Specific Natural Isotope Profile Studied by Isotope Ratio Mass Spectrometry (SNIP−IRMS): $^{13}C/^{12}C$ Ratios of Fructose, Glucose, and Sucrose for Improved Detection of Sugar Addition to Pineapple Juices and Concentrates

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The $\delta^{13}C$ values of fructose, glucose, and sucrose have been determined in authentic pineapple juices. The sugar fraction is separated from the organic acids by an anionic exchange process. Then the individual components (fructose, glucose, and sucrose) are isolated on a preparative HPLC device using a NH_2-type column. It is demonstrated that no significant isotope fractionation occurs when close to 100% of material is recovered and when the hydrolysis of sucrose is avoided. The control of the recovery rates and of the sucrose hydrolysis rate after purification is recommended for a reliable interpretation of the results. Correlations between the $\delta^{13}C$ values of fructose ($\delta^{13}Cf$), glucose ($\delta^{13}Cg$), and sucrose ($\delta^{13}Csu$) can be characterized by systematic differences between these values. For the set of measurements on authentic pineapple juices and concentrates, the mean and the standard deviation of the differences are $\delta^{13}Cf - \delta^{13}Cg = -0.6 \pm 0.6‰$, $\delta^{13}Cf - \delta^{13}Csu = -1.3 \pm 0.6‰$, and $\delta^{13}Cf - \delta^{13}Csu = -0.7 \pm 0.5‰$. The determinations of the ^{13}C content of fructose, glucose, and sucrose enable a refinement of the detection of added sugars in fruit juices, re-enforcing the SNIP−IRMS method.

Keywords: *Carbon-13; fructose; glucose; sucrose; pineapple; SNIP−IRMS; preparative HPLC; adulteration; authentication*

INTRODUCTION

The most common fraudulent practice of concern to the fruit juice industry is the addition of sugars. The optimum analytical approach for detecting such an adulteration relies on a combined use of isotopic and nonisotopic analytical methods (Brause, 1995; Rossmann et al., 1995; Lees et al., 1996; Martin et al., 1997). The nonisotopic analyses applied to pineapple can be separated in two categories. The first one concerns the composition of some characteristic constituents for which typical ranges have been defined for authentic samples (Krueger et al., 1992). The other one is based on the oligosaccharides profile (Low et al., 1994). Oligosaccharide analysis either by liquid chromatography or by capillary gas chromatography allows the detection of sweeteners at 10% levels of the total sugars. This approach is effective whatever the origin of the sugars added (C3 or C4 metabolism of the plant which is the source of the sugars). Unfortunately, all types of sweeteners are not detected. Thus, the addition of pure sucrose or clean invert sugar, which do not show characteristic marker peaks in the chromatogram, cannot be detected by this method (Stöber et al., 1998a,b). Isotopic analyses have been shown to be the most powerful techniques for detecting sugar addition in fruit juices. Thus the SNIF−NMR method (a trademark of Eurofins Scientific, Nantes, France) has been recognized as an official AOAC method (AOAC Official Method 995.15, 1996; Martin et al., 1996). It is routinely applied to detect the addition of beet-derived sweeteners in fruit juices, by measuring D/H ratios of the separate sites of the ethanol molecule obtained after fermentation of the fruit sugars. The IRMS technique provides complementary authenticity indicators (Bricout and Koziet, 1987; Doner, 1991; Krueger, 1995). It measures overall isotope ratios of whole molecules or products. Carbon isotope ratios thus provide an important means of differentiating between C3-derived sweeteners (beet, citrus fruits) and C4-derived sweeteners (cane, corn). The use of intermolecular isotope correlations has already been proposed (Parker, 1982; Bricout and Koziet, 1987; White and Winters, 1989). Recently, after initial publications by Schmidt and co-workers (Schmidt et al., 1993; Gensler and Schmidt, 1994), we introduced the SNIP−IRMS method (a trademark of Eurofins Scientific, Nantes, France). It uses the correlation of the $\delta^{13}C$ values between several metabolites isolated from fruit juices. This internal reference approach has been applied to the detection of added sugars, citric and malic acids in apple (Jamin et al., 1997a), pineapple (Jamin et al., 1997b), orange and mandarin (Jamin et al., 1998a), lemon (González et al., 1998), and grapefruit and strawberry (Jamin et al., 1998b) juices. Furthermore, the $\delta^{13}C$ value of the protein fraction of the fruit juice enables the absolute position on the scale of the $\delta^{13}C$ values of all extracted metabolites in the SNIP−IRMS method to be defined (Jamin et al., 1998b). The present paper describes the extension of this concept to the determination of $^{13}C/^{12}C$ ratios of the individual

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components, fructose, glucose, and sucrose, of the sugar fraction of a pineapple juice or concentrate in order to further improve the sensitivity of the isotopic techniques to detect C3-derived sweeteners or a double addition of C3 and C4 type sugars.

