Proteins As Intermolecular Isotope Reference for Detection of Adulteration of Fruit Juices

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The fruit proteins, defined as the fraction obtained after bentonite precipitation on a centrifuged juice or diluted concentrate, are proposed as internal standard for $\delta^{13}C$ measurements of fruit juices and concentrates. The range of the $\delta^{13}C$ of proteins for each studied fruit is set as the following: -26.0 ± 1.2‰ for orange, -27.7 ± 0.8‰ for grapefruit, -25.9 ± 1.3‰ for lemon, -12.5 ± 1.4‰ for pineapple, and -24.7 ± 1.3‰ for strawberry. A good correlation is found between $\delta^{13}C$ of proteins and $\delta^{13}C$ of sugars for a given fruit. The method presented is applicable to both fruit juices and concentrates. The $\delta^{13}C$ values of proteins are much less sensitive to industrial processes than $\delta^{13}C$ values of pulp, for instance. The $\delta^{13}C$ values of proteins are the first link in the chain of analyses performed in the SNIP-IRMS method. The $\delta^{15}N$ values of the protein fraction of fruit juices are also presented, and their interest for geographical discrimination is discussed.

Keywords: *Proteins; ^{13}C-IRMS; ^{15}N-IRMS; SNIP-IRMS; internal referencing; authentication; bentonite*

INTRODUCTION

Adulterations of fruit juices or concentrates can usually be best detected by the combined use of isotopic and nonisotopic analytical methods (Brause, 1995; Rossmann et al., 1995; Lees et al., 1996, Martin et al., 1997). Among the isotopic techniques, the combination of SNIF-NMR (a trademark of EUROFINS SCIENTIFIC) (Martin and Martin, 1981, 1995) and IRMS (Bricout and Koziet, 1987; Doner, 1991; Krueger, 1995) provides complementary information and improves the reliability of fruit juice authentication methods. However, as the natural variation of the isotopic distribution of fruit juice components is relatively large (AIJN, 1993), the detection of the addition of low amounts of illegal products is usually difficult. Recently, we have shown that the $^{13}C/^{12}C$ ratio determination by IRMS on several metabolites provides specific $\delta^{13}C$ profiles for a given fruit. The whole approach has been named SNIP-IRMS (a trademark of EUROFINS SCIENTIFIC). Using citric acid, malic acid, and sugars as isotopic probes, such a typical pattern has been found in apple (Jamin et al., 1997a), in pineapple (Jamin et al., 1997b), in orange and tangerine (Jamin et al., 1998), and in lemon (González et al., 1998). The interest of the above publications was also in the improvement of the detection limits of sugars or organic acids addition.

The results are interpreted on the basis of correlations between the $\delta^{13}C$ values of sugars, malic acid, and citric acid by taking one of these metabolites as internal reference. One drawback of this approach is the possibility that both sugars and organic acids have been added. Nevertheless, the internal reference technique reduces the variability of the authentic database. For a true intermolecular standardization, the internal reference should be a component that would not be added either because there is no economic interest or because it is not easily available. Several organic compounds have been proposed as standards for the $\delta^{13}C$ internal referencing method (Schmidt et al., 1993). Among them, pulp appeared to be the most convenient for fruit juices (Parker, 1982; Bricout and Koziet, 1987). In fact, the pulp fraction is defined as water and acetone-insoluble ingredients and therefore it is described to be cellulose, hemicelluloses, nitrogen-containing compounds, and some phenolic substances (Kornexl et al., 1996). It is supposed that the nitrogen of the pulp originates from proteins and/or amino acids. Such a complex composition of the pulp fraction could be altered by physical or chemical processes. For example, industrial processing may change the composition of the pulp fraction so that the isotopic content may not be comparable between laboratory-squeezed samples and commercial juices. Moreover, clarified juices or some concentrates do not contain any pulp fraction. For these reasons the pulp fraction does not appear to be suitable as a universal $\delta^{13}C$ internal reference. In addition, a recent collaborative study showed that the interlaboratory reproducibility (R) of the ^{13}C deviation values was much higher for pulp ($R = 1.89$‰) than for sugars ($R = 0.82$‰) (Rossmann et al., 1997). Experimental difficulties during purification of the pulp fraction have been described. There is therefore still a need for a truly universal internal standard reference for IRMS analysis of fruit products and especially for the $\delta^{13}C$ profile determination. Ideally, these new isotopic probes should be very stable compounds that could easily be isolated

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10.1021/jf980664g CCC: $15.00
Published on Web 11/11/1998

from most fruit products (juices or concentrates) without (or with strictly controlled) isotopic fractionation.

