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Analytical Methods

Natural origin of ascorbic acid: Validation by ^{13}C NMR and IRMS

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ABSTRACT

A new method for the extraction and purification of ascorbic acid from two tropical fruits (acerola and camu-camu) is presented. ^{13}C nuclear magnetic resonance and isotopic ratio mass spectroscopy ($^{13}C/^{12}C$) were used to recognize ascorbic acid coming from either natural or industrial sources. A quantitative ^{13}C NMR procedure was optimized to calculate isotopic relative abundances on each molecular site; data were treated by a multivariate method.
Samples were also analysed by IRMS coupled with gas-chromatography/combustion and elemental analysis devices. The combined use of these techniques enabled us to validate the origins of ascorbic acid batches issuing from different sources.

1. Introduction

Vitamin C, the most important water-soluble antioxidant in human diet, is mostly supplied by fruits and vegetables (Davey et al., 2000; Lee & Kader, 2007). L-Ascorbic acid, its main biologically active form, is reversibly oxidised to L-dehydroascorbic acid, which also exhibits biological activity. Further oxidation generates diketogulonic acid, that has no known biological function (Davey et al., 2000; Deutsch, 2000).

Dehydroascorbic and ascorbic acid content has to be determined in a dietary source in order to assess its overall vitamin C activity (Lee & Kader, 2007). L-Ascorbic acid is widely distributed in plants, where it plays crucial roles in metabolism and tissue growth. As the human body cannot synthesize the vitamin, we rely on dietary fruits and vegetables for the essential intake. Some tropical fruits such as acerola and camu-camu are outstanding in providing high vitamin C at a relatively low cost.

Camu-camu (*Myrciaria dubia*) is a low-growing shrub found throughout the Amazon rainforest, mainly in swampy or flooded areas. It bears round, light-orange-coloured fruits about the size of a lemon, which provide thirty times more vitamin C than oranges, as well as a full complement of minerals and amino acids that aid in the vitamin absorption. Hence a growing demand for this fruit in the natural products market; however, as with any vitamin C-rich fruit, the time elapsed between harvest and consumption is crucial, as up to one quarter of the vitamin content may be lost in less than a month.

Acerola (*Malphigia glabra*) is a small tree that grows in dry deciduous forests. Its red fruit, resembling the European cherry, contains about 80% juice and a large amount of vitamin C, as well as iron, calcium and phosphorus. Its vitamin content depends on the season, climate, growing site and especially on the stage of ripening: the riper the fruit the lower its vitamin content. Unripe fruit may contain up to 4.5%, 90-times more than peeled oranges.

As a measure against the fraudulent sale of industrially manufactured ascorbic acid in place of the natural product, the origin of vitamin batches may be authenticated by stable isotope analysis, a method which has become more and more reliable for food control quality over the past few years. The determination of stable isotope ratios for light elements (carbon, hydrogen and oxygen) is being increasingly applied to food for authenticity control and origin assessment (Rossmann, 2001). The isotopic ratio is defined as the relative amount of two isotopic forms of the same element in a given sample (e.g., $^{2}H/^{1}H$, $^{13}C/^{12}C$ and $^{18}O/^{16}O$). Since in animal and vegetal products these ratios are markedly influenced by the synthetic route and environmental factors affecting the organism, they can be used to trace back the origin of a food product and to reveal whether a compound from an alleged source has been replaced with a chemically identical molecule obtained from another source (Ogrinc, Kosir, Spangenberg, & Kidric, 2003).