The separation and purification of fructose, glucose, and sucrose from a fruit juice is the main difficulty of this problem. An HPLC technique was already proposed for the separation of the glucose−fructose fraction from the sucrose fraction, enabling the $^{18}O/^{16}O$ ratio and the $^{2}H/^{1}H$ ratio measurements on the latter (Doner et al., 1987). These studies did not cover the measurement of $^{13}C/^{12}C$ ratios of each collected fraction. The separation of fructose and glucose was not achieved. Recently, another approach using a size-exclusion HPLC technique was proposed for the analysis of orange juices and concentrates (Day et al., 1998). Data on the $^{13}C/^{12}C$ ratios of fructose, glucose, and sucrose from orange juices could thus be collected. The present paper presents the new methodology we have developed for the same purpose. The separation is achieved by a HPLC device equipped with a fraction collector, a refractive index detector, and a NH_2-type column. This stationary phase is well-known for its capability to separate the mono-, di-, and trisaccharides (Brandao et al., 1980; Shaw and Wilson, 1982). In the present work, it has been used at a preparative scale, allowing the separation of 10−100 mg of material. The reliability of the whole method is discussed. In particular, the isotopic effect of the sucrose hydrolysis is presented. This is of primordial importance since (i) pineapple presents naturally a large range of sucrose concentration (from 25 to 80 g/L; AIJN, 1993) and (ii) hydrolysis during sample preparation may occur. Therefore, the hydrolysis rate of sucrose should be taken into account in the interpretation of its ^{13}C content.

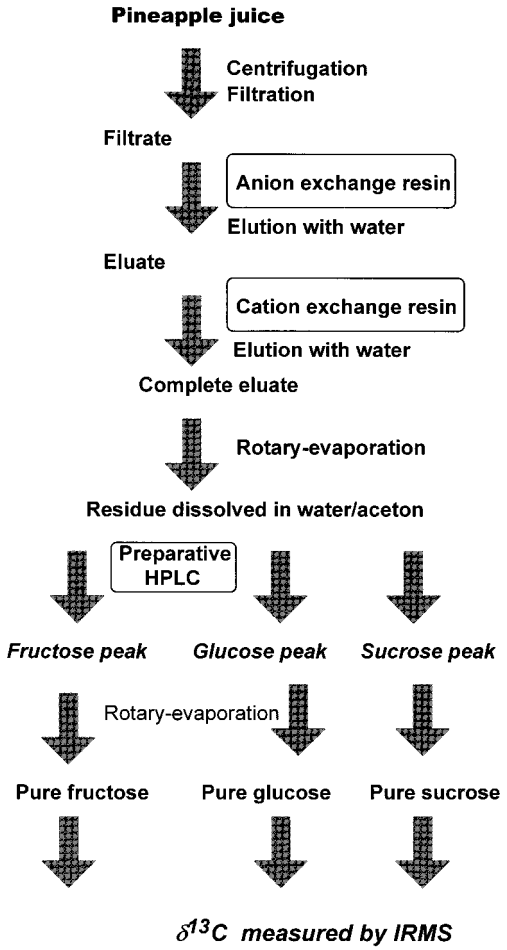

Figure 1. Flowchart of the preparation steps proposed for the determination of the $\delta^{13}C$ values of fructose, glucose, and sucrose in pineapple juices.

MATERIALS AND METHODS

Chemicals and Supplies. Cation-exchange resin Dowex 50 × 8-200 (H^+ form) was obtained from Sigma-Aldrich (St. Quentin, Fallavier, France); 1-cm i.d. glass columns were filled with 30 g of resin. Anion-exchange resin Dowex 1 × 8-200 (Cl^- form) was obtained from Sigma-Aldrich (St. Quentin, Fallavier, France); 1-cm i.d. glass columns 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 Hibar-NH_2 (250 × 10 mm) with 5-μm packing (Merck, Nogent-sur-Marne, France).

