Application of ^{2}H SNIF-NMR and ^{13}C SIRA-MS Analyses to Maple Syrup: Detection of Added Sugars

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The SNIF-NMR method may be used to measure the site specific isotope concentrations in a variety of organic compounds. The most common application of this technique involves quantitative deuterium nuclear magnetic resonance spectroscopy with appropriate interpretation of the accumulated spectra. SNIF-NMR together with SIRA-MS provides a powerful tool for food authentication and characterization. The concerted use of SNIF-NMR (deuterium) and SIRA-MS (carbon-13) on ethanol fermented from sugars has proven invaluable to the authentication of wines, spirits, and fruit juices. In this work, the analysis is extended to the authentication of maple syrup, a product rich in sucrose. Authentic maple syrup samples have been analyzed in detail to characterize the intrinsic variability of the isotopic ratios in this product. These data constitute a data base of authentic samples to which an unknown example of maple syrup can be compared. The isotopic ratios obtained from maple syrup are very different from those of both beet and cane or corn sugars. Therefore, the methodologies developed in this work are applicable for the detection of beet and cane or corn sugar added to a maple syrup. It can be shown that, through a simple statistical comparison between the data base and the unknown, a determination of the occurrence and/or extent of adulteration with added sugar can be made.

Keywords: *Maple syrup; adulteration; NMR; isotopic analysis; SNIF-NMR; sugar analysis*

INTRODUCTION

Maple syrup is produced from the xylem sap of the sugar maple, *Acer saccharum*. Tapped during the end of the winter season, this sap contains around 2% solids; these solids may be concentrated through evaporation of the water, leaving behind a syrup rich in sugars. The sugars, mostly sucrose, together with various trace metals (Whalen and Morselli, 1984), organic acids (Mollica and Morselli, 1984; Morselli and Whalen, 1986; Wassem et al., 1991), and various phenolics, furfurals (Kermasha et al., 1995), flavor components (Belford et al., 1991, 1992; Filipic et al., 1969), and chromophores, some of which are formed during the concentration process (Underwood and Filipic, 1964; Underwood, 1971), constitute maple syrup. Although great variability can exist in the sap content of various trees, around 35 L of sap is required to produce 1 L of pure syrup.

The major component of maple syrup, sucrose, can also be obtained from other, much less expensive sources (U.S. Department of Commerce, 1988): for example, corn, beets, and cane. These sources could be used to extend pure maple syrup with a concomitant increase in profit. Such adulteration has been reported widely in other industries (Patel, 1994), impacting not only the price but also consumer perceptions about the purity of the product. The ability to identify the botanical origin of any added sugars is a concern of the maple industry (Baggett and Morselli, 1982).

Background. Sophisticated isotope analyses are proving to be effective additions to the existing range of analytical tools utilized for food and beverage authentication. The SNIF-NMR (site specific nuclear isotope fractionation as measured by nuclear magnetic resonance) method (Martin and Martin, 1981; Martin et al., 1986, 1991, 1992) uses nuclear magnetic resonance spectroscopy to quantify the deuterium/hydrogen ratios on specific sites in a given molecule. After the technique was developed in the early 1980s, the original application of it was the detection of the chaptalization, or enrichment, in wines (Martin and Martin, 1988). Addition of sugars to a grape must prior to fermentation serves to increase the alcohol content and concomitantly the value of the wine. This addition of sugar is referred to as chaptalization. Both the deuterium and carbon-13 content on ethanol show a dependence on the botanical origin of the sugars from which the alcohol was fermented. Through isotopic analysis, it is possible to show the addition of non-grape sugars (beet, corn, cane, etc.) to a must *after* the solution is fermented into a wine. The most effective method currently in use to protect against chaptalization is SNIF-NMR. In the European Union (EU), chaptalization is either strictly limited or completely illegal. SNIF-NMR has been adopted in the EU as the official method for controlling chaptalization (*Official Journal of the European Communities*, 1990).

SNIF-NMR has been extended to products other than wine to show the addition of undeclared sugars. When this technique is applied to a product such as orange juice, the sample is fermented under strictly controlled conditions. The resulting alcohol is removed through a computer-controlled distillation apparatus. The quantitative removal of alcohol is assured through monitoring the alcohol grade of the fermented juice and the

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Table 1. $\delta^{13}C_{PDB}$ (‰) Ranges Observed for the C-3 and C-4 Pathways Together with Examples of Plant Sources of Foods and Sweeteners Found in Each Pathway[a]

C-3	C-4
$-24‰ < \delta^{13}C < -30‰$	$-9‰ < \delta^{13}C < -12‰$
apple	cane
beet	corn
citrus fruits	
maple syrup	

[a] The overall carbon isotope ratio of ethanol is expressed in the δ scale: $\delta^{13}C_{PDB}$ (‰) = 1000 $[(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{PDB}]/(^{13}C/^{12}C)_{PDB}$ (PDB being the international reference for carbon).

distillate. Finally, SNIF-NMR is used to measure the D/H ratios on the ethanol. This application of SNIF-NMR has been collaboratively studied for both the European Normalization Committee (CEN/TC174/WG 1) and the AOAC International (Martin et al., 1996a,b).