From the literature, the phenolic compounds fraction was proposed for the study of the ^{13}C composition of apples (Lee and Wrolstad, 1988). More recently, amino acids have been used for origin assignment of fruit juices (Kornexl et al., 1996) and wines (Giménez Miralles et al., 1997). The use of proteins as an internal standard for carbon isotope ratio analysis makes IRMS a powerful tool for detecting adulteration of honeys (AOAC, 1995; White et al., 1998). Soluble proteins, because of their large size, are more likely to be preserved throughout industrial processes and analytical separation and purification. They are therefore less subject to isotope fractionation during industrial processing and laboratory manipulations than free amino acids.

The aim of the present study is to explore the potential interest of proteins as internal reference for establishing $\delta^{13}C$ profiles of organic compounds in fruit juices. A few studies have dealt with $\delta^{13}C$ values of proteins but without further development (Whelan et al., 1970; Gaffney et al., 1979). In this paper, a fast and simple method for the isolation of proteins from fruit juices is presented, and the reliability of this new probe as ^{13}C internal reference is discussed. This approach was tested on the main economically significant fruit products (orange, grapefruit, pineapple, lemon, and strawberry) with the exception of apple and grape. The method also works on these fruits, but for apple, the amount of proteins is very low and the juice is often clarified by enzymatic treatments (Wall et al., 1996), making the technique less attractive. The application to grape juice will be discussed elsewhere. Preliminary work has shown that $\delta^{15}N$ values could be indicative of the nature of the soil where the plant was grown (Danho et al., 1992; Kornexl et al., 1996, 1997; Remaud et al., 1997). Therefore, the interest in the $^{15}N/^{14}N$ ratio in proteins for determining the geographical origin of fruit juices is also addressed.

MATERIALS AND METHODS

Sample Description. Authentic fruit juices (orange, grapefruit, pineapple, lemon, and strawberry) were obtained from fresh fruits squeezed in the laboratory. The origins of the fruits under consideration were representative for the main areas of production worldwide [(orange) Brazil, Cuba, Israel, United States, etc.; (grapefruit) Argentina, Israel, United States, etc.; (lemon) Argentina, Italy, Spain, United States, etc.; (pineapple) Costa Rica, India, Indonesia, Kenya, South Africa, Thailand, western Africa, etc.; (strawberry) western Europe, eastern Europe, United States, etc.]. Authentic concentrates were also used as references. Some commercially available fruit juices and concentrates were analyzed to test the method.

Extraction of Proteins. Juices or diluted concentrates were centrifuged (5000*g*, 10 min) and filtered to remove the pulp fraction and insoluble pectins. Two hundred milliliters of juice equivalent was used in most cases. Three procedures proposed for preparing proteins from honey (White et al., 1998) have been tested and compared in the case of fruit juices. Several steps were combined to obtain the protein fraction.

Dialysis. After removal of the pulp fraction, the juices or diluted concentrates were concentrated under vacuum to ~60 °Brix and poured into dialysis bags retaining molecules of molecular weight >12 000 (Sigma, St. Quentin Fallavier, France). An 18-h dialysis against tap water was performed.