Sample Description. This work is based on the study of different pineapple samples from different origins representative of the main areas of production worldwide. Pure pineapple juices were obtained from fresh fruit squeezed in the laboratory. Authentic concentrates were also used as references. Pure fructose, glucose, and sucrose were purchased from Merck (Nogent-sur-Marne, France).

Sample Preparation. The analytical procedure has been adapted from previous studies devoted to the separation of the main organic acids from fruit juices and nectars (see, for example, Jamin et al., 1997b). The entire procedure is described in Figure 1. It allows the simultaneous isolation of pure fructose, glucose, and sucrose from fruit juices. The fructose, glucose, and sucrose contents of all samples were determined by enzymatic titration (using International Federation of Fruit Juice Producers (IFU) methods; AIJN, 1993), and an aliquot containing about 5 g of total sugars was submitted to the separation procedure. About 50 mL of juices or diluted concentrates (5-fold weight/weight dilution) was centrifuged (7500*g*, 10 min) and filtered. The resulting solution was applied to the anion- and cation-exchange resin as previously described (Jamin et al., 1997b). The resulting solution was rotary-evaporated, and the residue was submitted to fructose, glucose, and sucrose content determination, to estimate the recovery rates of individual sugars and the hydrolysis rate of sucrose. If there is no significant hydrolysis, the above residue is dissolved in 50 mL of water/acetone (50−50%, v−v). The final solution is obtained by filtering through a 0.22-μm filter; 1 mL of the resulting solution was injected into the preparative high-performance liquid chromatography (HPLC) device. The eluant composition was defined according to Müller and Siepe (1980). The fractions corresponding in the order of elution to the fructose, glucose, and sucrose peaks were collected separately (a baseline separation of the peaks has to be achieved). The complete elution time was about 60 min. One injection was sufficient to obtain enough material for isotopic analyses (^{13}C and/or ^{18}O IRMS). The fructose, glucose, and sucrose fractions were all concentrated under vacuum. A flow of nitrogen gas was applied to remove all organic traces. Finally, the pure molecules were dissolved in a tiny amount of water, prior to $\delta^{13}C$ determination.

IRMS Measurements. The protocol for the measurement of $\delta^{13}C$ of the organic materials has been adapted from Koziet et al. (1993). The carbon isotope ratios were determined using a Finnigan Mat DeltaS mass spectrometer (Orsay, France) associated with a Carlo Erba NA1500C-N elemental analyzer (Rueil Malmaison, France). Fructose, glucose, and sucrose were introduced into the elemental analyzer as a liquid. Residual water was removed after combustion. The $^{13}C/^{12}C$ ratios are expressed by the ^{13}C deviations $\delta^{13}C$. All $\delta^{13}C$ values are

Table 1. Accuracy and Precision of the HPLC Separation[a]

mixture	δ^{13}Cf	δ^{13}Cg	δ^{13}Csu
ref	−10.5	−11.2	−24.7
A1	−10.6	−10.7	−24.4
A2	−11.0	−10.8	−24.5
A3	−10.9	−11.1	−24.3
A4	−10.7	−11.0	−24.8
A5	−11.0	−10.9	−24.1
mean	−10.8	−10.9	−24.4
SD	0.2	0.2	0.3
ref	−25.6	−11.2	−11.4
B1	−25.2	−11.5	−11.3
B2	−25.0	−11.1	−11.6
B3	−25.1	−11.6	−11.4
B4	−25.6	−10.9	−11.5
B5	−25.6	−11.0	−11.9
mean	−25.3	−11.2	−11.5
SD	0.3	0.3	0.2

[a] ^{13}C deviations (in ‰) of fructose (δ^{13}Cf), glucose (δ^{13}Cg), and sucrose (δ^{13}Csu) after separation on HPLC column (see Materials and Methods) of mixtures A and B. ref stands for the pure individual component before being mixed. SD is the standard deviation of the mean value obtained from five independent measurements.

related to the Vienna Pee Dee Belemnite (V. PDB) carbonate standard. Suitable quality control references were included in each batch.