The SNIF-NMR technique, when applied to beverages, consists of quantitative nuclear magnetic resonance together with measurement of the overall $^{13}C/^{12}C$ ratios of the ethanol through mass spectroscopy. This latter technique can be used to distinguish between the two main photosynthetic pathways, C-3 and C-4, utilized by plants to produce sugars (Bender, 1971; Park and Epstein, 1960). For fruit juice authentication, the measurement of overall isotope ratios carried out on ethanol or directly on the extracted carbohydrates has been used to differentiate between fruit sugar and exogenous sugars (Bricout, 1978; Marell et al., 1978; Doner and Bills, 1981; Krueger and Reesman, 1982; Dunbar and Schmidt, 1984; Bricout and Koziet, 1987; Doner et al., 1987; Koziet et al., 1986; Lee and Wrolstad, 1988; Widmer et al., 1992). The analysis can be extended further through measurement of the D/H and $^{18}O/^{16}O$ ratios on the water from the juice (Nissenbaum et al., 1974; Brause et al., 1984; Cohen and Saguy, 1984). Table 1 shows the typical ranges observed in various food products as a function of their photosynthetic pathway.

While ^{13}C mass spectrometry permits differentiation between the two pathways, quantitative NMR goes one step further. SNIF-NMR permits discrimination of botanical origin *within* a pathway. Adulteration of wines and juices often occurs through the addition of sugars derived from beet. Since beet utilizes the same photosynthetic pathway as grape, maple syrup, orange juice, and a variety of other products, detection of added sugar is essentially impossible using ^{13}C mass spectroscopy alone. Some other techniques have been applied to infer the presence of beet sugars. These are based on the occurrence of high-molecular weight sugars characteristic of the type of beet sugar used in the adulteration (Dupuy, 1978; Vialle, 1981). When sugars derived from beet are used to adulterate C-3 fruit juices such as orange, the sucrose must be chemically inverted to produce the sucrose/fructose/glucose profile found in these fruits. This inversion leads to the production of oligosaccharides which are not found in the pure juice. The presence of these oligosaccharides can then be used as a strong indication of adulteration with inverted sugars (Swallow et al., 1991; Wudrich et al., 1993; Stuckel et al., 1995).

Since maple syrup consists of mostly pure sucrose, typically over 95% (Morselli et al., 1984), it may be adulterated through the addition of pure sucrose and *not* inverted sugar syrups. Oligosaccharides characteristic of sucrose inversion are not present in the sucrose used as an adulterant. This powerful chromatographic approach to the detection of adulteration is rendered less effective. As will be shown later in this paper, SNIF-NMR can be used not only to detect added sugars but also to quantify the amount added.

Chart 1

CH_2DCH_2OH	CH_3CHDOH	CH_3CH_2OD
I	II	III

The steps involved in the detection and quantification of added sugars using these isotopic methods involve a comparison of the values obtained from the suspect product with those parameters measured on authentic, representative samples. Critical to the proper interpretation of the data is the development of (i) a set of appropriate rules to guide this comparison and (ii) a representative data base of the product in question. This paper addresses the creation of a comprehensive, isotopic data base for maple syrup samples gathered from the major producing regions in North America. This work shows, for the first time, a complete NMR and mass spectroscopic analysis of maple syrup samples.

METHOD

Authentic maple syrup samples were collected from the major producing regions of North America, with a particular emphasis placed on Québec and Vermont. Three methods were utilized for the collection of syrup samples. A series of 33 samples was accumulated by Mapleville USA, Inc., a maple syrup distribution company. Mapleville utilized an independent agency which followed sample collection practices published by the U.S. Department of Agriculture (USDA). These samples were gathered in 1991, 1992, and 1993. During the 1995 season, Eurofins Laboratories purchased samples of maple syrup, at random, throughout the state of Vermont directly from small, independent producers. Finally, Agriculture and Agri-Food Canada supplied 20 authentic samples from the main Canadian producing regions. These samples were gathered in accordance with standard sample collection procedures practiced by Agriculture Canada.

Sample Treatment. Maple syrup consists primarily of sucrose. To conduct SNIF-NMR this sugar must first be fermented into ethanol. This AOAC International method used to analyze the samples has been described in detail in a previous publication (Martin et al., 1996a). The steps involved prior to this fermentation include measurement of the °Brix of the syrup followed by dilution to a standard 12° Brix with water. The isotopic content of the water was previously determined through mass spectroscopy. This point will be discussed in more detail in the next section. Fermentation of the resulting solution is carried out with a standard yeast strain for about 3 days (Martin et al., 1983a). The solution is carefully monitored using a commercial enzyme kit to determine when all sugars have been converted to alcohol. At this point, the alcohol grade of the solution is measured. This step is followed by distillation with a computer-controlled Cadiot column fitted with a Teflon spinning band, the automatic distillation control or ADCS system. The precise determination of the water content of the recovered ethanol is obtained by the Karl Fischer method. The yield of the extracted ethanol is always greater than 95% by mass.

The D/H ratios are measured by recording the intensities of the NMR peaks corresponding to the three isotopomers listed in Chart 1 (Martin and Martin, 1988). A typical NMR spectrum is shown in Figure 1. The alcohol is mixed with a known quantity of an official internal standard, *N*,*N*-tetramethylurea (TMU), supplied by the Institute for Reference Materials and Measurements at Geel, Belgium. An internal ^{19}F reference is also introduced to lock the magnetic field. A detailed description of typical spectrometer settings is provided elsewhere (Martin et al., 1983b).

