Detection of Exogenous Sugars or Organic Acids Addition in Pineapple Juices and Concentrates by ^{13}C IRMS Analysis

Eric Jamin,*,† Javier Gonzalez,† Gérald Remaud,† Norbert Naulet,‡ and Gilles G. Martin†

Eurofins Laboratories, rue Pierre Adolphe Bobierre, B.P. 42301, F-44323 Nantes Cedex 03, France, and Laboratoire d'Analyse Isotopique et Electrochimique de Métabolismes, CNRS UPRES-A 6006, Université de Nantes, 2 rue de la Houssinière, B.P. 92208, F-44322 Nantes Cedex 03, France

When pineapple concentrate is adulterated, organic acids are often added to "naturalize" the chemical composition. To improve isotopic methods devoted to the study of pineapple product authenticity, three major components (sugars, malic acid, and citric acid) have been used as individual probes for the determination of carbon-13 isotope ratios. After a cleanup step, sugars and organic acids were separated from each other by an anion exchange process, and pure malic and citric acids were isolated by preparative reversed-phase HPLC. This method has been applied to the stable isotope analysis of pineapple juice samples from different locations and production years . A correlation between the carbon isotope ratios of sugars and organic acids has been observed, and cutoff points concerning the difference of δ^{13}C values between those metabolites have been defined. In the case of the addition of sugar from C3 plants (such as beet), it has been demonstrated that the detection limit can be as low as 10% or even 5%, whereas it is often >20% when using the conventional carbon-13 method on the whole juice. Malic acid and citric acid addition also becomes detectable using these individual measurements.

Keywords: *Pineapple juice; carbon 13; sugars; organic acids; authentication*

INTRODUCTION

Although they are not going to be thoroughly reviewed in this paper, nonisotopic chemical methods, devoted to authenticity assessment of pineapple juices and concentrates according to natural concentration and ratio ranges, have an interesting contribution in the detection of adulteration (Association of German Fruit Juice Industries, 1987; AIJN, 1993; Krueger et al., 1992; Low et al., 1994). However, sophisticated formulators of fraudulent juice add cocktails of inexpensive sweeteners, organic acids, and mineral salts, so that adulteration becomes undetectable using compositional analysis by itself. Therefore, the combined use of isotopic and nonisotopic analytical methods is usually the most effective way to detect adulteration (Lees et al., 1996). SNIF-NMR (Site-Specific natural Isotope Fractionation determined by Nuclear Magnetic Resonance, a trademark of Eurofins Laboratories, Nantes, France; AOAC, 1996; Martin et al., 1996a) and isotope ratio mass spectrometry (IRMS) (Dunbar and Schmidt, 1984; Brause et al., 1984; Doner et al., 1987; Bricout and Koziet, 1987; Rossmann et al., 1990; Widmer et al., 1992; Koziet et al., 1993; Krueger, 1995; Jamin et al., 1997) can be used to differentiate between fruit sugar and various types of exogenous sugars, such as cane and beet sucrose, high-fructose corn syrup, and beet invert syrup. Since carbon and hydrogen isotope ratios provide complementary information, the combination of these two techniques improves the reliability of fruit juice authentication methods (Martin et al., 1996b).

The carbon-13 content of pineapple juice, derived from a Crassulacean acid metabolism (CAM) plant, is very high compared to that of products from plants as-similating carbon dioxide through the C3 photosynthetic pathway (O'Leary, 1988). A mean value in the vicinity of −12‰ is commonly accepted for sugars from pineapple (Krueger et al., 1992). Therefore, the addition of a C3 product (typically around −25‰) into pineapple juices or concentrates lowers the δ^{13}C of the final product, thus enabling adulteration proof. Nevertheless, the natural distribution of carbon isotope ratios of CAM plants is highly influenced by environmental conditions, which results in a wide natural range for this parameter. Hence, for δ^{13}C of the sugars, cutoff points of −15‰ and −11‰ have been defined (AIJN, 1993; Low et al., 1994). Therefore, there is a need for new isotopic probes to enhance the information provided by the δ^{13}C of the sugars. Moreover, when the standardized (CEN) method (Koziet et al., 1993) for measuring δ^{13}C is performed correctly, the sugars have to be purified before IRMS analysis. Unfortunately, in practice, the published δ^{13}C values often correspond to the overall organic material of the pineapple product (sugar, pulp, organic acids, etc.). Therefore, measurement on isolated sugars may be preferable because it should improve the precision and accuracy of the carbon isotope ratio determination.

The use of internal standards for carbon isotope ratio analysis makes IRMS a powerful tool for detecting adulteration of food products such as honeys (White and Winters, 1989; AOAC, 1995). In the case of fruit juices, the pulp has been proposed as a convenient internal reference (Parker, 1982; Bricout and Koziet, 1987). Nevertheless, in the case of commercial products, industrial treatments (concentration, clarification) sometimes eliminate most of the pulp or may change the composition of the pulp fraction. On the other hand, soluble components are more likely to be preserved throughout industrial processes, so that they are more reliable in terms of internal referencing, provided that addition of these soluble products can also be checked. The use of intermolecular isotope correlations between

* Author to whom correspondence should be sent [fax (33) 2 51 83 21 11; e-mail Eurofinsfr@aol.com].
† Eurofins Laboratories.
‡ Université de Nantes.