RESULTS AND DISCUSSION

Evaluation of the Method. *Accuracy.* The NH_2-type was chosen as the stationary phase because (i) it is available for preparative HPLC separation, (ii) it has high capability for separating saccharides, disaccharides, and trisaccharides, (iii) organic solvents can be used as eluant, leading to neutral solutions of collected products, which makes it possible to measure the ^{13}C and ^{18}O contents on fructose, glucose, and sucrose, and (iv) such a device is reasonably priced. The isotope effects of the resin preparation steps have already been shown previously (see, for example, Jamin et al., 1997a), and they will not be commented on further here. We have focused our interest on the HPLC separation. The accuracy (the isotopic recovery) of the chromatographic method was evaluated using a test mixture of pure commercial fructose, glucose, and sucrose of known δ^{13}C values. The results are compiled in Table 1. The experiment was conducted on two types of solutions with different isotopic profiles. Thus, in one case, mixture A consists of C4 fructose and glucose added to a C3 sucrose (−10.5‰, −11.2‰, and −24.7‰, respectively). In another case, mixture B was made using C3 fructose added to C4 glucose and sucrose (−25.6‰, −11.2‰, and −11.4‰, respectively). The mean and the standard deviation values of five independent HPLC separations show no significant shift from the original value of each pure molecule. The components we employed for each mixture have a very different δ^{13}C value, so that even a limited amount of mixing between the fractions due to an overlap of peaks would have resulted in a significant change of the δ^{13}C value of each component. We have also verified that the molecules constituting the head of the chromatographic peak are ^{13}C enriched with respect to the tail, this phenomenon being particularly sensitive for fructose and glucose. Therefore, it is clear that a complete separation should be achieved. Furthermore, mixtures A and B were alternatively injected in order to assess a possible memory effect. The results of Table 1 confirm that this effect is negligible. It can be concluded that, provided that yields close to 100% are reached, the whole purification procedure depicted in Figure 1 is reliable for the δ^{13}C value determination of fructose, glucose, and sucrose from fruit juices.

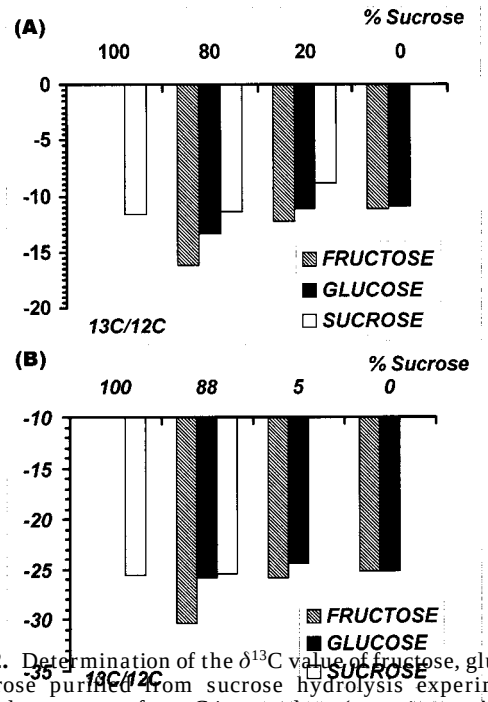

Figure 2. Determination of the δ^{13}C value of fructose, glucose, and sucrose purified from sucrose hydrolysis experiments performed on sucrose from C4 type plant (experiment A) and from C3 type plant (experiment B); % sucrose corresponds to the percentage of the sucrose in weight remaining after partial hydrolysis.

Sucrose Hydrolysis. The sucrose hydrolysis generates one molecule of fructose and one molecule of glucose. This may occur during the laboratory handling of the sample. A partial hydrolysis is expected to be associated with isotopic fractionations. Our goal was to evaluate this isotope effect and its importance to the reliability of the proposed analytical method. For this purpose, chemical hydrolyses in boiling acidic media were monitored on solutions of C4 and C3 type sucrose (bearing a δ^{13}C value of −11.6‰ and −25.6‰, respectively). The evolution of the carbon-13 content of each component which could be determined, with respect to the percentage of the remaining sucrose, is shown in Figure 2. It appears that, for both cane and beet sucrose (histograms A and B, in Figure 2), the hydrolysis leads to isotopic effects since the generated fructose and glucose show lower δ^{13}C values than the remaining sucrose. Conversely, sucrose is enriched toward the end of the hydrolysis. Interestingly, fructose undergoes a larger isotope fractionation than glucose, since it is always the more impoverished, whatever the origin of the initial sucrose. But fructose and glucose have the same δ^{13}C value when the hydrolysis is complete and similar to that of the starting sucrose. This study shows that knowledge of the hydrolysis rate of sucrose when handling the fruit juice sample is pertinent. This is the reason for the presence of this control step in the whole methodology (Figure 1).