Bentonite Precipitation. Bentonite (Générale De Technologie, Le Pallet, France) was added in excess (200 mg/100 mL) to the centrifuged juice and dispersed by stirring. This suspension was centrifuged (7500*g*, 10 min) and the supernatant discarded. Approximately 100 mL of water was added and mixed with the pellet to extract residual sugars. Washing of the pellet with water was repeated several times, until all sugars had been eliminated (controlled by colored reaction). Because sugars are the major components of fruit juices, it was assumed that all other soluble molecules had been eliminated at this stage. The washed pellet was transferred to a small crystallizing dish, 5 mL of concentrated HCI was added, and the samples were heated to boiling on a hot plate. This removed completely aluminum hydroxide (which interferes with recovery of CO_2 during combustion) and residual carbonates that could have introduced a bias in the measurement of the carbon isotope ratio of proteins. The resulting powder constituted the proteins fraction.

Table 1. Comparison of the $\delta^{13}C$ Values of Proteins (‰) Extracted from Several Fruits, According to Three Procedures (See Materials and Methods)

fruit	dialysis + tungstic acid	dialysis + bentonite	direct bentonite precipitation
orange	−27.9	−27.4	−27.4
orange	−28.6	−28.4	−28.2
strawberry	−25.2	−24.4	−24.6
pineapple	−13.6		−13.1
lemon	−27.1		−26.5

Tungstic Acid Precipitation. On the clarified juice or diluted concentrate (200 mL) a 10% sodium tungstate solution was added (5 mL), followed immediately by 5 mL of a 0.67 N H_2SO_4 solution. This mixture was put in a 80 °C water bath until visible floc forms were observed, with clear supernatant. If no floc forms were visible, 5 mL portions of 0.67 N H_2SO_4 were added. The suspension was centrifuged (7500*g*, 10 min) and the supernatant discarded. The solid was washed at least five times with water. The resulting pellet was then dried in the oven.

The results shown in the present work were obtained using the direct bentonite precipitation method. It should be noted that this overall method enabled the supernatant to be used for the $\delta^{13}C$ determinations on organic acids or sugars (the SNIP-IRMS method).

Isolation of Pulp. The purification of the pulp fraction from lemon juices and concentrates was achieved according to the method of Rossmann et al. (1997).

IRMS Measurements. The carbon and nitrogen isotope ratios were determined separately (Barrie and Lemley, 1989) using a Finnigan MAT DeltaE mass spectrometer or simultaneously using a Finnigan MAT DeltaS mass spectrometer, equipped with an open split interface (ConFlo II) (Orsay, France), associated with a Carlo Erba NA1500C-N elemental analyzer (Rueil Malmaison, France). Samples placed in tin containers were submitted to a flash combustion in a stream of helium enriched with pure oxygen. The carbon and nitrogen results obtained by IRMS are expressed in δ‰ with respect to the PDB (Pee Dee Belemnite) international standard for carbon and atmospheric nitrogen for nitrogen.

RESULTS AND DISCUSSION

Comparison of Possible Methods of Protein Extraction. Three alternative procedures have been tested for various fruit juices and concentrates: (i) dialysis followed by bentonite precipitation, (ii) dialysis followed by tungstic acid precipitation, and (iii) direct bentonite precipitation. The results are given in Table 1. There is no significant difference between results obtained by dialysis followed by bentonite precipitation and direct bentonite precipitation, which demonstrates that all potential interfering compounds are removed by washing the pellet. Thus, the dialysis step is useless when the bentonite precipitation procedure is used. Higher differences appear between the tungstic acid and bentonite procedures, but these are always smaller than the reproducibility of 0.7−0.8‰ previously found in the case of the $\delta^{13}C$ measurements of the sugars fraction

Table 2. δ^{13}C and δ^{15}N Values of Proteins (δ^{13}Cp and δ^{15}Np, in ‰) Extracted from a Commercial Pineapple Juice According to Several Experimental Tests[a]

test	bentonite added (mg)	δ^{13}Cp	δ^{15}Np	test	bentonite added (mg)	δ^{13}Cp	δ^{15}Np
A	100	−13.6	1.5	F	200	−13.5	1.6
B	150	−13.2	1.5	G	400	−13.0	1.2
C	200	−13.2	1.5	H	600	−14.0	0.9
D	250	−13.3	1.5	I	800	−15.7	0.8
E	300	−13.1	1.4	J	1000	−17.8	0.9

[a] From test A to E, several amounts of bentonite were added to 200 mL of centrifuged juice. From test F to J, 200 mg of bentonite was added successively to 200 mL of centrifuged juice.