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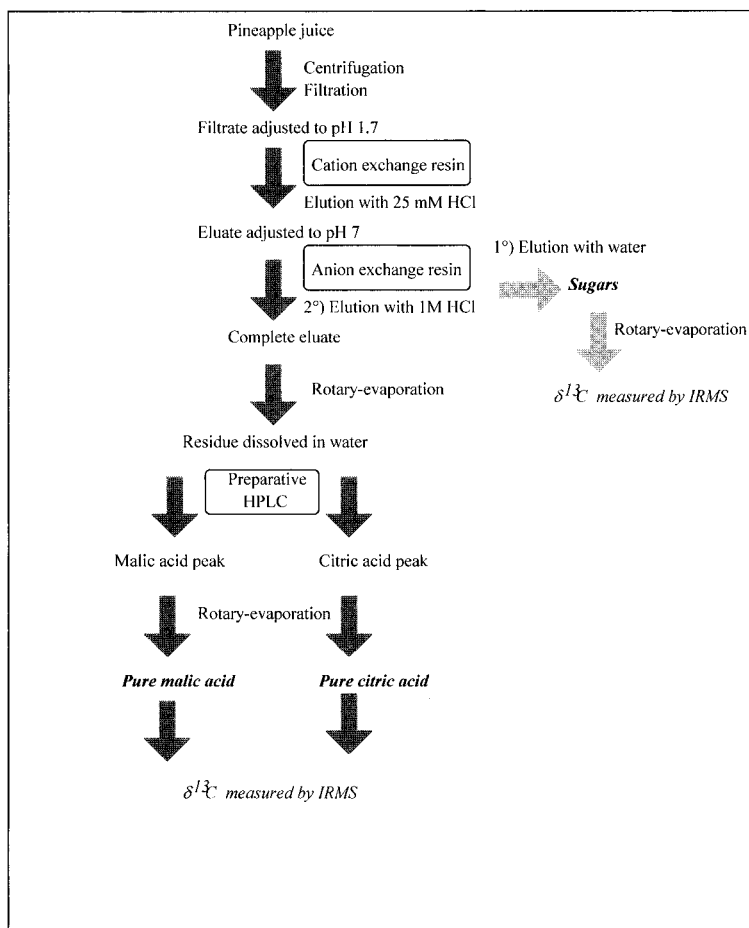
Figure 1. Flow chart of the analytical protocol used for the isolation of sugars, malic acid, and citric acid from pineapple juice.

sugars and organic acids in fruit juices has been suggested as a criterion for authenticity assessment (Schmidt et al., 1993; Gensler et al., 1994; Rossmann et al., 1995; Jamin et al., 1997). The simultaneous isolation of sugars, malic acid, and citric acid, as described in this paper, leads to a characteristic carbon-13 fingerprint of authentic pineapple products. It will be examined to what extent the assessment of characteristic ranges of carbon isotope ratios improves the detection of sugars or organic acids addition in pineapple juices and concentrates.

MATERIALS AND METHODS

Chemicals and Supplies. Dowex cation exchange resin AG50WX8 (200–400 mesh, H$^+$ form) was obtained from Sigma-Aldrich (St. Quentin Fallavier, France). Glass columns (1 cm i.d.) were filled with 10 g of resin. Anion exchange resin AG1X8 (200–400 mesh, Cl$^-$ form) was obtained from BioRad (Ivry-sur-Seine, France). Glass columns (1 cm i.d.) were filled with 20 g of resin.

Chromatographic Equipment. The chromatographic system consisted of a Merck Model L-6250 pump, a Rheodyne Model 7725i injector, and a Precision Instruments Iota2 RI detector (all elements purchased from Merck, Nogent-sur-Marne, France). The column used for preparative chromatography was a Lobar Lichroprep RP-8 size 6 (440 × 37 mm) with 40–63 μm packing (Merck).

Samples Description. This work is based on the study of 35 different pineapple samples from different origins. Pure pineapple juices were obtained from fresh fruits squeezed in the laboratory immediately after their arrival using a Centri Bistrot robot (Robot Coupe S.A., Bagnolet, France) after removal of the peel. Some authentic pineapple juices or concentrates were also analyzed. The years of production

taken into account are 1993, 1994, and 1996. The origins of the fruits are representative of the three main production places around the world: most of the samples came from either Africa (Benin, Cameroon, Ivory Coast, Kenya), South and Central America (Brazil, Costa Rica, Honduras, Martinique, Puerto Rico), or Asia (China, Japan, Indonesia, Philippines, Thailand). One sample from Hawaii and one from Australia were also included in the study. Finally, five pineapple concentrates industrially produced in Thailand were analyzed.