Although different approaches to the problem have been developed (Reid, O'Donnell, & Downey, 2006), only two techniques are currently being used to measure isotopic ratios: site-specific natural isotopic fractionation nuclear magnetic resonance (SNIF-NMR)

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and isotopic ratio mass spectroscopy (IRMS). Depending on its origin and production technique, a molecule will carry different proportions of two isotopes for each molecular position of the element. IRMS can determine the overall isotope ratio for a specific element; in food chemistry it is applied to the study of $^{13}C/^{12}C$, $^{18}O/^{16}O$ and $^{15}N/^{14}N$ ratios. On the other hand magnetic resonance is site-specific and can measure the relative isotopic ratios of a given element for each molecular site. SNIF-NMR was first developed by Martin, Martin, Mabon, and Michon (1983) who, in the early 1980s, studied the adulteration of wines by analyzing site-specific $^{2}H/^{1}H$ ratios for ethanol. This became the official method within the European Community (EC Regulation No. 2676/90, 1990) for detecting the addition of sugar in wines. Thanks to advancements in NMR spectrometers and related software, this technique was extended to the analysis of site-specific $^{13}C/^{12}C$ ratios (Zhang, Trierweiler, Jouitteau, & Martin, 1999). ^{13}C NMR spectroscopy has the advantage of limited peak overlaps, allowing simultaneous determinations on several carbon positions, without the inconvenience of solvent overlap. In order to obtain accurate quantitative ^{13}C spectra, lengthy experimental sessions are required, due to long longitudinal relaxation times; NOE effects must also be avoided.

The present study reports a new procedure for extracting ascorbic acid from tropical fruits (acerola unripe berries and camu-camu fruits); it also describes a convenient method for determining site-specific ^{13}C relative abundances in a vitamin C specimen. The ability of the latter to discriminate between samples of different origins was assessed by subjecting a combination of ^{13}C NMR data to multivariate analysis. IRMS also was used to discriminate synthetic from natural ascorbic acid.

2. Materials and methods

All solvents used were of analytical reagent grade and were purified according to published procedures (Amarengo & Perrin, 1998). Calcium carbonate, calcium oxide, sulphuric acid, MSTFA (*N*-methyl-*N*-trimethylsilyltrifluoroacetamide), ethanethiol, ammonium iodide, octadecane were purchased from Sigma–Aldrich and used as received without further purification.

2.1. Samples

As many as 27 independent samples of ascorbic acid of various origins were investigated: two sets were industrial (7 supplied by Sigma-Aldrich and 8 supplied by Carlo Erba); two sets were extracted from camu-camu (7) and acerola (5), both provided as frozen fruits by MB Med S.r.l. (Rivalta, Torino). Camu-camu fruits and acerola berries are produced in the State of Pará (Brazil).

2.2. Extraction and purification of ascorbic acid from acerola and camu-camu

One kilogram of unripe acerola berries was centrifuged in a domestic device (RoboDiet, De Longhi), then pressed with a screw press. The crude juice, after filtration under vacuum through a Celite® pad, yielded 750 ml of clear liquid. Likewise from 1 kg of camu-camu pulp, 940 ml of clear liquid were obtained by the same procedure.

Samples of these liquids were analysed by HPLC after filtration through a 45 μm syringe filter. Injection volume: 25 μl; column: Waters XTerra (4.6/150, 5 μm); elution gradient (solvents: A = 0.1% aqueous trifluoroacetic acid, B = 0.1% trifluoroacetic acid in acetonitrile) with the following profile: isocratic elution at B = 1% for 11 min; linear gradient up to B = 100% for the next 15 min; isocratic elution at B = 100% for further 10 min; flow rate: 1.4 ml/min. A Waters HPLC system, consisting of a 1525EF binary pump, a 717plus autosampler, a 2996 diode array detector and a WFC III fraction collector was used for analysis. The diode array detector was set at λ = 215, 230, and 254 nm. Quantitative determinations, after system calibration with 5 ascorbic acid standard samples, indicated that camu-camu juice contained 3% vitamin C, whereas acerola juice contained only 1%.