(Koziet et al., 1993; Rossmann et al., 1997). Consequently, all of the proteins were extracted by a direct bentonite precipitation, which was found to be a very simple and efficient method. This is in good agreement with the physicochemical properties of bentonites. These clays are aluminosilicates. The aluminum cations are bonded to a specific arrangement of oxygen anions, leading to several structures such as montmorillonite, illite, and kaolinite. Other cations, such as calcium, sodium, and magnesium, take part in the structure (Maujean, 1993). Montmorillonite is the main component of bentonite used for must and wine protein haze prevention (Hsu and Heatherbell, 1987; Blade and Boulton, 1988). Physicochemical characteristics of several types of bentonites were studied for wine clarification purposes. It was shown that the maximum adsorbable protein content by unit of adsorbing material increases with the sodium/calcium cations ratio (Marchal et al., 1995). Therefore, we have used sodium bentonites for the present work.

Evaluation of the Bentonite Precipitation Method. The proteins fraction is described as the solid obtained after centrifugation followed by an enological bentonite precipitation on a fruit juice. The chemical composition of this fraction reveals the presence of mainly proteins with some amino acids. Some phenolic substances are also precipitated, but their amounts are too small to affect significantly the final δ^{13}C value of the fraction. Two experiments have been designed to assess the influence on δ^{13}C and δ^{15}N parameters of the amount of bentonite required for the precipitation. For the first one, different amounts of bentonite were added to a commercial pineapple juice (tests A−E in Table 2), and for the second the proteins were run out of a pineapple juice by adding successively five times 200 mg of bentonite (tests F−J in Table 2). It appears, on the one hand, that the amount (up to 300 mg for 100 mL of juice) of bentonite does not affect the δ^{13}C and δ^{15}N values and, on the other hand, that there are almost no proteins after the second addition of 200 mg of bentonite: the ^{13}C and ^{15}N signals are very low, and the values themselves cannot be trusted, because of both a poor combustion (due to a large amount of mineral material) and a very low signal intensity during the IRMS experiment. Consequently, the ratio of the amount of bentonite versus the volume of juice has been set to 200 mg for 100 mL of juice. To evaluate the method and before an interlaboratory comparison was considered, an internal reproducibility of the ^{13}C and ^{15}N isotope determination was estimated. The standard deviations of the δ^{13}C and δ^{15}N values measured on eight replicates of the bentonite precipitation of a commercial pineapple juice made from concentrate are shown in Table 3. The standard deviation of the δ^{13}C and δ^{15}N values values is within the expected range for the ^{13}C/^{12}C and ^{15}N/^{14}N ratio determinations (Koziet et al., 1993). The overall procedure is therefore reliable.

Table 3. Repeatability of δ^{13}C and δ^{15}N Measurements of Proteins (δ^{13}Cp and δ^{15}Np)

repetition	δ^{13}Cp (‰)	δ^{15}Np (‰)
1	−12.6	1.2
2	−12.8	1.4
3	−12.6	1.4
4	−12.9	1.3
5	−12.7	1.3
6	−13.1	1.3
7	−12.9	1.2
8	−13.1	1.2
mean[a]	−12.8	1.3
SD[a]	0.2	0.1

[a] The mean value and the standard deviation (SD) were obtained from eight replicates.