Sample Preparation. The analytical procedure has been adapted to the case of pineapple juice from a general method devoted to the isolation of the main organic acids from fruit juices and nectars (Gensler and Schmidt, 1994). The entire procedure is described in Figure 1. It allows the simultaneous isolation of sugars and of pure malic and citric acids from pineapple juices.

The L-malic acid and citric acid contents of all samples were determined by enzymatic titration [using International Federation of Fruit Juice Producers (IFU) Methods 21 and 22; AIJN, 1993], and an aliquot containing 500 mg of citric acid was submitted to the separation procedure. Juices or diluted concentrates (5-fold w/w dilution) were centrifuged (7500g, 10 min) and filtered. The filtrate was adjusted to pH 1.7 and applied to the cation exchange resin (previously conditioned at the same pH). Amino acids and other cationic substances were retained, and elution was done with 200 mL of 25 mM HCl. A rapid test (color reaction) was done at this stage to check that all sugars had been eluted, and if not, elution was carried out further. The complete eluate was adjusted to pH 7 with 5 M NaOH and applied to the anion exchange resin (previously conditioned at the same pH). Organic acids and other anionic substances were retained, and the sugars were removed by washing with 250 mL of water. A rapid test (color reaction) was done at this stage to check that all sugars had been eluted, and if not, elution was carried out further. Then the organic acids were eluted with 200 mL of 1 M HCl. This

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Table 1. Isotopic Recovery of Sugars, Malic Acid, and Citric Acid, Analyzed on a Test Mixture

	δ^{13}C found	δ^{13}C shift from original value
cane sugar	−11.1	−0.2
malic acid	−26.0	+0.2
citric acid	−11.5	−0.2

eluate was concentrated to 100 mL, and its malic acid and citric acid contents were determined by enzymatic titration. The recovery yield for each acid should be, at this stage, >95%.

The resulting solution was rotary-evaporated, and the residue was dissolved in 20 mL of water. Five milliliters of the resulting solution was injected into the preparative high-performance liquid chromatography (HPLC) device. Elution was performed with 5.8 mM HCl (10 mL/min) at room temperature, and the malic and citric acid peaks were collected separately. The elution times were from 40 to 54 min after injection for malic acid and from 56 to 90 min after injection for citric acid. Since chromatography is a source of isotopic fractionation (Gensler and Schmidt, 1994), a baseline separation of the two peaks has to be achieved and the peaks have to be collected completely. The sugars, malic acid, and citric acid fractions were all concentrated under vacuum.

IRMS Measurements. The carbon isotope ratios were determined using a Finnigan Mat DeltaE mass spectrometer (Orsay, France) associated with a Carlo Erba NA1500C-N elemental analyzer (Rueil Malmaison, France). Sugars and organic acids were introduced into the elemental analyzer as liquid. Residual water was removed after combustion. All δ^{13}C values are related to the Pee Dee Belemnite (PDB) carbonate standard:

$$\delta^{13}\text{C (\textperthousand)} = [(R_i/R_{\text{ref}}) - 1] \times 1000 \qquad (1)$$

R_i and R_{ref} are, respectively, the isotopic ratios ^{13}C/^{12}C of the sample and of the analytical reference.

RESULTS AND DISCUSSION

Reliability of the Method. *Accuracy.* The isotopic recovery of the isolation procedure has been determined using a test mixture of cane sugar, malic acid, and citric acid of known isotopic content. The concentrations of cane sugar, malic acid, and citric acid have been set to 100, 2, and 8 g/L, respectively, which correspond to the mean values in the literature for total sugars and organic acids (Dillon, 1995). Sixty-five milliliters of this solution was submitted to the analytical process depicted in Figure 1. The shift of the analyzed δ^{13}C value from the original value was not >0.2‰ for any of the three components (Table 1). Therefore, it can be assumed that the method we employed is free of isotopic effects, provided that yields close to 100% are reached. This has been checked for each component at each stage of the preparation procedure (see Materials and Methods). Moreover, the malic and citric acids we employed have δ^{13}C values of −26.2 and −11.3, respectively, so that even a very limited amount of mixing between the fractions due to an overlap of chromatographic peaks would have resulted in a significant change of the δ^{13}C of each acid. Therefore, this test shows that the HPLC separation used in this study allows a complete separation of malic and citric acids.