Reproducibility. Before considering an interlaboratory reproducibility study, an internal reproducibility of the complete process can be assessed, by performing five independent analyses on the same pineapple juice, by

Table 2. Mean ^{13}C Deviations (in ‰) of Fructose (δ^{13}Cf), Glucose (δ^{13}Cg), and Sucrose (δ^{13}Csu) Isolated from Pineapple Samples from Various Production Areas[a]

	δ^{13}Cs	δ^{13}Csc	δ^{13}Cf	δ^{13}Cg	δ^{13}Csu	δ^{13}Cf − δ^{13}Cg	δ^{13}Cf − δ^{13}Csu	δ^{13}Cg − δ^{13}Csu
mean	−12.1	−12.0	−12.9	−12.3	−11.6	−0.6	−1.3	−0.7
SD	1.0	1.1	1.1	1.2	1.2	0.6	0.6	0.5

[a] δ^{13}Cs and δ^{13}Csc stand for the ^{13}C deviations of the total sugar fraction and the calculated value obtained from the linear combination of the individual components of the sugar fraction δ^{13}Cf, δ^{13}Cg, and δ^{13}Csu. The mean and standard deviation (SD) have been calculated from the whole population of 30 samples.

Table 3. ^{13}C Deviations (in ‰) of Fructose (δ^{13}Cf), Glucose (δ^{13}Cg), Sucrose (δ^{13}Csu), Sugars (δ^{13}Cs), Malic Acid (δ^{13}Cm), and Citric Acid (δ^{13}Cc) Isolated from Pineapple Samples from Various Origins[a]

sample	δ^{13}Cf	δ^{13}Cg	δ^{13}Csu	δ^{13}Cf − δ^{13}Cg	δ^{13}Cf − δ^{13}Csu	δ^{13}Cg − ^{13}Csu	δ^{13}Cs
a	−13.3	−12.9	−12.8	−0.4	−0.5	−0.1	−13.0
b	−14.8	−12.9	−12.4	***−1.9***	−2.3	−0.5	−12.7
c	−12.9	−13.2	−13.7	0.3	***0.8***	***0.5***	−13.2
d	−12.9	−12.5	−13.2	−0.4	***0.3***	***0.7***	−12.8

[a] a, authentic sample; b, sample a with 13% (in weight with respect to the total amount of fructose) of added fructose bearing a δ^{13}C value of −25.4‰, corresponding to an addition of 3% of the whole sugar fraction; c, sample a with 9% (in weight with respect to the total amount of sucrose) of added sucrose bearing a δ^{13}C value of −25.7‰, corresponding to an addition of 5% of the whole sugar fraction; d, sample a with 28% (in weight with respect to the total amount of glucose) of added glucose bearing a δ^{13}C value of −10.8‰, with 28% (in weight with respect to the total amount of fructose) of added fructose bearing a δ^{13}C value of −10.5‰, and with 6% (in weight with respect to the total amount of sucrose) of added sucrose bearing a δ^{13}C value of −25.7‰, the total of these three sugar additions corresponding to an addition of 18% of the whole sugar fraction. The figures in bold italics point out the parameter out of range defined by using a 95% confidence level from the standard deviation values computed in Table 2, indicating an adulteration.

different operators and at different periods of time. The standard deviation of the mean value calculated from the five experiments is below 0.3‰ for the δ^{13}C value of fructose, glucose, and sucrose. This is consistent with the standard deviation of the reproducibility determined for the method of ^{13}C analysis of sugars in fruit juices (Koziet et al., 1993). It should be noticed that we have obtained similar results when the protocol was applied to other fruits (citrus, strawberry, etc.) or to other industrial matrixes (purées, concentrates, juices made from concentrates). All these results show that the present methodology is reliable.