In order to obtain a fraction enriched in vitamin C, the clear juice (500 ml) was treated with a 5:1 mixture of calcium carbonate and calcium oxide so as to bring the pH to a value between 8 and 8.5. Precipitation was accelerated by adding acetone (600 ml). The white solid was then filtered off and dried under reduced pressure to yield a product enriched in vitamin C (20 g and 18 g from camu-camu and acerola juice, respectively). Each solid was suspended in water (150 ml) and pH was adjusted to 2.5 with 1 N sulphuric acid. The precipitate of calcium sulphate was filtered off and filtrates were lyophilized to yield brownish powders (16 g from acerola and 14 g from camu-camu). HPLC analysis showed that their ascorbic acid contents were 20% (acerola) and 53% (camu-camu).

Pure ascorbic acid was obtained from them by preparative HPLC-MS (Waters Fraction Link autopurification system equipped with Waters Micromass ZQ and Waters 2996 diode array detector). Each powder was dissolved in water, loaded on a Waters Atlantis dC18 column (19/100, 5 μm) and eluted with 0.1% aqueous trifluoroacetic acid. The ascorbic acid peak, identified by the diode array detector and the ESI-MS detector, was automatically collected. After freeze-drying it yielded pure ascorbic acid; ^{1}H NMR spectrum was used to check the absence of impurities.

^{1}H NMR 600 MHz, (D_2O): δ 3.68 (dd, $^{2}J_{H-H}$ = 11 Hz, $^{3}J_{H-H}$ = 7 Hz, 1H); δ 3.71 (dd, $^{2}J_{H-H}$ = 11 Hz, $^{3}J_{H-H}$ = 9 Hz, 1H); δ 4.00–4.02 (m, 1H); δ 4.90 (d, $^{3}J_{H-H}$ = 7 Hz, 1H).

2.3. NMR measurements

Either 150 mg of industrial product or 50 mg of natural product were dissolved in 0.55 ml of D_2O. Due to the high solubility in water of ascorbic acid (33 g per 100 ml), the signal-to-noise ratio in the ^{13}C spectrum exceeded 500 for industrial samples.

^{13}C NMR spectra were recorded at 25 °C using a Bruker Avance 600 spectrometer operating at 14 T and equipped with a 5 mm broad-band probe. For the quantitative ^{13}C NMR study the following experimental parameters were used: 90° pulse width (14 μs, –3.0 dB), pulse delay of 38 s, spectral width of 21186.44 Hz (120 ppm), 64 K sampled points with 1.5 s to acquire them; 120 (for industrial samples) or 1140 (for natural samples) transients were acquired. In order to avoid the Overhauser effect, inverse-gated decoupling techniques were applied (WALTZ-16, 100 μs, –3.50 dB). The decoupler offset was set at 5 ppm in the proton chemical-shift domain. The Fourier transform was applied to the acquired FIDs using an exponential window with a line-broadening factor of 2 Hz.

Fig. 1 shows the ^{13}C NMR spectrum of an industrial sample with the assignments.

T_1 values were calculated by inversion recovery sequence with 9 τ values ranging from 1 ms to 64 s. Analysis of the experimental data afforded for the different carbon nuclei T_1 values lying in the range between 0.915 s and 7.271 s (Table 1).

2.4. EA-IRMS measurements

The stable carbon isotopic composition of industrial samples was analysed by means of a ThermoFinnigan Delta Plus XP mass spectrometer using a Costech ECS4010 elemental analyzer as inlet. This is the so-called "Dumas combustion" approach for converting samples to CO_2, N_2, and water.