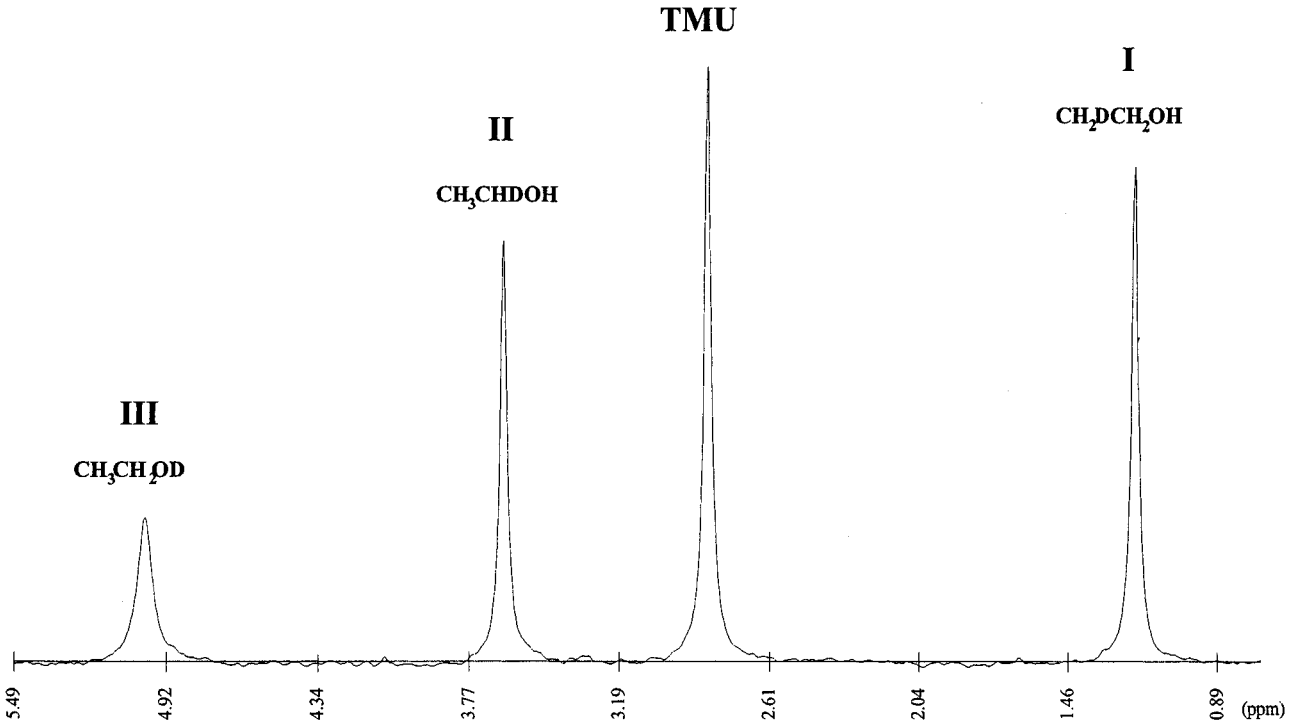

Figure 1. Deuterium spectrum of ethanol fermented from maple syrup.

The site specific isotope ratios measured on a given molecule are dependent on the isotopomeric content of that species. In the case of ethanol, CH_3CH_2OH, for instance, three monodeuterated isotopomers are considered at natural abundance.

Deuterium NMR provides direct access to the isotope ratios associated with the methyl, $(D/H)_I$, and methylene, $(D/H)_{II}$, sites of ethanol. These parameters are obtained by referring the areas of the ^2H NMR signals, I and II, to that of a working standard of tetramethylurea, TMU, the isotope ratio of which has been previously calibrated (Martin et al., 1985). A relative parameter, R, has also been defined as

$$R = 2[(D/H)_{II}/(D/H)_I] = 3S_I/S_{II} \quad (1)$$

where S_I and S_{II} are the intensities of signals I and II. This parameter, which can be obtained without the use of a standard, represents the isotope content of site II corresponding to an isotope content in the methyl site arbitrarily characterized by the probability factor 3.

These parameters are determined on ethanol obtained from the fermentation of the syrup appropriately diluted with water of known isotopic content and in accordance with well-defined experimental protocol (Martin et al., 1996a).

Normalization of the Isotopic Parameters. Determination of the isotopic makeup of the water used in dilution of the syrup is important. It turns out that, during fermentation, hydrogen (or deuterium) from the water is introduced to a small extent into the methyl position of the ethanol and to a much larger extent on the methylenic site (Martin et al., 1986). It was shown in an earlier study (Martin et al., 1986) that, during fermentation, nonexchangeable protons from sites 1, 6, and 6′ of glucose and fructose transfer almost quantitatively to the CH_3 site in the resulting ethanol. The influence of the isotopic makeup of the medium $(D/H)^s_w$ as it pertains to SNIF-NMR has been the subject of intense investigation, and it has been shown that the water used for the fermentation has only a small though not negligible influence on the isotopic makeup of the methyl position on ethanol. When water with varying D content was used for the fermentation of the same juices (where D ranged from 150 to 170 ppm), the isotopic parameters of ethanol varied linearly ($r > 0.99$) as a function of the $(D/H)^s_w$ value of the fermentation medium. Since during fermentation the nonexchangeable protons transfer quantitatively to the methyl position in the ethanol (Zhang et al., 1995), this D/H ratio is used for identification of the botanical origin of the sugars. Since the final results depend to some small extent on the water used for fermentation, the practice of normalizing all results to those obtained from the Vienna international standard V.SMOW (Martin et al., 1996b) was adopted in this work. This reference which is internationally agreed as the most suitable (Craig, 1961) has an isotope ratio $(D/H)^{V.SMOW}$ of 155.76 ppm. This standardization facilitates direct comparison of work carried out in different laboratories using slightly different waters for the dilution of the syrup.