Repeatability. The overall repeatability of the complete process has been determined by performing 10 independent analyses on the same pineapple juice sample. The standard deviation of repeatability Sr is 0.1‰ for sugars, malic acid, and citric acid δ^{13}C values, which means that the repeatability is below 0.3‰. These results are consistent with the repeatability determined for the method of ^{13}C analysis of sugars in fruit juices recently adopted by CEN, involving precipi-

Table 2. ^{13}C Deviations of Sugars (δ^{13}Cs), Malic Acid (δ^{13}Cm), and Citric Acid (δ^{13}Cc) Isolated from Pineapple Samples from Various Production Areas[a]

origin	sugars δ^{13}Cs	malic acid δ^{13}Cm	citric acid δ^{13}Cc	diff δ^{13}Cs − δ^{13}Cm	diff δ^{13}Cs − δ^{13}Cc	diff δ^{13}Cm − δ^{13}Cc
Benin	−13.9	−14.2	−14.4	0.3	0.5	0.2
Cameroon	−12.9	−13.6	−13.7	0.7	0.8	0.1
Ivory Coast	−12.5	−12.8	−13.4	0.3	0.9	0.6
Ivory Coast	−13.7	−13.5	−14.5	−0.2	0.8	1.0
Ivory Coast	−13.4	−14.1	−14.8	0.7	1.4	0.7
Kenya	−10.8	−9.6	−10.4	−1.2	−0.4	0.8
Brazil	−11.5	−12.1	−12.7	0.6	1.2	0.6
Costa Rica	−12.1	−12.3	−13.1	0.2	1.0	0.8
Costa Rica	−12.2	−11.9	−12.7	−0.3	0.5	0.8
Puerto Rico	−12.2	−11.9	−12.7	−0.3	0.5	0.8
Honduras	−11.5	−11.2	−12.7	−0.3	1.2	1.5
Martinique	−12.1	−11.2	−12.5	−0.9	0.4	1.3
Martinique	−11.4	−10.5	−12.2	−0.9	0.8	1.7
Martinique	−11.6	−11.5	−12.1	−0.2	0.5	0.6
Martinique	−11.6	−10.7	−12.1	−0.9	0.5	1.3
Martinique	−12.0	−11.9	−12.0	−0.1	0.0	0.1
Martinique	−11.7	−11.3	−13.0	−0.4	1.3	1.7
Martinique	−12.0	−11.3	−12.3	−0.7	0.3	1.0
Martinique	−11.5	−11.2	−11.8	−0.3	0.3	0.6
China	−11.6	−11.9	−13.0	0.3	1.4	1.1
Japan	−11.9	−12.0	−13.1	0.1	1.2	1.1
Thailand	−12.7	−13.2	−13.0	0.5	0.3	−0.3
Thailand	−12.7	−12.3	−12.9	−0.4	0.2	0.6
Thailand	−12.6	−12.3	−12.8	−0.3	0.2	0.5
Indonesia	−13.2	−13.4	−13.6	0.2	0.4	0.2
Indonesia	−13.1	−13.4	−13.4	0.3	0.3	0.0
Philippines	−11.5	−2.1	−12.3	0.6	0.8	0.2
Philippines	−12.4	−12.0	−12.3	−0.4	−0.1	0.3
Australia	−12.5	−11.9	−12.4	−0.6	−0.1	0.5
Hawaii	−11.3	−10.4	−11.1	−0.9	−0.2	0.7
mean	−12.20	−12.06	−12.76	−0.15	0.56	0.71
SD	0.76	1.11	0.92	0.53	0.49	0.50
min	−13.9	−14.2	−14.8	−1.2	−0.4	−0.3
max	−10.8	−9.6	−10.4	0.7	1.4	1.7
cutoff min	−13.7	−14.3	−14.6	−1.2	−0.4	−0.3
cutoff max	−10.7	−9.8	−10.9	0.9	1.5	1.7

[a] The minimum, maximum, mean, and standard deviation (SD) have been calculated from the whole population of 30 samples. The proposed cutoff points (cutoff min and cutoff max) were calculated as the 95% confidence interval limits of each parameter.

Table 3. Standard Deviation (SD), Minimum, and Maximum of ^{13}C Deviations of Sugars (δ^{13}Cs), Malic Acid (δ^{13}Cm), and Citric Acid (δ^{13}Cc) Isolated from Seven Authentic Industrial Pineapple Concentrates from Various Production Places

	sugars δ^{13}Cs	malic acid δ^{13}Cm	citric acid δ^{13}Cc	diff δ^{13}Cs − δ^{13}Cm	diff δ^{13}Cs − δ^{13}Cc	diff δ^{13}Cm − δ^{13}Cc
SD	0.64	0.92	0.57	0.52	0.26	0.45
min	−13.2	−13.4	−13.6	−0.9	−0.1	0.0
max	−11.5	−10.7	−12.1	0.6	0.8	1.3

tation of organic acids and amino acids as calcium salts, recovery of sugars from the supernatant, and lyophilization (Koziet et al., 1993).

δ^{13}C Values of Sugars and Organic Acids from Authentic Pineapple Juices and Concentrates. The carbon isotope ratios of sugars, malic acid, and citric acid of 30 authentic samples have been determined according to the analytical procedure described in Figure 1. The δ^{13}C values and the minimum, maximum, mean, and standard deviation for the whole population of 30 samples are presented in Table 2. The mean values for sugars, malic acid, and citric acid are close to previously published results obtained when the organic acids were precipitated as calcium salts and then further separated by HPLC, while the sugars were obtained by lyophilization of the supernatant (Gensler

et al., 1994). The minima and maxima values of $\delta^{13}C$ computed from the whole set of measurements on individual samples confirm the large natural ranges of carbon isotope ratios in pineapple juice (AIJN, 1993; Low et al., 1994). Therefore, the only way to improve the detection of sugar addition is the use of intermolecular standardization.