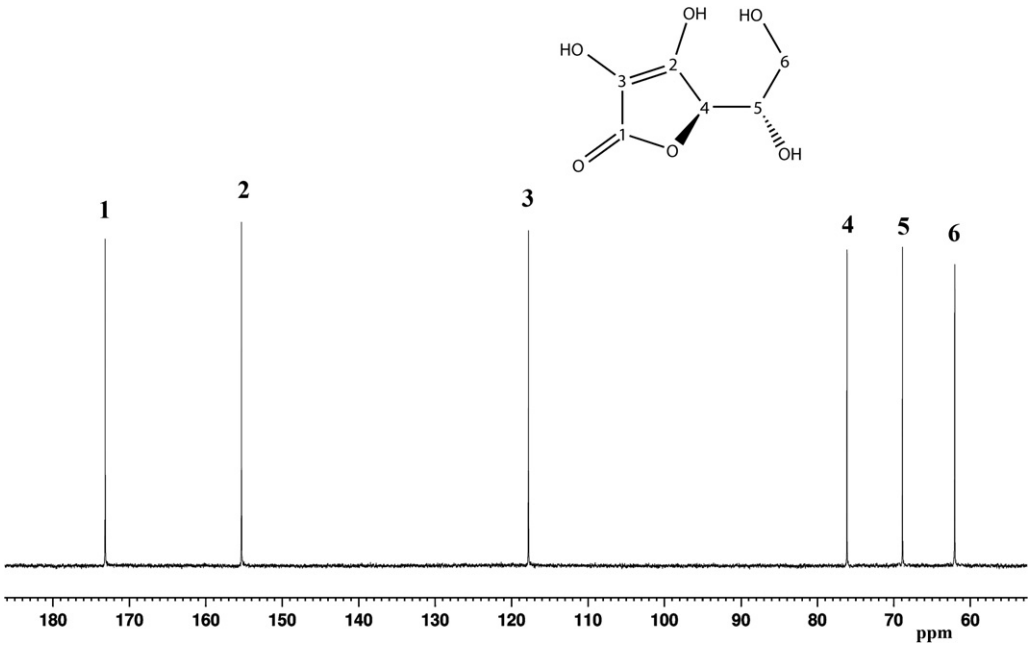

Fig. 1. ^{13}C NMR spectrum of an industrial sample recorded at 150.921 MHz in D_2O (273 mg/mL). Above: the structure of ascorbic acid.

Table 1
^{13}C NMR chemical shifts and longitudinal relaxation times (T_1) for a solution of ascorbic acid in D_2O (273 mg/mL) at 25 °C

Site (see formula in Fig. 1)						
	1	2	3	4	5	6
Chemical shift (ppm)	173.10	155.22	117.77	76.12	68.85	62.03
T_1 (s)	6.31	4.53	7.27	0.97	0.91	0.94

2.5. GC-C-IRMS measurements; sample preparation

Overall carbon isotope ratios were measured with a Trace GC Ultra (Thermo, Italy) equipped with a 95% polydimethylsiloxane capillary column, (50 m × 0.2 mm i.d., 0.33 μm coating tickness). They were expressed in $\delta^{13}C‰$, corresponding to:

$$1000 \times \frac{^{13}C/^{12}C_{sample} - ^{13}C/^{12}C_{std}}{^{13}C/^{12}C_{std}}$$

where the suffix std refers to the international carbonate standard V-PDB (Coplen, 1994; Coplen, 1995).

Ascorbic acid contains highly polar functional groups. Problems arising when such compounds are analysed by GC have been discussed (Klee, 1985). Prior to GC analysis they must be derivatized. Usually, alcohols and polyalcohols (including ascorbic acid) are analysed as trimethylsilyl (TMS) ethers. TMS derivatives combine good chemical and thermal stability with a high volatility and can be prepared in quantitative yields.

A mixture of 20 ml of MSTFA (*N*-methyl-*N*-trimethylsilyltrifluoroacetamide), 80 mg of ethanethiol and 40 mg of ammonium iodide was used as the silylating reagent. Ascorbic acid derivatives were prepared as follows: 10 μl of a methanolic solution of dried ascorbic acid (5 mg/ml) containing octadecane (1 mg/ml) as internal standard were placed in a vial and evaporated to dryness under a nitrogen stream; then 100 μl of MSTFA were added to the residue. The mixture was stirred for 4 h at room temperature in the dark. After derivatization, excess silylating agent was removed by a stream of argon and the residue was dissolved in 100 μl of methanol. Mass spectra of derivatives (Fig. 2) were in accordance with those reported in the literature (De Wilt, 1971).