Normalization of the results is performed by using eqs 2−4 below. These equations also consider previous published (Martin et al., 1986, 1996b) and unpublished work and the results of several other series of measurements carried out in different institutes.

$$(D/H)_I^{Norm.V.SMOW} = (D/H)_I - 0.19[(D/H)^s_w - 155.76] \quad (2)$$

$$(D/H)_I^{Norm.V.SMOW} = (D/H)_{II} - 0.78[(D/H)^s_w - 155.76] \quad (3)$$

$$R^{Norm.V.SMOW} = R - 0.011[(D/H)^s_w - 155.76] \quad (4)$$

Determinations of Overall Isotope Ratios of Sugar, Ethanol, and Water by SIRA-MS. The ^{13}C determinations may be carried out on the syrup or directly on the alcohol obtained after fermentation. When the fermentation is carried out under rigorously controlled conditions, a strong correlation exists between the results obtained using each of these methods. Table 3 contains the results obtained with both methods on some of the authentic samples presented in Table 2 as well as on commercial maple syrups purchased during the 1995 season. From these data, it appears that, when the same sample is examined using each of the two methods, the ^{13}C value obtained on the sugar is about 0.4‰ less negative than that obtained from the ethanol. The standard deviation on the difference is 0.24‰. This difference is the result of isotopic fractionation which takes place during the evolution of CO_2 while fermentation occurs.

The $\delta^{13}C$ deviations observed following direct measurements on the sugars (Table 3) have almost the same mean value (−24.1‰) as the literature value of −24.2‰ (Morselli et al., 1984). The value reported here is also identical to that in an

Table 2. Isotopic Parameters of Maple Syrup from the United States and Canada Produced in the 1992, 1993, and 1995 Seasons[a]

no.	year	region[b]	$\delta^{13}C$(‰)	$(D/H)_I^{Norm.V.SMOW}$	$(D/H)_{II}$	$R_I^{Norm.V.SMOW}$
1	92	VT	−23.7	101.5	127.6	2.513
2	92	NY	−23.2	101.1	128.1	2.535
3	92	NH	−23.5	102.6	131.5	2.565
4	92	VT	−23.5	100.1	128.0	2.559
5	92	MI	−23.4	100.6	127.2	2.530
6	92	OH	−22.7	101.2	127.2	2.515
7	92	NH	−23.5	100.9	126.3	2.505
8	92	ME	−23.8	100.4	127.0	2.530
9	92	ME	−23.9	100.1	127.0	2.538
10	92	O	−23.2	99.8	126.5	2.535
11	92	NB	−23.9	101.1	127.4	2.520
12	92	NB	−23.1	100.4	127.6	2.543
13	92		−23.6	100.9	127.1	2.519
14	92	QB	−23.2	100.1	127.6	2.549
15	92	QB	−23.5	100.8	128.6	2.552
16	92	QB	−23.6	100.6	127.1	2.527
17	92	QB	−23.3	99.7	128.1	2.569
18	92	QB	−23.5	101.0	127.2	2.518
19	92	QB	−23.2	102.0	127.3	2.496
20	92	QB	−23.6	100.7	127.4	2.532
21	92	QB	−23.5	100.6	128.1	2.546
22	92	QB	−23.5	100.5	126.6	2.521
23	92	QB	−23.4	100.7	126.8	2.519
24	92	QB	−23.4	100.9	126.9	2.514
25	92	QB	−23.9	100.9	126.5	2.506
26	93	QB	−23.6	101.6	129.9	2.556
27	93	VT	−23.7	101.2	129.3	2.555
28	93	VT	−23.8	100.8	129.3	2.565
29	93	VT	−23.6	101.3	128.4	2.535
30	93	VT	−23.5	101.4	129.9	2.562
31	93	QB	−23.3	100.9	129.5	2.566
32	93	ME	−24.1	100.9	129.1	2.559
33	93	OH	−23.3	99.5	127.9	2.571
34	93	OH	−23.2	100.0	128.8	2.576
35	95	VT	−24.0	101.5	129.9	2.560
36	95	VT	−24.2	101.3	128.9	2.544
37	95	VT	−24.1	101.1	129.8	2.566
38	95	VT	−24.0	101.7	129.7	2.551
39	95	VT	−24.1	101.2	128.7	2.545
40	95	VT	−23.9	101.4	129.9	2.563
41	95	VT	−23.2	101.5	129.2	2.546
42	95	VT	−23.6	101.5	128.9	2.539
43	95	VT	−24.3	101.0	129.8	2.571
44	95	VT	−24.3	101.7	130.0	2.556
45	95	VT	−24.0	101.1	130.3	2.575
46	95	VT	−24.0	101.4	129.9	2.564
47	95	VT	−24.0	100.9	129.2	2.560
48	95	ON	−23.4	102.5	130.2	2.541
49	95	NS	−23.9	100.8	129.4	2.565
50	95	ON	−23.6	100.0	128.0	2.559
51	95	NS	−23.7	101.1	129.1	2.555
52	95	QB	−24.5	100.7	128.7	2.557
53	95	NB	−23.7	100.4	129.2	2.574
54	95	QB	−23.9	102.2	128.4	2.512
55	95	QB	−23.8	100.9	129.1	2.560
56	95	QB	−23.6	100.3	129.1	2.573
57	95	QB	−23.3	102.0	129.9	2.548
58	95	QB	−24.0	101.4	130.4	2.573
59	95	QB	−23.3	101.3	130.2	2.571
60	95	QB	−23.4	101.3	129.5	2.556
61	95	QB	−24.1	101.1	130.6	2.584
62	95	QB	−23.3	101.4	129.5	2.555
63	95	QB	−23.6	101.2	130.0	2.567
64	95	QB	−23.7	101.8	129.6	2.545
65	95	NB	−23.0	102.7	129.8	2.528
mean			−23.63	101.03	128.67	2.547
standard deviation			0.35	0.66	1.25	0.021