Proteins as Internal Standard for the SNIP-IRMS Method. Table 4 shows the mean and standard deviation of the δ^{13}C values of proteins (δ^{13}Cp), sugars (δ^{13}Cs), malic acid (δ^{13}Cm), and citric acid (δ^{13}Cc) isolated from orange, grapefruit, lemon, pineapple, and strawberry samples and their respective differences (δ^{13}Cp − δ^{13}Cs, δ^{13}Cs − δ^{13}Cm, δ^{13}Cs − δ^{13}Cc, δ^{13}Cm − δ^{13}Cc). The standard deviation of the δ^{13}Cp − δ^{13}Cs parameter is smaller than the standard deviation of each individual component (δ^{13}Cp or δ^{13}Cs). This indicates a good correlation between the carbon-13 content of proteins and sugars in fruit juices. The correlation observed between proteins and other metabolites is comparable to that found between sugars and organic acids (Table 4). A larger standard deviation is found for lemon and strawberry when the δ^{13}C values of malic acid are involved in the differences (δ^{13}Cs − δ^{13}Cm and δ^{13}Cm − δ^{13}Cc). This is due to a relatively large dispersion of the δ^{13}Cm value for these fruits. An explanation of this dispersion could be found in the low (sometimes very low) concentration of malic acid in lemon and strawberry juices, leading to possible isotopic fractionation in the metabolism of this molecule. This has been found for other molecules such as ascorbic acid (Gensler et al., 1995) and glycerol (Weber et al., 1997). Interestingly, the δ^{13}C values of proteins are very close to those of the main metabolite of the juice (i.e., sugars for most fruits, citric acid for lemon juice). Moreover, the mean δ^{13}C value of proteins is always the lowest of all fractions studied except in the case of pineapple, which undergoes a different metabolism (Crassulacean acid metabolism instead of C3 metabolism). A significant enrichment of the δ^{15}N values of proteins isolated from citrus fruits as compared to pineapple and red fruits can also be observed in Table 4.

To illustrate the interest of internal δ^{13}C correlations between proteins and other metabolites of fruit juices, controlled amounts of HFCS (δ^{13}C = −10.2‰) have been added to an orange juice bearing δ^{13}C values of −24.1‰ for sugars and −24.9‰ for proteins. The carbon 13 contents of sugars has been monitored in the spiked samples. The expected values (δ^{13}Cexp) for given amounts of C4 product added (Am%) have been estimated according to

$$\delta^{13}\mathrm{Cexp} = \frac{(\mathrm{Am}\%)(\delta^{13}\mathrm{Cadd}) + (100 - \mathrm{Am}\%)(\delta^{13}\mathrm{Cini})}{100} \quad (1)$$

Table 4. Carbon-13 Deviations (‰) of Proteins (δ^{13}Cp), Sugars (δ^{13}Cs), Malic Acid (δ^{13}Cm), and Citric Acid (δ^{13}Cc) and Nitrogen-15 Deviation (‰) of Proteins (δ^{15}Np) Isolated from Several Fruit Samples of Various Origins

fruit		δ^{13}Cp	δ^{15}Np	δ^{13}Cs	δ^{13}Cm	δ^{13}Cc	δ^{13}Cp − δ^{13}Cs	δ^{13}Cs − δ^{13}Cm	δ^{13}Cs − δ^{13}Cc	δ^{13}Cm − δ^{13}Cc
orange	mean[a]	−26.0	5.0	−25.4[b]	−24.8[b]	−23.7[b]	−1.0 (64)	−0.6[b]	−1.6[b]	−1.1[b]
	SD[a]	1.2	1.2	0.8	0.9	0.8	0.7	0.6	0.4	0.5
grapefruit[c]	mean	−27.7	4.7	−26.5	−25.1	−25.1	−1.3 (27)	−1.3	−1.4	−0.1
	SD	0.8	1.8	0.8	0.9	0.7	0.5	0.5	0.4	0.4
lemon	mean	−25.9	5.9	−24.7[d]	−22.9[d]	−25.5[d]	−0.5 (50)[e]	−1.8[d]	0.8[d]	2.5[d]
	SD	1.3	2.0	1.2	0.9	1.1	0.7	0.8	0.5	0.7
pineapple	mean	−12.5	2.1	−12.2[f]	−12.1[f]	−12.8[f]	−0.1 (42)	−0.2[f]	0.6[f]	0.7[f]
	SD	1.4	1.3	0.8	1.1	0.9	0.5	0.5	0.5	0.5
strawberry[c]	mean	−24.7	0.7	−24.0	−23.2	−24.2	−0.8 (24)	−0.8	0.2	1.1
	SD	1.3	2.0	0.8	0.9	0.6	0.6	0.9	0.6	0.7

[a] The mean and standard deviation (SD) have been calculated from the whole population indicated by the number in parentheses. [b] From Jamin et al. (1998). [c] This work. [d] From González et al. (1998). [e] δ^{13}Cp − δ^{13}Cc, instead of δ^{13}Cp − δ^{13}Cs. [f] From Jamin et al. (1997b).