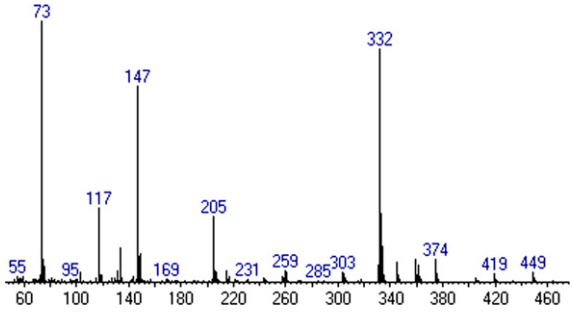

Fig. 2. MS spectrum of derivatized ascorbic acid.

GC-C-IRMS analyses were performed as follows. Natural ascorbic acid derivatives were injected splitless, whereas those of industrial samples were injected with a 1:20 split ratio at 280 °C. For all samples the GC oven was programmed as follows: from 80 °C (isothermal for 2 min) to 315 °C at the rate of 20 °C min^{-1}, then a final isothermal step at 315 °C for 3 min. Helium was used as carrier gas and CO_2 as reference. The effluent from the column was combusted at 940 °C in an alumina tube (0.55 mm inner diameter, 1.55 mm outer diameter, 320 mm long) which served as an oxidation reactor. The tube contained three wires made of CuO, NiO, and Pt respectively (all 240 mm long and 0.125 mm thick). Nitrogen oxides were reduced to N_2 at 640 °C in a reduction furnace made by three twisted copper wires of 0.125 mm diameter. A hygroscopic Naphion membrane, interposed just before the spectrometer inlet, eliminated excess water, avoiding CO_2 protonation and consequent formation of HCO_2^+ ($m/z = 45$).

Each sample was analysed three times and results were averaged. CO_2 reference was calibrated using alkane mixtures supplied by Arndt Schimmelmann of Indiana University with certified $\delta^{13}C$ values against V-PDB. The $\delta^{13}C$ of 15 linear alkanes, ranging from C_{16} to C_{30}, were calculated by Schimmelmann according to the literature (Coplen et al., 2006).

However, because in converting ascorbic acid to the *tetrakis*trimethylsilyl derivative carbon atoms are added to the molecule, the $\delta^{13}C$ value of the trimethylsilyl group had to be subtracted from

$\delta^{13}C$ values obtained for derivatized ascorbic acids (see par. 3.2). For this correction we used a method described in the literature (Jones, Carter, Eglinton, Jumeau, & Fenwick, 1991).

2.6. Analysis of data

Data treatment by principal component analysis (PCA) was used to process table-crossing ascorbic acid samples (as individuals) and specific isotopic deviations (as variables). This mathematical procedure transforms a number of (possibly) correlated variables into a smaller number of uncorrelated variables called principal components. The first principal component accounts for as much as possible of the variability in the data, and each succeeding component for as much of the remaining variability as possible. Multivariate analysis was performed by using the chemometrical package V-PARVUS (Forina, Lanteri, Armanino, Casolino, & Casale, 2007).

3. Results and discussion

Experimental data obtained by NMR and IRMS were used separately to classify ascorbic acid samples coming from natural and industrial sources. ^{13}C NMR data were used to obtain site-specific relative ^{13}C contents for each carbon site (Tenailleau, Lancelin, Robins, & Akoka, 2004). IRMS data were used as an additional tool for discriminating the sample origin.

3.1. ^{13}C NMR

Relative site-specific contents X_i obtained by the analysis of NMR spectra were calculated according to the procedure reported by Tenailleau et al. (2004) with a slight modification:

$$X_i = \frac{f_i}{F_i}$$

where f_i is the fraction of the isotopomer monolabelled in position i and F_i is the population of the i site supposing isotope distribution to be random.