A slight geographical discrimination can be observed. Hence, $\delta^{13}C$ values < -13 for all three components taken into account are only observed for samples from western Africa (Benin, Ivory Coast) and Indonesia. The photosynthetic activity of CAM plants is directly influenced by water availability in the field where these plants are grown: when water is available during the day, the carbon metabolism changes from C4 to C3, so that $\delta^{13}C$ values are lowered (O'Leary, 1988). Therefore, the low $\delta^{13}C$ values observed in western Africa and Indonesia may come from a higher relative humidity in those countries. Thus, the use of ^{13}C deviations, together with measurement of nitrogen (Kornexl et al., 1996) and deuterium isotope ratios (Martin et al., 1996b) using other molecular probes, could improve the recognition of geographical origins.

Table 2 also displays the values of the differences between individual $\delta^{13}C$ values of sugars ($\delta^{13}Cs$), malic acid ($\delta^{13}Cm$), and citric acid ($\delta^{13}Cc$). It appears that the ranges for these differences are lower than those observed for the individual components. These results clearly indicate the occurrence of an isotopic correlation between the organic acids and sugars. Indeed, the correlation coefficients between $\delta^{13}C$ values are as follows: r (sugars, malic acid) = +0.90, r (sugars, citric acid) = +0.85, and r (malic acid, citric acid) = +0.90. Therefore, a characteristic pattern of ^{13}C distribution between sugars, malic acid, and citric acid in authentic pineapple juices can be built. To improve the authentication of pineapple juice products, the use of these systematic differences as an authenticity criterion can be recommended.

The consequence of the mixing of large amounts of pineapples in the fruit-processing industry is that commercial juices and concentrates do not show such extreme $\delta^{13}C$ values as those given in Table 2 for isolated batches pressed from 1 kg of fruits or less. This was verified by experiments on industrially made pineapple concentrates (Table 3). Therefore, the risk of making a false positive conclusion of adulteration corresponding to a natural outlier sample is null. Such averaging effects of industrial processing of fruit juices have also been shown elsewhere (Martin et al., 1996b). The results on authentic samples presented in Table 2 being representative for a large number of production areas around the world, it can be supposed that all of the unadulterated commercial samples will fall within the $\delta^{13}C$ values intervals centered on the whole population mean plus or minus twice the larger standard deviation observed for the analysis of the samples reported in this paper, made from single laboratory squeezed fruits. The cutoff points of Table 2 have been defined using this statistical approach. It is worth noting that for all three components, the calculated $\delta^{13}C$ cutoff points are quite close to the minima and maxima values observed on this population of laboratory-made juices. The minima and maxima values of the differences between two parameters are even comprised within the intervals defined by the corresponding cutoff values.

$\delta^{13}C$ Values of Sugars and Organic Acids from
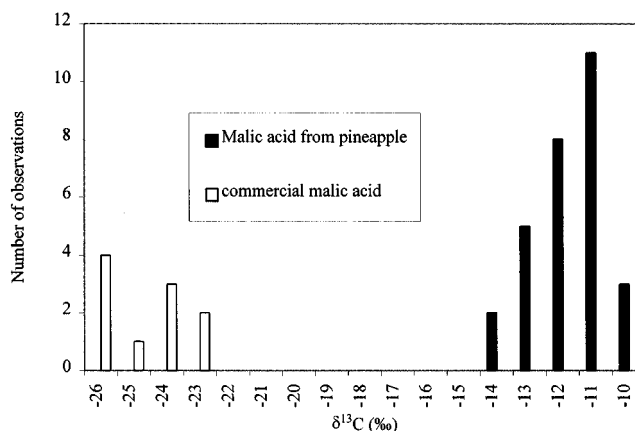
Figure 2. Frequency distribution histogram of $\delta^{13}C$ values of malic acids isolated from authentic pineapple juices and purchased from chemical suppliers.

