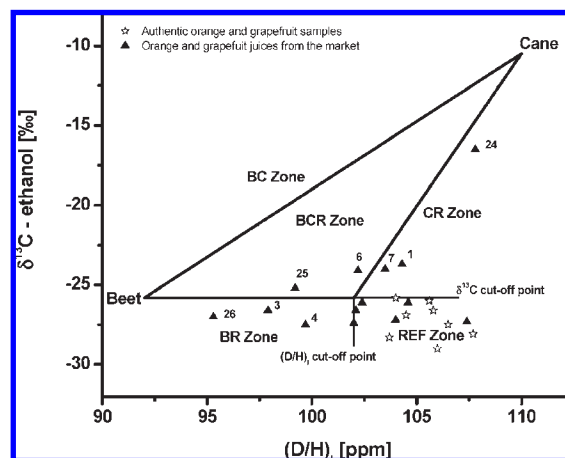


Figure 2. Adulteration triangle. Samples represent the plane of $\delta^{13}\text{C}$ content and of the hydrogen isotope ratio $(\text{D}/\text{H})_I$ of ethanol from beet, cane, and authentic fruit juices.

Table 2. Isotopic Parameters Determined in Ethanol Fermented from the Authentic Fruit Juices from Cyprus

botanical origin	$(\text{D}/\text{H})_I$ (ppm)	$(\text{D}/\text{H})_{II}$ (ppm)	R	$\delta^{13}\text{C}$ (‰ ethanol)
orange	105.6	129.9	2.462	-26.0
orange	107.7	130.6	2.425	-28.1
orange	105.8	129.6	2.448	-26.6
orange	104.5	127.5	2.439	-26.9
orange	104.0	128.8	2.477	-25.8
mandarin	101.0	123.1	2.436	
mandarin	106.0	127.7	2.409	-29.0
mandarin	106.5	129.9	2.439	-27.5
grapefruit	104.9	126.3	2.409	
grapefruit	103.7	126.0	2.430	-28.3
grapefruit	105.1	127.2	2.420	
mean	105.0	127.9	2.436	-27.3
SD	1.7	2.2	0.021	1.2

are quite close to the minimum and maximum values observed in laboratory-made juices and are comparable with those reported by Martin et al. (4). The adulteration triangle, based on the data from the Cyprus orange and grapefruit fruit juices, is presented in **Figure 2**. The addition of cane sugar is indicated for samples 1, 7, and 24; samples 6 and 25 fall in the zone where added beet and cane sugar are both detected, whereas samples 3, 4, and 26 fall in the zone of beet sugar addition. Addition of sugar was declared only in sample 24. Apple juices were excluded from this discussion, because we do not have data from authentic samples, and most of the data are from Slovenia. In addition, $(\text{D}/\text{H})_I$ values ranged between 91.6 and 102.6 ppm with an average value of 96.7 ± 3.6 ppm and are different from the data obtained from orange or grapefruit juices, but similar to the data reported by Martin et al. (4). The cut-off points for the apple reference group based on the literature data were -25.0% for $\delta^{13}\text{C}$ values and 97.0 ppm for $(\text{D}/\text{H})_I$ (4). These data indicated that apple juice samples 14, 16, 21, and 22 contain added beet sugar, which was also declared by the producer.

The stable isotope data from commercially available fruit juices from the Slovenian and Cypriot markets indicate instances of mislabeling on packages. Of 11 Slovenian samples, one orange juice (sample 8, labeled "100% pure orange juice, no added sugar") was found to be adulterated with cane sugar. Samples from Cyprus were found to be described incorrectly in 40% of cases: five orange juices, two grapefruit juices, one apple juice,

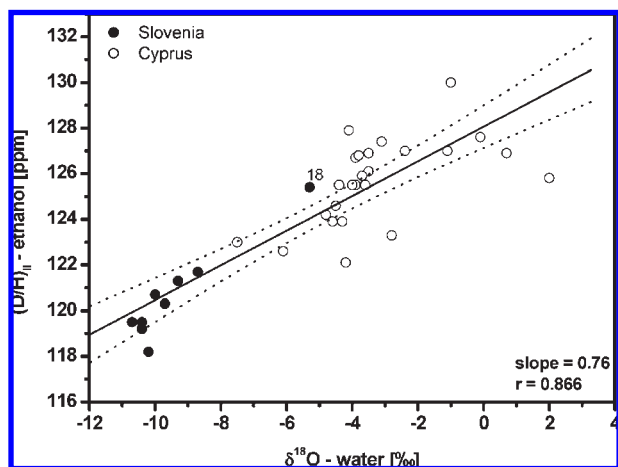


Figure 3. Correlation between the $\delta^{18}\text{O}$ values determined in fruit juice water and the $(\text{D}/\text{H})_{\text{II}}$ values of fruit juice ethanol. Dotted lines represent 95% confidence intervals about the regression. Natural single-strength juice made from apples is marked (sample 18).