[a] The overall carbon isotope ratio of ethanol is expressed in the δ scale: $\delta^{13}C_{PDB}$ (‰) = 1000 $[(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{PDB}]/(^{13}C/^{12}C)_{PDB}$ (PDB being the international reference for carbon); $(D/H)_I^{Norm.V.SMOW}$ and $(D/H)_{II}^{Norm.V.SMOW}$ are the hydrogen isotope ratios of the methyl, I, and methylene, II, sites of ethanol, and $R_I^{Norm.V.SMOW}$ is defined in eqs 1−4. [b] The following abbreviations for states and provinces are used in this table: ME, Maine; MI, Michigan; NB, New Brunswick; NH, New Hampshire; NS, Nova Scotia; NY, New York; OH, Ohio; ON, Ontario; QB, Québec; VT, Vermont.

earlier report on Vermont maple syrups, −24.05‰ (Hilaire-Marcel, 1977). The mean value reported here for Québec samples is also relatively similar though slightly more negative than that reported previously (Hilaire-Marcel, 1977). With

Table 3. Differences Observed between the $\delta^{13}C$ Measured on the Sugars of Maple Syrup and the $\delta^{13}C$ Measured on the Ethanol Resulting from the Fermentation of the Same Samples[a]

type	$(D/H)_I$ ethanol	$\delta^{13}C$(‰) sugars	$\delta^{13}C$(‰) ethanol	enrichment
authentic	101.3	−24.3	−24	0.3
authentic	101.3	−24.2	−24.2	0
authentic	100.8	−24	−24.1	-0.1
authentic	101.4	−24.2	−24	0.2
authentic	100.9	−24.2	−24.1	0.1
authentic	101.1	−24.2	−23.9	0.3
authentic	101.3	−23.6	−23.2	0.4
authentic	101.4	−24.1	−23.6	0.5
authentic	100.7	−24.6	−24.3	0.3
authentic	101.4	−24.6	−24.3	0.3
authentic	100.8	−24.7	−24	0.7
authentic	100.9	−23.8	−24	-0.2
authentic	101.8	−23.5	−23.4	0.1
authentic	100.5	−24.4	−23.9	0.5
authentic	99.6	−24	−23.6	0.4
authentic	100.8	−24.1	−23.7	0.4
authentic	100.2	−24.6	−24.5	0.1
authentic	99.9	−24	−23.7	0.3
authentic	102	−23.7	−23.9	-0.2
authentic	100.7	−24.4	−23.8	0.6
authentic	100.1	−24.1	−23.6	0.5
authentic	101.7	−23.9	−23.3	0.6
authentic	101.1	−24.5	−24	0.5
authentic	101	−23.9	−23.3	0.6
authentic	100.7	−24.5	−24.1	0.4
authentic	101.2	−24	−23.3	0.7
authentic	100.9	−24.4	−23.6	0.8
authentic	101.5	−24.1	−23.7	0.4
authentic	102.4	−23.3	−23	0.3
commercial	100.6	−24	−23.4	0.6
commercial	100.6	−24.3	−23.9	0.4
commercial	100.3	−24.1	−23.6	0.5
commercial	100.9	−24.1	−23.6	0.5
commercial	101.9	−24.2	−23.6	0.6
commercial	101	−24.1	−23.6	0.5
commercial	101.3	−23.9	−23.3	0.6
commercial	100.9	−24.2	−23.8	0.4
commercial	100.7	−23.9	−23.4	0.5
commercial	101	−24.2	−23.5	0.7
commercial	101.2	−24.1	−23.6	0.5
commercial	100.2	−24.1	−23.5	0.6
mean of all samples	100.98	−24.12	−23.73	0.40
SD of all samples	0.57	0.30	0.34	0.24
mean of authentic	101.01	−24.13	−23.80	0.34
SD of authentic	0.61	0.35	0.37	0.26
mean of commercial	100.88	−24.10	−23.57	0.53
SD of commercial	0.46	0.12	0.17	0.09

[a] One can observe a small enrichment (0.39‰) of the ^{13}C content of ethanol. The standard deviation of this enrichement is 0.24‰. The standard deviations of $\delta^{13}C$ and $(D/H)_I$ are significantly lower for commercial syrups than for the authentic data base samples.

consideration of the standard deviation for the direct measurements reported here on the $\delta^{13}C$ for maple syrup, the acceptable range of two standard deviations is −24.8 to −23.4‰. In fact, the minimum value observed was −24.7‰, while the maximum value obtained was −23.3‰. The cutoff points reported here are in excellent agreement with the maximum value of −23.49‰ proposed elsewhere (Morselli, 1984) and the range of −24.3 to −23.7‰ reported by Leavitt (1985).