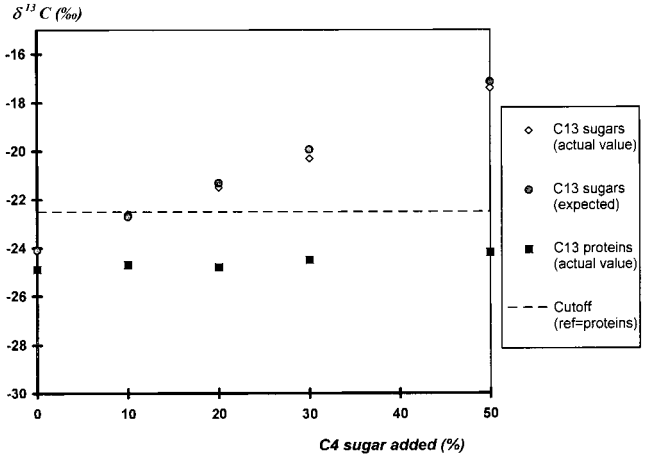

Figure 1. Plot of δ^{13}C values of proteins from an orange juice and sugars versus percentage of C4 sugar (HFCS, $\delta^{13}C = -10.2‰$) addition. The proposed cutoff values using proteins (ref = proteins) as internal reference have been computed at 95% confidence interval, leading to a maximum difference between δ^{13}C values of sugars and proteins equal to 2.4‰.

where δ^{13}Cini is the initial δ^{13}C value and δ^{13}Cadd is the δ^{13}C value of the corresponding adulterant. Figure 1 shows that the increase of the δ^{13}C value of sugars against the amount of C4 sugar added is linear and in agreement with the expected values. The δ^{13}Cp − δ^{13}Cs difference enables the quantification of the amount of sugars added, without any knowledge of the origin of the fruit or of the $^{13}C/^{12}C$ ratios of other metabolites. This experiment shows also the robustness of the extraction method of proteins, because the δ^{13}C value of proteins is not significantly affected by even 50% addition of C4 sugars.

The interest of the proteins fraction is further illustrated by using the SNIP-IRMS approach on commercial samples. The results compiled in Table 5 clearly show that an internal standard such as proteins allows an unambiguous determination of the type and the extent of the adulteration. Typically, sample "pineapple 1" (Table 5) is adulterated because the δ^{13}C values of sugars, on the one hand, and organic acids, on the other hand, do not fit the correlation observed for authentic products (Jamin et al., 1997b). The contribution of the ^{13}C content of proteins to the interpretation of the analytical results makes it possible to conclude that a double addition of citric and malic acids has taken place and to quantify this addition without any bias because it can be safely assumed that proteins are not potential undeclared additives.

Comparison of Results between Pulp and Protein Determination. A change of the ^{13}C content associated with industrial treatments was suspected because of a low correlation between pulp and other metabolites when studying lemon concentrates (González et al., 1998). To compare the robustness of pulp and proteins as ^{13}C internal references, their δ^{13}C values have been compared before and after industrial concentration of lemon juice. Table 6 shows significant differences between the carbon-13 content of pulp (δ^{13}CPu) according to the type of product: pure juice from fresh lemon fruits squeezed in the laboratory and the corresponding industrial concentrate made from lemon fruits harvested during the same period and from the same production area (Tucuman, Argentina). It is clear that the correlation, which can be established between the ^{13}C content of pulp and citric acid in pure lemon juice, is not retained when one is working with the corresponding concentrates. Moreover, the δ^{13}CPu − δ^{13}Cc difference is out of the range of the 95% confidence interval established from the cutoff point calculated from the pure juices. This is not the case when the proteins are used as internal standard. The δ^{13}C value of proteins seems to be more preserved than that of pulp throughout industrial processes involved in the making of concentrates. This validates the extrapolation of the proteins' δ^{13}C values from a database of laboratory-squeezed fruits to any authentic fruit product, including commercial ones.