and two pineapple juices. All of these juice samples were declared as 100% natural juices without sugar addition. On the basis of $\delta^{13}\text{C}$ and $(\text{D}/\text{H})_{\text{I}}$ values it was found that beet sugar was added in three citrus samples (samples 3, 4, and 26) and four apple juice samples (samples 14, 16, 21, and 22), whereas in two samples (samples 6 and 25) a mixture of beet and cane sugar addition was detected.

On the basis of the results obtained in this paper it is evident that commercial Slovenian and Cypriot fruit juices do not always correspond to declarations on their packaging. Therefore, correct labeling should be enforced through legislation to protect the rights of both consumers and producers.

Geographical Origin Assignment Using $\delta^{18}\text{O}$ Values. The $\delta^{18}\text{O}$ values were different and, on average, higher in Cypriot than in Slovenian fruit juices. The meteoric and consequently tap waters used for redilution of fruit juices are isotopically different. The reason for this lies primarily in the higher annual average temperatures and more arid climate conditions typical for the island of Cyprus and the consequent enrichment in heavier isotopes in precipitation and water. The $\delta^{18}\text{O}$ values for Cyprus fruit juices ranged from -6.1 to $+0.7\text{‰}$, with the lowest observed in sour cherry fruit juice. The $\delta^{18}\text{O}$ values in Slovenian fruit juices ranged between -10.7 and -5.3‰ . The highest $\delta^{18}\text{O}$ value of -5.3‰ was observed in pure apple juice (sample 18). These data, together with the $\delta^{13}\text{C}$ values of sugars, indicate that the juice is authentically made from apples. The $(\text{D}/\text{H})_{\text{II}}$ value correlates with the $\delta^{18}\text{O}$ value (Figure 3), and two groups of samples can be readily identified. The fruit juices from the colder, continental Slovenia are concentrated in the lowest part of the diagram, whereas those from Cyprus, as a warmer, Mediterranean country, are in the upper zone enriched in ^{18}O . The wide range of $\delta^{18}\text{O}$ values obtained by Calderone and Guillou (22) also shows the effect of the origin of samples, and clear separation was found between continental and peninsular countries. A projection of the first two principal components representing 82.8% of the total variance ($\text{PC1} = 54.8\%$, $\text{PC2} = 28.0\%$) of data for samples produced in Slovenia and Cyprus is presented (Figure 4). Very good separation of samples from different countries was found, no matter what their botanical origin was. Such a good separation of juices can be expected due to the different precipitation regimens and climates. In addition, the common practice in the production of fruit juices is to concentrate pressed juices for easier and cheaper storage and then dilute with tap water just before

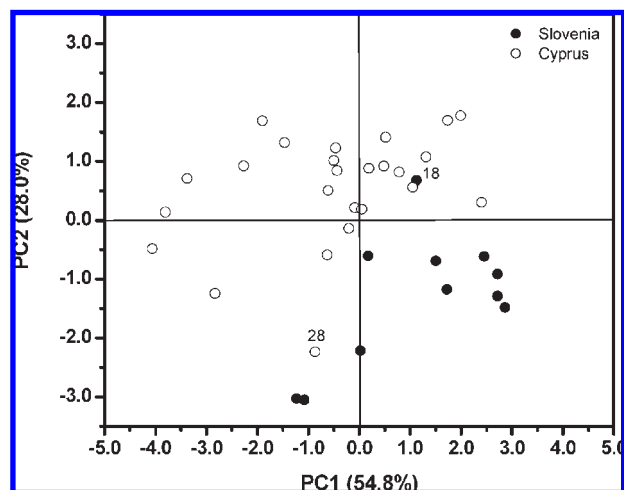


Figure 4. PCA performed with isotopic ratios $(\text{D}/\text{H})_{\text{I}}$, $(\text{D}/\text{H})_{\text{II}}$, $\delta^{13}\text{C}$ of ethanol, $\delta^{13}\text{C}$ of sugars, $\delta^{18}\text{O}$ of water, and ratio R for the samples of fruit juices produced in Slovenia and Cyprus. The plane constituted by the first two principal components $\text{PC1}/\text{PC2}$ represents 82.8% of the total variance for six variables. Samples 18 and 28 are not classified properly; one is natural single-strength juice and the other pineapple juice with cane sugar addition, respectively.