With the exception of those values summarized in Table 3, columns 1 and 2, all the $\delta^{13}C_{PDB}$ values reported in this publication have been measured on ethanol fermented from maple syrup under the strictly controlled conditions described earlier. The deuterium content of the starting fermentation water $(D/H)^{s}_{w}$ has also been measured by SIRA-MS (Koziet et al., 1995).

RESULTS AND DISCUSSION

The isotopic parameters obtained from the maple syrup samples are shown in Table 2. When consideration is given to the mass spectroscopic measurement on ethanol, the $\delta^{13}C$ results are in agreement with an earlier report on maple syrup (Morselli and Baggett, 1984; Carro et al., 1980). In this work, the $\delta^{13}C$ is measured on ethanol after fermentation is completed, which explains the shift observed with previous work where the $\delta^{13}C$ was measured on the entire syrup sample. On the basis of the ^{13}C results alone, there does not appear to be any statistical difference between the two main producing regions: Québec and Vermont. Figure 2 shows a plot of the ^{13}C as a function of the D/H ratio at the methyl position. The open circles represent samples gathered in the United States, while the closed squares are the result of analysis on Canadian syrup samples. A simple visual inspection of this diagram suggests that the $\delta^{13}C$ ratio for Canadian samples is slightly higher than those observed in the United States. Indeed, a simple Student's *t* test comparison of the means observed in each country does show a difference at the 95% confidence level. There are, however, some mitigating factors which must be considered before a final judgment can be made. In the current state of this data base, United States producing regions are somewhat under-represented when compared to those of Canada. Further, the samples were taken during different years. Since it has been shown previously that climatic conditions can alter the $(D/H)_I^{Norm.V.SMOW}$ ratio slightly, the difference observed in this study requires further, more intensive, investigation (Martin et al., 1988).

No attempt was made to measure the sugars in sap directly. It has been shown previously that processing has no statistical effect on the isotopic makeup of carbohydrates (Zhang et al., 1985). Further, this product is sold as a syrup rather than as a sap; consequently, to assist in the detection of adulteration, an isotopic data base must be developed for the *final* product, maple syrup, rather than for its sap.

One of the more interesting observations that can be made on these data is the very low variability of both $\delta^{13}C$ and $(D/H)_I^{Norm.V.SMOW}$. The standard deviation for each of these measurements is 0.35 and 0.66, respectively. These statistics are smaller than those observed from analysis on other food products, e.g. orange juice, grapefruit, etc. This difference is particularly pronounced for the $\delta^{13}C$ measurements. The most likely explanation for the comparatively low variability observed in maple syrup is the size of the region and the short length of the producing season. Fruits such as apples and oranges are produced in many regions of the earth with a much longer season. As will be discussed later, this small variability lowers the threshold for detection of added sugars.

While maple syrups from Vermont and Québec are compared, the geographical effect is more obvious. Moreover, it is also possible to observe that the isotopic variability is smaller in Vermont than in Québec. One possible explanation for this effect might be the difference in size of the producing regions for each of these origins. As shown in Figure 2, the geographical effect on the isotopic parameters is not as strong as to allow definitive geographic identification. However, this effect significantly increases the precision of the detection of possible adulteration when the geographic origin of the sample is known. The geographic effect observed in this study is so small that it was not possible to attribute differences to temperature, humidity, or latitude. The two factors discussed previously are usually more significant. No significant year to year variation was