Geographical Discrimination. The interest in the $^{15}N/^{14}N$ ratio of proteins in fruit juices for geographical dicrimination has been studied according to the approach defined by Kornexl et al. (1996). The data shown in the present work are a compilation of several factors affecting the isotope ratios in fruits (region, harvest period, and variety). There being not enough samples from the same location and the same harvest period, a complete exploration of the interest of $^{15}N/^{14}N$ ratio of proteins for geographical discrimination was not undertaken. Nevertheless, as preliminay results, the combination of δ^{15}N and δ^{13}C values of proteins of pineapple have been plotted in Figure 2. Valuable information is brought by the $^{15}N/^{14}N$ ratios from Figure 2. Thus, it is on the basis of the δ^{15}N values of proteins that the distinction between pineapple juices from South Africa and those from Kenya can be achieved. In addition, the western Africa origin (Ivory Coast, Guinea, and Ghana) is well separated from the other locations, using both δ^{13}C and δ^{15}N values of proteins. These observations are possible on pineapple when a small number of samples is used because of the restricted location of the production area of this fruit. $^{15}N/^{14}N$

Table 5. Carbon-13 Deviations (‰) of Proteins (δ^{13}Cp), Sugars (δ^{13}Cs), Malic Acid (δ^{13}Cm), and Citric Acid (δ^{13}Cc) and Nitrogen-15 Deviation (‰) of Proteins (δ^{15}Np) Isolated from Commercial Fruit Products (Juice or Concentrate)

fruit	origin	δ^{13}Cp	δ^{15}Np	δ^{13}Cs	δ^{13}Cm	δ^{13}Cc	δ^{13}Cp − δ^{13}Cs	δ^{13}Cs − δ^{13}Cm	δ^{13}Cs − δ^{13}Cc	δ^{13}Cm − δ^{13}Cc
orange	Argentina	−27.4	8.0	−23.9	−24.9	−24.6	***−3.5***	1.0	***0.7***	−0.3
lemon	Sicily	−24.8	1.7	−24.6	−23.8	−20.0	***−4.9***	0.8	***−4.6***	***−3.8***
pineapple 1	unknown	−12.6	3.1	−12.0	−14.8	−15.6	−0.6	***2.8***	***3.6***	0.8
pineapple 2	unknown	−14.1	3.0	−18.6	−15.5	−15.9	***4.5***	***−3.1***	***−2.7***	0.4
pineapple 3	Thailand	−13.8	2.0	−14.7	−12.7	−13.6	***0.9***	***−2.0***	***−1.1***	0.9
strawberry	unknown	−24.2		−25.2	−22.4	−24.4	***1.0***	***−2.8***	***−0.8***	2.0

[a] 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 4, indicating an adulteration. [b] δ^{13}Cp − δ^{13}Cc, instead of δ^{13}Cp − δ^{13}Cs.

Table 6. Carbon-13 Deviations of Pulp (δ^{13}CPu), Proteins (δ^{13}Cp) and Citric Acid (δ^{13}Cc) Isolated from Pure Lemon Juice (Samples 1−7) and from the Corresponding Industrial Concentrates (Samples 1′−7′)