Table 4. ^{13}C Deviations of Sugars ($\delta^{13}Cs$), Malic Acid ($\delta^{13}Cm$), and Citric Acid ($\delta^{13}Cc$) Isolated from Five Pineapple Concentrates Processed in Thailand

sample	sugars $\delta^{13}Cs$	malic acid $\delta^{13}Cm$	citric acid $\delta^{13}Cc$	diff $\delta^{13}Cs - \delta^{13}Cm$	diff $\delta^{13}Cs - \delta^{13}Cc$	diff $\delta^{13}Cm - \delta^{13}Cc$
1	−12.3	−12.4	−13.7	0.1	1.4	1.3
2	−12.3	−12.0	−12.5	−0.3	0.2	0.5
3	−12	−11.8	−17.0	−0.2	5.0	5.2
4	−12.6	−12.5	−14.2	−0.1	1.6	1.7
5	−11.5	−11.8	−11.4	0.3	−0.1	−0.4

Commercial Pineapple Concentrates. Since the Southeast Asia is the most important place of production for pineapple concentrates used in the fruit juice industry, we have analyzed some commercial pineapple concentrates made with fruits grown in Asia and processed in Thailand. Table 4 displays the results for these five samples. According to the limits defined on authentic samples (Table 2), no addition of exogenous sugars or malic acid can be detected in these samples. However, it appears that citric acid has been added to sample 3: the $\delta^{13}C$ values of citric acid and of the differences with the other components are outside the accepted range. In the case of sample 4, since the difference between sugars and citric acid $\delta^{13}C$ values is 0.1‰ higher than the cutoff maximum, addition of citric acid having a low $\delta^{13}C$ value may be suspected. In the case of sample 5, since the difference between malic and citric acid $\delta^{13}C$ values is 0.1‰ lower than the cutoff minimum, addition of citric acid having a low $\delta^{13}C$ value may also be suspected.

These observations illustrate the usefulness of ^{13}C fingerprinting in the study of pineapple product authenticity. In some countries, citric acid addition to pineapple juice may be authorized under some circumstances, but it must be declared. The IRMS analysis of carbon isotope ratios may then be used to check that the regulations are respected.

$\delta^{13}C$ Values of Commercial Organic Acids. Figure 2 is a histogram representation of frequencies associated with the $\delta^{13}C$ values observed for 10 commercially available pure malic acid samples from 10 different batches and 8 different suppliers, as well as for the 30 malic acids isolated from pineapple mentioned in Table 2. It appears that all commercial products have $\delta^{13}C$ values < -23.7, indicating that the starting products used for chemical synthesis probably have their ^{13}C in the C3 plant range. Since the minimum value acceptable for malic acid from authentic pineapple juice is -14.3‰, the detection of malic acid addition should

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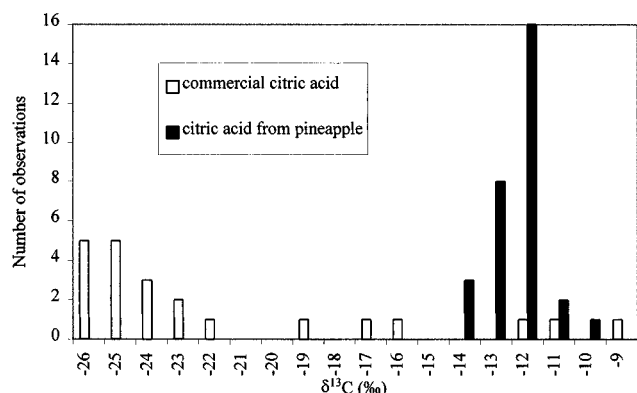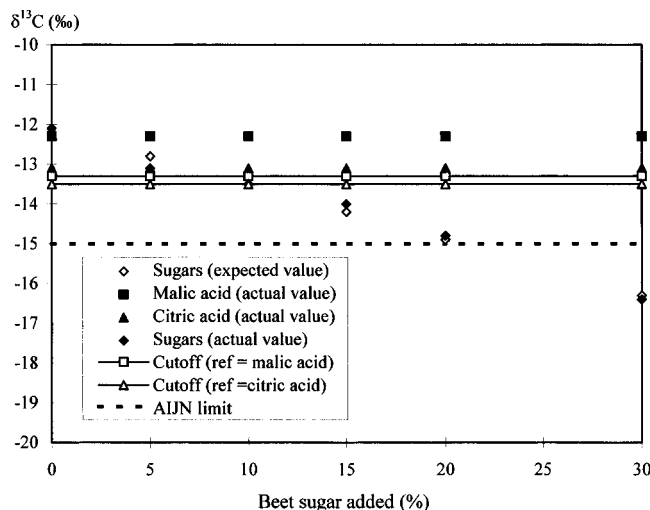

Figure 3. Frequency distribution histogram of $\delta^{13}C$ values of citric acids isolated from authentic pineapple juices and purchased from chemical suppliers.

Figure 4. Plot of $\delta^{13}C$ values of sugars, malic acid, and citric acid versus percentage of beet sugar ($\delta^{13}C = -26.1$) addition. The proposed cutoff values have been computed using the cutoff min values defined in Table 2.

be feasible in many instances. Indeed, by taking into account mean values of $-25.3‰$ and $-12.1‰$ for each type, it appears that the $\delta^{13}C$ value of malic acid will go out of the accepted range after a 17% commercial malic acid addition. Moreover, this detection limit is even lower when sugars or citric acid can be used as internal reference.