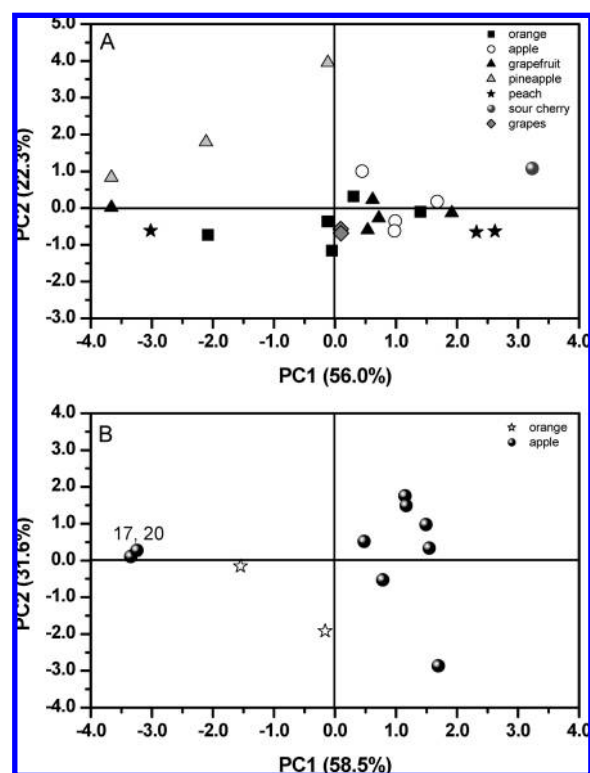


Figure 5. (A) PCA performed with isotopic ratios $(\text{D}/\text{H})_{\text{I}}$, $(\text{D}/\text{H})_{\text{II}}$, $\delta^{13}\text{C}$ of ethanol, $\delta^{13}\text{C}$ of sugars, $\delta^{18}\text{O}$ of water, and ratio R for the samples of fruit juices produced in Cyprus. The plane constituted by the first two principal components $\text{PC1}/\text{PC2}$ constitutes 78.3% of the total variance for six variables. (B) PCA performed with isotopic ratios $(\text{D}/\text{H})_{\text{I}}$, $(\text{D}/\text{H})_{\text{II}}$, $\delta^{13}\text{C}$ of ethanol, $\delta^{13}\text{C}$ of sugars, $\delta^{18}\text{O}$ of water, and ratio R for the samples of fruit juices produced in Slovenia. The plane constituted by the first two principal components $\text{PC1}/\text{PC2}$ represents 90.1% of the total variance for six variables. Two samples, 17 and 20, highly diluted with cane sugar addition are identified.

packaging. There are only two samples that fall in the wrong group. The first sample, which was declared as 100% natural apple juice produced in Slovenia (sample 18), gave the highest $\delta^{18}\text{O}$ value of all of the Slovenian juices. For this sample the production technology is different, because it is not concentrated but only pressed from the fruit, without redilution (water addition). The second sample was the juice from pineapple produced in Cyprus (sample 28), which falls in the group of Slovenian juices. As already mentioned, in this sample the presence of C_3 product could be detected using $\delta^{13}\text{C}$ values in the sugar. This assumption is further supported by the difference between the $\delta^{13}\text{C}$ values of the pulp and the sugar, which was found to be 3.8‰.

Further statistical evaluation was used to differentiate between fruit juices of different botanical origins. The projections of the first two principal components for fruit juice samples produced in Cyprus (Figure 5A) and in Slovenia (Figure 5B) are presented. Only pineapple juices could be distinguished from the other juices in the case of Cypriot juices. The reason for that is that pineapple belongs to the CAM group of plants, whereas the other juices are produced from the fruits of the C_3 group of plants. A better picture could be seen in the case of Slovenian samples, in which only orange and apple juices were evaluated. There are actually three different groups of samples: orange juices, apple juices, and another group of two apple juices (Figure 5B). A more detailed evaluation of these two samples (samples 17 and 20) showed that they were both highly diluted (15 and 50%). After this dilution, exogenous cane sugar was added, which shifted isotope ratios to higher values.

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