sample	δ^{13}C Pu (‰)	δ^{13}Cc (‰)	δ^{13}Cp (‰)	δ^{13}C Pu − δ^{13}Cc	δ^{13}Cp − δ^{13}Cc
			Pure Juices		
1	−26.3	−26.0	−26.3	−0.3	−0.3
2	−25.4	−24.9	−25.6	−0.5	−0.7
3	−25.6	−26.7	−26.7	1.1	0.0
4	−24.8	−25.0	−25.9	0.2	−0.9
5	−26.4	−26.8	−26.6	0.4	0.2
6	−25.4	−25.3	−25.0	−0.1	0.3
7	−26.0	−26.4	−26.7	0.4	−0.3
mean	*−25.5*	*−25.8*	*−26.4*	*0.2*	*−0.2*
SD	*0.7*	*0.8*	*0.7*	*0.5*	*0.4*
			Concentrates		
1′	−26.6	−26.4	−26.1	−0.2	0.3
2′	−27.2	−26.2	−26.3	−1.0	−0.1
3′	−26.9	−26.7	−26.3	−0.2	0.4
4′	−26.9	−24.8	−24.5	−2.1	0.3
5′	−26.9	−25.6	−25.0	−1.3	0.6
6′	−28.0	−26.4	−26.9	−1.6	−0.5
7′	−27.4	−26.4	−26.7	−1.0	−0.3
mean	*−27.0*	*−25.9*	*−26.3*	*−1.1*	*0.1*
sd	*0.6*	*0.7*	*0.8*	*0.7*	*0.4*

[a] The mean and the standard deviation (SD) reflect the dispersion of the values.

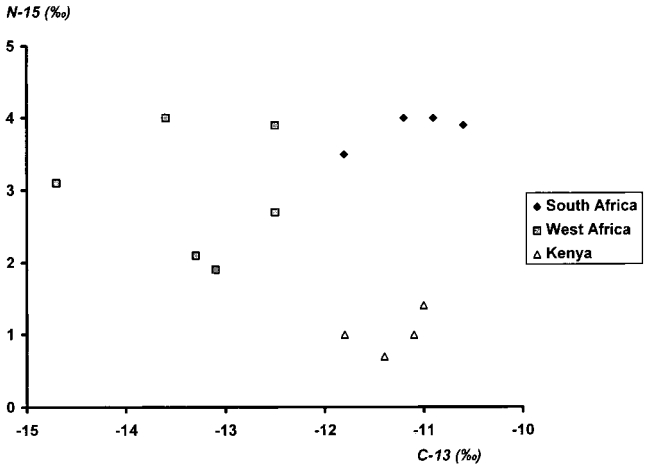

Figure 2. Plot of δ^{15}N values versus the δ^{13}C values of proteins from pineapple juices of various origins.

ratios are also potentially a good tool to observe differences between agricultural practices on rather small agricultural regions (Kornexl et al., 1996). We are currently collecting more data on the other fruits to further evaluate the interest in the δ^{15}N values of proteins for geographical assignment.

Conclusion. $^{13}C/^{12}C$ ratios of proteins in fruit juices enable the absolute position on the scale of δ^{13}C values of all metabolites in the SNIP-IRMS method to be defined. Isotopic correlations between proteins and other metabolites isolated from fruit juices have been found. Proteins are supposed to undergo a lower metabolic turnover than metabolites such as sugars and organic acids, which should result in more stable isotopic values (Melzer and O'Leary, 1991). For all types of fruits included in this study, the addition of proteins provides no commercial benefit to adulterators because this minor component is not taken into account when the price of a given product is defined. Therefore, proteins can be used as a reliable internal standard for estimating the amount of sugar and/or organic acid addition even in the case of a simultaneous addition of sweeteners, malic acid, and citric acid. A quantification of the double adulteration is possible without knowledge of the origin of the fruit. A second application of proteins isolated from industrial fruit products is to get unbiased δ^{13}C and δ^{15}N values, which can provide additional indicators for origin recognition.

ABBREVIATIONS USED

AIJN, Association of the Industry of Juices and Nectars; AOAC, Association of Official Analytical Chemists; HFCS, high-fructose corn syrup; IRMS, isotope ratio by mass spectroscopy; NMR, nuclear magnetic resonance; SNIP-IRMS, specific natural isotope profile studied by IRMS; SNIF-NMR, specific natural isotope fractionation studied by NMR.

ACKNOWLEDGMENT

We are grateful to Pr. N. Naulet for his contribution to the IRMS experiments and to M. Messina and S. Rodríguez for their help in the implementation of the protein purification.

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Received for review June 18, 1998. Revised manuscript received September 14, 1998. Accepted September 18, 1998.

JF980664G