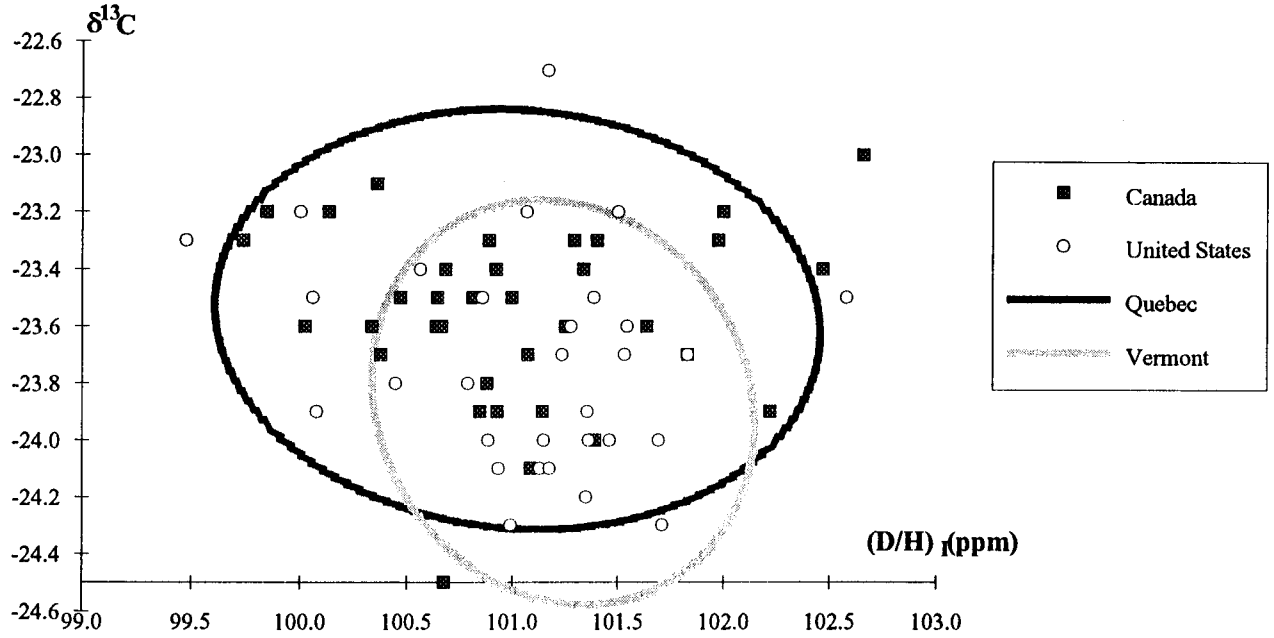

Figure 2. Plot of the $\delta^{13}C$ results as a function of the $(D/H)_I$ ratio for each of the samples examined in this study. The 95% confidence interval of maple syrup from Québec and Vermont allows the evaluation of the geographic effect on the isotopic parameters.

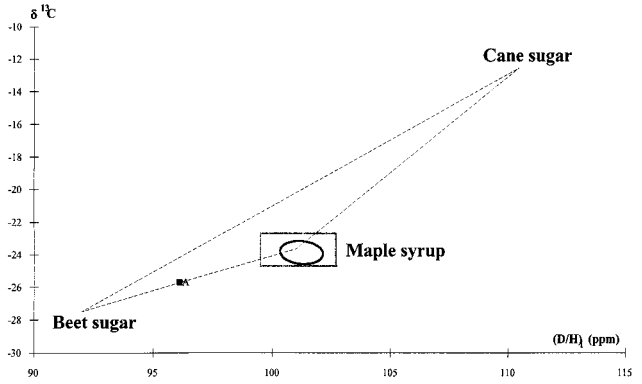

Figure 3. Adulteration triangle showing an adulterated "market sample" A, the rectangle defined by the cutoff points used to detect samples, and the 95% confidence eclipse for authentic maple syrup samples from Vermont. Using formula 5, sample A [$\delta^{13}C = -25.7$, $(D/H)_I = 96.1$] was found to contain at least 41% added beet sugar. It appears clearly from the graph that this formula and the cutoff points proposed provide a very conservative estimate of the amount of added sugar.

observed in the isotopic analysis of maple syrup reported in this study.

The major sources of commercial sucrose are beet, cane, and corn. The sugar beet, sugar cane, and properly converted corn can yield a product that is essentially pure sucrose. The isotopic parameters for cane and corn sucrose are similar; therefore, to effect clarity in the following discussions, the term "cane and/or corn sugar" will be abbreviated as simply cane sugar.

Since each of these plants uses a different photosynthetic pathway, Table 1, it is necessary to consider both the $\delta^{13}C_{PDB}$ values and the $(D/H)_I^{Norm.V.SMOW}$ when analyzing maple syrup. Figure 3 shows the optimal representation for these two sources of sugar. The values obtained are plotted in the plane of $\delta^{13}C_{PDB}$ values (vertical axis) and of the V.SMOW-normalized $(D/H)_I^{Norm.V.SMOW}$ values (horizontal axis). The isotopic parameters for authentic reference alcohols from beet, cane, and maple syrup (Table 2) form a triangle, which is illustrated in Figure 3. Pure beet, corn, and maple syrup samples lie at each apex. The ellipse drawn for maple syrup represents a 95% confidence interval for syrups from Vermont. Any mixture of these sugars lies within this triangle, the isotopic composition of a mixture correlates directly with the concentration of each component in that mixture. Hence, a sample consisting of a 50/50 mixture of beet and cane should have a $\delta^{13}C_{PDB}$ and $(D/H)_I^{Norm.V.SMOW}$ value which falls halfway between each of the respective apexes. This triangle is often referred to as an "adulteration triangle", since any sample which falls upon the triangle but outside the ellipse encompassing genuine syrup samples is considered to be adulterated.

Sugar addition can be quantified using eqs 5−9 below. The index, COP, refers to the "cutoff point" for the geographic origin of maple syrup under consideration. The subscripts beet and cane stand for the mean values of the beet and cane reference groups, respectively. The superscript P denotes the result obtained for the tested product. Although the index PDB and Norm.V.SMOW are omitted for the sake of clarity, the $(D/H)_I$, $(D/H)_{II}$, and R values must be corrected beforehand for the minor effect due to the fermentation water (eqs 2−4). Equation 5 or 6 allows the quantification of adulteration with beet or cane sugar only. In the case of adulteration with a mixture of cane and beet sugars, the total amount of added sugar could be simply calculated with formula 7, using eqs 8 and 9.

$$\%\text{adulteration}_{Min} = \%\text{beet}_{Min} = 100\frac{(D/H)_I^{P} - (D/H)_I^{COP}}{(D/H)_I^{beet} - (D/H)_I^{COP}} \quad (5)$$

$$\%\text{adulteration}_{Min} = \%\text{cane}_{Min} = 100\frac{\delta^{13}C^{P} - \delta^{13}C^{COP}}{\delta^{13}C^{cane} - \delta^{13}C^{COP}} \quad (6)$$

$$\%\text{adulteration}_{Min} = \%\text{cane}'_{Min} + \%\text{beet}'_{Min} \quad (7)$$