Figure 3 is a histogram representation of frequencies associated with the $\delta^{13}C$ values observed for 22 commercially available pure citric acid samples from 22 different batches and 9 different suppliers, as well as for the 30 citric acids isolated from pineapple mentioned in Table 2. Although most of the commercial citric acids have $\delta^{13}C$ values in the C3 plant range, higher values can also be found. Unlike malic acid, citric acid is in most cases produced by fermentation of sugars or sometimes paraffin hydrocarbons so that the $\delta^{13}C$ value observed for citric acid reflects that of the fermentation medium (Doner, 1985). Since C3 or C4 molasses as well as mixtures of C3 and C4 sugar products can be used, the resulting range for citric acid ^{13}C deviation goes from $-9‰$ to $-30‰$ (Figure 3). However, since the ranges defined for citric acid from authentic pineapples (Table 2) are much narrower than those found for commercial citric acids, the detection of added citric acid should be feasible in many cases.

Detection of an Addition of a C3 Product in Pineapple Juice. The European guidelines concerning pineapple juices state that $\delta^{13}C$ values of sugars < -15 are indicative of addition of C3 products (AIJN, 1993). Taking into account this minimum value and a mean $\delta^{13}C$ value for endogenous sugars of -12.2 (Krueger et al., 1992), the detection limit corresponds to a 2.8‰ shift. Since the mean $\delta^{13}C$ value for beet sucrose is of the order of -26.2 (Rossmann et al., 1995), the detection limit is not better than 20% when the ^{13}C deviation of sugars is used by itself. Moreover, the addition of citric or malic acid, even in large amounts, will not result in a significant change of the $\delta^{13}C$ value of the whole juice (often measured instead of that of sugars), because their concentrations are much lower than that of sugars.

The usefulness of sugars and organic acids ^{13}C fingerprinting was tested by two spiking experiments. Controlled amounts of beet sucrose ($\delta^{13}C = -26.1$, experimental) and citric acid of C3 origin ($\delta^{13}C = -25.0$, experimental) were added to a pineapple juice bearing typical $\delta^{13}C$ values of -12.1, -12.3, and -13.1 for sugars, malic acid, and citric acid, respectively. The resulting samples have been analyzed according to the procedure depicted in Figure 1, and the $\delta^{13}C$ values of sugars and citric acid have been measured. The ex-

pected values ($\delta^{13}C_{exp}$) for given amounts of C3 product added (Am%) have been computed according to

$$\delta^{13}C_{exp} = \frac{(Am\%)(\delta^{13}C_{add}) + (100 - Am\%)(\delta^{13}C_{ini})}{100} \quad (2)$$

where $\delta^{13}C_{ini}$ is the initial $\delta^{13}C$ value and $\delta^{13}C_{add}$ is the $\delta^{13}C$ value of the corresponding adulterant.

Figure 4 shows that the decrease of the $\delta^{13}C$ value of sugars against the amount of C3 sugar added is linear and in agreement with the expected values. By taking into account the cutoff minimum values defined in Table 2 for differences between the ^{13}C contents of sugars and organic acids, two cutoff points can be calculated. In this example, while the $\delta^{13}C$ value of sugars conforms to AIJN guidelines (AIJN, 1993) up to a 20% addition, the detection limit is close to 10% when malic acid is used as the internal reference and only slightly higher than 10% when citric acid is used as the internal reference. This experiment demonstrates that detection levels reached by using organic acids as internal references are significantly lower than those obtained from the use of the sugar $\delta^{13}C$ value by itself, so that more sensitive detection and proof of adulteration can be made using the additional information provided.

Figure 5 shows that the decrease of the $\delta^{13}C$ value of citric acid against the amount of added exogenous acid is linear and conforms to the expected values. By taking into account cutoff maximum values defined in Table 2 for differences between the ^{13}C contents of sugars and organic acids, two cutoff points can be calculated. In this example, the detection limit is around 5% when sugars are used as the internal reference and around 10% when malic acid is used as the internal reference.

Detection of an Addition of a C4 Product. Because of its low price, high-fructose corn syrup (HFCS) is often used to raise the sugar content of fruit juices. Like other products from C4 plants, HFCS has a $\delta^{13}C$ value of about -10, so that adding this product increases the $\delta^{13}C$ value of the juice. Similar results are obtained in the case of a cane sugar addition. Detecting such adulterations is obviously much more difficult since the $\delta^{13}C$ values of sugars from C4 plants largely overlap those of pineapple sugar. Nevertheless, the use of internal referencing can also improve the detection limit