^{13}C Content of Fructose, Glucose, and Sucrose in Pineapple Samples. The δ^{13}C values of fructose, glucose, and sucrose in authentic samples of pineapple have been determined according to the analytical procedure depicted in Figure 1. The δ^{13}C values (mean and standard deviation) of fructose, glucose, and sucrose (δ^{13}Cf, δ^{13}Cg, and δ^{13}Csu, respectively) are presented in Table 2. In addition, we indicate the mean values of the sugar fraction (δ^{13}Cs), measured as shown previously (Jamin et al., 1997b), and of the calculated sugar fraction (δ^{13}Csc), obtained from the linear combination of the molar fraction (% weight) of fructose, glucose, and sucrose and their individual δ^{13}C values. There is a good agreement between δ^{13}Cs and δ^{13}Csc. This is a confirmation that the measurement of the individual component of the whole sugars fraction is reliable. The variations of the δ^{13}C values of each fraction from the whole set of measurements in pineapple are in agreement with the natural ranges of ^{13}C content of the other metabolites (Jamin et al., 1997b). The highest δ^{13}C values (especially for the sucrose fraction) are found for pineapple from Kenya and to a lesser extent from South Africa, while the more negative δ^{13}C values correspond to samples from Western Africa. This was already observed for the other metabolites in pineapple (especially malic acid; Jamin et al., 1997b, 1998b). Since all secondary metabolites are derived from a glucose molecule formed at the end of the photosynthesis process in the plant, it is logical that any water stress leading to a ^{13}C enrichment of glucose would be visible for all constituent molecules of the fruit. The isotopic fractionations occurring along the metabolic pathways provide further differences between δ^{13}C values for each component. Thus it is sucrose which shows the highest ^{13}C content of the sugar fraction. The values of the differences between δ^{13}Cf, δ^{13}Cg, and δ^{13}Csu are also displayed in Table 2 (δ^{13}Cf − δ^{13}Cg, δ^{13}C − δ^{13}Csu, and δ^{13}Cg − δ^{13}Csu). The standard deviations (SD) of these differences are lower than those calculated for the individual δ^{13}C values. This is an indication of the occurrence of correlations between the ^{13}C content of fructose, glucose, and sucrose. Correlations were also observed for orange (Day et al., 1998; Martin et al., 1998). Therefore, characteristic patterns of ^{13}C distributions between fructose, glucose, and sucrose are likely to occur in each fruit. These systematic differences can be used as authenticity criteria for pineapple juices and concentrates as shown below.

Detection of Adulterations. The SNIP−IRMS method, using proteins, sugars, and malic and citric acids as molecular probes for ^{13}C/^{12}C ratio determination, is very efficient for the detection of addition of sugars and malic and citric acids from C3 metabolism type sources to pineapple. The detection threshold can be easily set below 10% for sugars (Jamin et al., 1997b), although a double addition of C3 and C4 type sweeteners is possible and could affect this detection limit. Therefore, an improvement in the determination of the amount of C3 type sugars would limit the importance of a double addition. Adulterations by sweeteners can be achieved by adding different types of commercial products. The measurement of ^{13}C/^{12}C ratios of the individual component is more sensitive for detecting sugar additions than the whole sugar fraction. This can be demonstrated by performing spiking experiments. Table 3 shows the results of three types of exogenous sugars added to a genuine pineapple juice (sample a). The sugar fraction of this sample is constituted 23% fructose and glucose and 54% sucrose. The SNIP−IRMS analysis of sample a indicates a ^{13}C profile in agreement with an authentic product. Samples b and c correspond to an addition of 13% pure C3 fructose (in weight with respect to the total amount of fructose) and 9% pure C3 sucrose (in weight with respect to the total amount of sucrose), respectively. The δ^{13}C values of these components changed as expected. The rules established in

Table 4. ^{13}C Deviations (in ‰) of Proteins ($\delta^{13}Cp$), Fructose ($\delta^{13}Cf$), Glucose ($\delta^{13}Cg$), Sucrose ($\delta^{13}Csu$), Sugars ($\delta^{13}Cs$), Malic Acid ($\delta^{13}Cm$), and Citric Acid ($\delta^{13}Cc$) Isolated from Commercial Pineapple Concentrates[a]

sample	$\delta^{13}Cp$	$\delta^{13}Cf$	$\delta^{13}Cg$	$\delta^{13}Csu$	$\delta^{13}Cs$	$\delta^{13}Cm$	$\delta^{13}Cc$
e	−12.1	−13.8	−13.6	***−14.4***	***−14.3***	−11.4	−12.3
f	−11.6	−12.8	−11.5	−11.4	−11.7	−11.6	***−14.4***
g	−13.6	***−14.8***	−13.2	***−14.5***	***−14.1***	−12.4	−12.9

[a] The figures in bold italics point out the parameter out of range from the expected values (Table 2), indicating an adulteration.