with

$$\%\text{beet}'_{\text{Min}} = 100 \times \{[(D/H)_{\text{I}}^{\text{P}} - (D/H)_{\text{I}}^{\text{COP}}](\delta^{13}\text{C}^{\text{cane}} - \delta^{13}\text{C}^{\text{COP}}) - [(D/H)_{\text{I}}^{\text{cane}} - (D/H)_{\text{I}}^{\text{COP}}](\delta^{13}\text{C}^{\text{P}} - \delta^{13}\text{C}^{\text{COP}})\}/\{[(D/H)_{\text{I}}^{\text{beet}} - (D/H)_{\text{I}}^{\text{COP}}](\delta^{13}\text{C}^{\text{cane}} - \delta^{13}\text{C}^{\text{COP}}) - [(D/H)_{\text{I}}^{\text{cane}} - (D/H)_{\text{I}}^{\text{COP}}](\delta^{13}\text{C}^{\text{beet}} - \delta^{13}\text{C}^{\text{COP}})\} \quad (8)$$

$$\%\text{cane}'_{\text{Min}} = 100 \times \{[(D/H)_{\text{I}}^{\text{beet}} - (D/H)_{\text{I}}^{\text{COP}}](\delta^{13}\text{C}^{\text{P}} - \delta^{13}\text{C}^{\text{COP}}) - [(D/H)_{\text{I}}^{\text{P}} - (D/H)_{\text{I}}^{\text{COP}}](\delta^{13}\text{C}^{\text{cane}} - \delta^{13}\text{C}^{\text{COP}})\}/\{[(D/H)_{\text{I}}^{\text{beet}} - (D/H)_{\text{I}}^{\text{COP}}](\delta^{13}\text{C}^{\text{cane}} - \delta^{13}\text{C}^{\text{COP}}) - [(D/H)_{\text{I}}^{\text{cane}} - (D/H)_{\text{I}}^{\text{COP}}](\delta^{13}\text{C}^{\text{beet}} - \delta^{13}\text{C}^{\text{COP}})\} \quad (9)$$

$$\text{beet sugar (mean value) } \delta^{13}\text{C}_{\text{PDB}} = -27.5‰ \quad (D/H)_{\text{I}} = 92.0 \text{ ppm} \quad (10)$$

$$\text{cane sugar (mean value)} = \delta^{13}\text{C}_{\text{PDB}} = -12.5‰ \quad (D/H)_{\text{I}} = 110.5 \text{ ppm} \quad (11)$$

This approach provides the minimum amount of added sugar. A more accurate assessment of the percentage of added sugar can be calculated using the mean values corresponding to the syrup origin instead of the cutoff points for the reference groups. However, when these mean values are used, it is sometimes possible to slightly overestimate the percentage of added sugar.

Determining appropriate criteria for adulteration can often prove challenging. The most straightforward method is to assume that any sample lying beyond a given number of standard deviations from the mean has a very low probability of being genuine. Various approaches resulting in setting the cutoff points 2, 3, and sometimes 4 standard deviations from the mean can be taken. Choosing 3 standard deviations leads to a very low probability of rejecting an authentic product. Few genuine syrup products lie this far from the mean. So, in order to detect the adulteration with sugar of a single sample of maple syrup, the cutoff points should be set at around 3 standard deviations from the mean. However, if the sample is a blend of different origins, the product is likely to be adulterated when the isotopic parameters are only 2 standard deviations from the mean. Also, when used in monitoring or for the case of quality control for a provider, the cutoff points should be defined at 2 standard deviations from the mean in order to detect small but systematic adulteration.

If the 3 standard deviation rule is adopted, this selection criterion would then create cutoff points at 99 and 103 ppm for $(D/H)_{\text{I}}^{\text{Norm.V.SMOW}}$ and −24.7 to −22.7‰ for the δ^{13}C data. These cutoff points provide a high degree of confidence (>99.7%) that a sample is adulterated. On the basis of the 65 genuine samples reported in this work, the minimum value for $(D/H)_{\text{I}}^{\text{Norm.V.SMOW}}$ observed is 99.5 ppm, and the maximum observed is 102.7 ppm. For δ^{13}C, the highest value obtained is −22.7‰. Consequently, any sample lying beyond these later limits must be at least considered highly suspect. This situation is particularly true for industrial syrup, since the practice of blending averages differences from batch to batch. Consequently, the variability observed in the isotopic makeup on such syrups is smaller than the values reported in this work. For example, the standard deviations of the isotopic parameters measured on commercial maple syrup samples purchased in the second half of 1995 were $^1/_3$ smaller for $(D/H)_{\text{I}}$ and $^1/_2$ of the values for δ^{13}C obtained from authentic, data base samples (Table 3). These observations confirm that cutoff points set at ±2 standard deviations from the mean provide sufficient certainty for not mistaking as adulterated a genuine maple syrup sample.

Further, the $(D/H)_{\text{I}}$ and δ^{13}C values should not only be examined independent from each other. A bivariate analysis of the results is advisable, particularly when both variables lie far from the mean values for authentic maple syrups. Indeed, it is extremely unlikely that *both* parameters are *simultaneously* 2 standard deviations from the mean values obtained for authentic syrups. In such cases, bivariate probability ellipses should be used as discussed elsewhere (Martin et al., 1996b).

From all these observations, we can conclude that, depending on the isotopic values of the original, pure maple syrup, SNIF-NMR will normally detect any added beet sugar, when such sugar represents between 5 and 20% of the total solids. Prior knowledge of the geographic origin of the syrup improves the detection limit slightly. The threshold for detection of added sugar in a blend is lower than that for a nonblended maple syrup sample.

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