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δ^{13}C (‰)
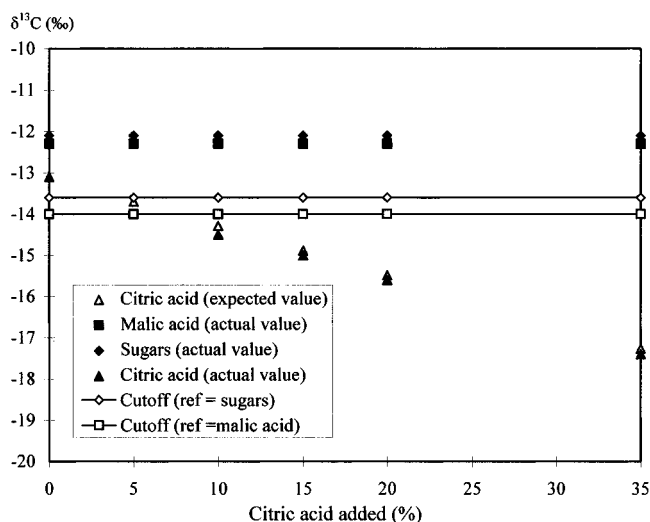
Figure 5. Plot of δ^{13}C values of sugars, malic acid, and citric acid versus percentage of commercial citric acid (δ^{13}C = −25.0) addition. The proposed cutoff values have been computed using the cutoff max values defined in Table 2.

Table 5. Expected Values for Sugars (δ^{13}Cexp) and for the Difference between Those and Organic Acids after Addition of Sugar with a δ^{13}C Value of − 10 into a Pineapple Juice with an Initial δ^{13}C Value of Sugars of −13.7[a]

C4 sugar added Am%	sugars δ^{13}Cexp	malic acid δ^{13}Cm	citric acid δ^{13}Cc	diff δ^{13}Cexp − δ^{13}Cm	diff δ^{13}Cexp − δ^{13}Cc
0	−13.7	−13.5	−14.3	−0.2	0.6
10	−13.3	−13.5	−14.3	0.2	1.0
20	−13.0	−13.5	−14.3	0.5	1.3
30	−12.6	−13.5	−14.3	0.9	1.7
40	−12.2	−13.5	−14.3	1.3	2.1
50	−11.9	−13.5	−14.3	1.7	2.5
60	−11.5	−13.5	−14.3	2.0	2.8
70	−11.1	−13.5	−14.3	2.4	3.2

[a] The δ^{13}C values of malic acid (δ^{13}Cm) and citric acid (δ^{13}Cc) have been calculated from the mean differences of Table 2.

of ^{13}C measurements and allow adulteration proof in some favorable cases.

Table 5 displays a calculation of expected results in the case of a sample with a starting δ^{13}C value of sugars equal to the cutoff min of Table 2, i.e. −13.7. Expected values for malic and citric acids have been computed from the mean differences displayed in Table 2. Expected values for sugars resulting from the addition of various amounts of a sweetener with a value of −10‰ have been computed according to eq 2. In this example, the δ^{13}C value of the sugars conforms to AIJN guidelines (AIJN, 1993) up to a 70% addition, while the cutoff points of the differences between sugars and organic acids are reached after a 30% addition. Thus, it appears that in the case of samples with a low initial ^{13}C content, adulteration with C4 sugars might also be detected.

CONCLUSION

The present study shows that in the case of an addition of exogenous sugar in pineapple, be it a C3 sugar or a mixture with sugar from C4 plants, the detection level of the existing isotopic methods could be significantly lowered by using the information provided by the simultaneous IRMS measurement of sugars and organic acids isolated from the juice. A correlation has been shown between the δ^{13}C values of these components, whatever the origin of pineapples, and cutoff

values for the differences between δ^{13}C values of sugars, malic acid, and citric acid are proposed. In the meantime, the use of the same analytical procedure enables the detection of organic acids addition by defining natural δ^{13}C ranges for these components.

Because of the overlap between δ^{13}C ranges of pineapple and C4 plants (cane, corn), the combined use of SNIF-NMR and ^{13}C IRMS fingerprinting is the best way to minimize the detection level of C4 sugar addition. Since SNIF-NMR makes use of deuterium isotope ratios measurements, these two techniques elicit complementary information about the authenticity of pineapple products.

In practice, the first step of the interpretation of an unusual ^{13}C profile observed for an unknown sample is to identify which components could have been added and which ones can be regarded as internal references. Most of the time, adulteration practices tend to raise the soluble solids content of the product by adding inexpensive sweeteners. Addition of citric acid can also be used to keep a satisfactory Brix/acid ratio. In both cases, malic acid will be a fair internal reference. The possibility of a simultaneous addition of inexpensive sweeteners together with citric and L-malic acid to keep a satisfactory compositional profile of the product has to be taken into account (Low et al., 1994). However, such sophisticated adulteration would be detected because the δ^{13}C value of malic acid would be too low.

This improvement of isotopic detection of adulterations using new probes as internal references can probably be extended as a general principle for other fruit juices. For example, in the case of citrus juices, malic and citric acid are also available in quantities compatible with a simultaneous isolation as pure compounds using preparative HPLC. The use of the pulp as a common internal reference could even offer the opportunity to detect a simultaneous adulteration of sugars and organic acids. In the future, application of the SNIF-NMR method to organic acids (Remaud et al., 1995) should also provide complementary information for the detection of the addition of products from the same metabolic type.

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