Table 2 are not anymore respected, indicating that the two samples (b and c) are adulterated. It should be noticed that the above additions were not detected on the basis of the $\delta^{13}C$ values of the whole sugar fraction. In fact, the amount of sugar added when considering the whole sugar fraction is below 5% in samples b and c in Table 3 (3% and 5%, respectively). The ^{13}C profile of fructose, glucose, and sucrose is therefore very useful for detecting adulterations at low levels. Its interest is further shown by the results obtained for sample d (Table 3), on which specific additions aimed to mask a double adulteration were performed. Thus, a blend of C4 fructose and glucose with C3 sucrose, adequately combined, cannot be detected by the ^{13}C content of the whole sugar fraction ($\delta^{13}Cs$), although about 18% of total sugars were added. The relationship between $\delta^{13}Cf$ and $\delta^{13}Csu$ or $\delta^{13}Cg$ and $\delta^{13}Csu$ of sample d clearly indicates that addition of beet sucrose was performed. The quantification of the actual amount of added sugars remains difficult, but this sample could not be classified as authentic.

Finally, the measurement of $\delta^{13}Cf$, $\delta^{13}Cg$, and $\delta^{13}Csu$ can be included in the SNIP−IRMS procedure applied to the analysis of pineapple. Table 4 illustrates the potential of the method. Three commercial pineapple concentrates (samples e−g) were analyzed by the present extended SNIP−IRMS method. Thus, the ^{13}C deviations of malic acid ($\delta^{13}Cm$), citric acid ($\delta^{13}Cc$), and whole sugar fraction ($\delta^{13}Cs$) were determined as described by Jamin et al. (1997b), and the $^{13}C/^{12}C$ ratio of the protein fraction ($\delta^{13}Cp$) was measured as shown previously (Jamin et al., 1998b). From the results of Table 4, it can be concluded that sample e is not authentic because of an addition of exogenous sucrose (beet sucrose), citric acid from C3 plants was added in sample f, and sample g was subjected to a double addition of fructose and sucrose from C3 plants. The adulterations could not be detected using the determination of the ^{13}C content of the whole sugar fraction only.

CONCLUSION

When the adulteration of pineapple is achieved using pure materials as sweeteners (pure beet sucrose, for example) the Cap-GC oligosaccharide profile method is inoperative (Stöber et al., 1998a). Isotope analyses only may make it possible to detect this addition. The present study shows that the $\delta^{13}C$ values of individual sugars (fructose, glucose, and sucrose) allow the detection of small adulterations by C3 sweeteners (pure or mixed) and of double adulterations by C3 and C4 sweeteners (pure or mixed). The detection threshold depends mainly on the molar fraction of each component within the sugar fraction. Thus, the highest sensitivity of the method applied on pineapple would be reached on fructose and glucose, since these molecules are usually present at lower amounts than sucrose. The $\delta^{13}C$ determination carried out on fructose, glucose, and sucrose is therefore a valuable component of the SNIP−IRMS method. This is exemplified in the case of an addition of medium invert beet sugar. This adulteration can be detected by using the ^{13}C measurement of all six metabolites (proteins, malic acid, citric acid, fructose, glucose, and sucrose) because (i) the composition of the sugar fraction of pineapple is not usually similar to that of medium invert beet sugar (1/3, 1/3, and 1/3), (ii) the $\delta^{13}C$ values of fructose and glucose in medium invert beet sugar should not be identical to that of sucrose, since there is an isotope fractionation associated to the hydrolysis, and (iii) even when the initial correlation between the $\delta^{13}C$ values of fructose, glucose, and sucrose is still fulfilled after addition, the relationship between the $\delta^{13}C$ values of the sugar fraction and the protein fraction would not exist anymore.

ABBREVIATIONS USED

AIJN, Association of the Industry of Juices and Nectars; AOAC, Association of Official Analytical Chemists; HPLC, high-performance liquid chromatography; IRMS, isotope ratio by mass spectrometry; NMR, nuclear magnetic resonance; SNIP−IRMS, specific natural isotope profile studied by IRMS; SNIF−NMR, specific natural isotope fractionation studied by NMR.

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