F_i and f_i were calculated as follows:

$$F_i = \frac{P_i}{\sum_i P_i}$$

$$f_i = \frac{I_i}{\sum_i I_i}$$

where P_i is the number of equivalent carbons for site i and I_i is the area of the carbon NMR peak corresponding to the same site. For our compound P_i equals 1 and F corresponds to 1/6 for each molecular site. NMR signals were evaluated according to their integrals. In Table 2 NMR site-specific patterns for each source are reported with the standard deviation. These data revealed, for natural ascorbic acids, a general enrichment of ^{13}C in positions 1 and 6 and an impoverishment in 2, whereas industrial samples showed the opposite trend, in agreement with Versini, Ziller, Frassanito, Mattivi, and Rossmann (2005).

PCA was computed in the space of the six variables, one for each carbon site, calculated from 210 NMR spectra. Each value resulted as an average of 10 (in the case of industrial samples) or 5 (for natural samples) ^{13}C spectra.

In Fig. 3 data are plotted in the reduced space of principal components (PC1 and PC2), whose relative contributions to the total variance are indicated in parentheses. Analysis of variance showed that for PC1 the major contributions arise from sites 5 (26.0%), 6 (20.4%) and 2 (18.3%); for PC2 from sites 3 (28.3%) and 4 (18.6%). Other sites contribute less than 18%.

Fig. 3 clearly shows that industrial and natural samples lie in different regions of the plot. Camu-camu and acerola samples are easily distinguishable from each other and from industrial ones. On the other hand, it is not possible to discriminate between Sigma–Aldrich and Carlo Erba samples, because they form a spread-out, homogeneous group. Ascorbic acid extracted from acerola juice exhibited a high ^{13}C content in positions 5 and 6, whereas samples from camu-camu berries showed a relatively high ^{13}C content in positions 3 and 4. Some of the industrial samples exhibited high levels of ^{13}C in C_2, a carbonylic carbon, but lower contents in position 4.

3.2. IRMS

Although a multivariate treatment of NMR data proved effective for discriminating between natural and industrial ascorbic acid samples, we found that IRMS analysis was required for a precise distinction between industrial samples. In order to obtain the $\delta^{13}C$ of TMS groups, we applied the method reported by Jones et al. (1991) with a slight variation. We analysed both derivatized (by GC-C-IRMS) and underivatized (by EA-IRMS) industrial samples and treated data as follows:

$$6 \times \delta_A + 12 \times \delta_T = 18 \times \delta_D$$

$$\delta_T = \frac{18 \times \delta_A - 6 \times \delta_A}{12}$$

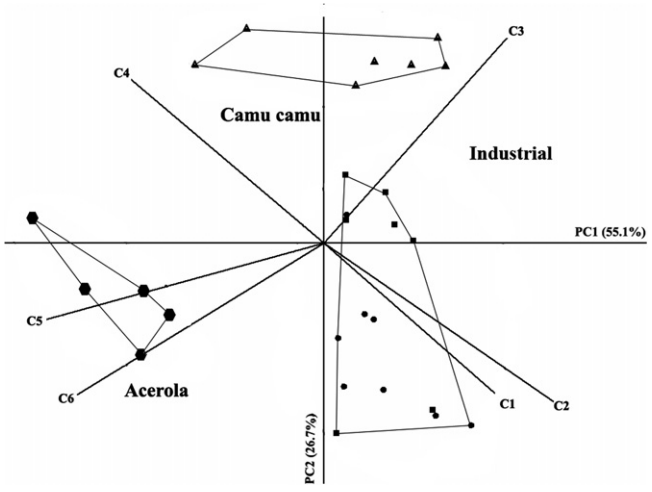

Fig. 3. 2D representation of PCA calculated from ^{13}C NMR data. Samples (⬣ Acerola, ▲ Camu-camu, ■ Sigma–Aldrich, ● Carlo Erba) are plotted in the plane of the two principal components. Scores and loadings are reported.

Table 2
Site-specific ^{13}C pattern (mean and standard deviations are reported for each of four sources)

Source	X_1	SD	X_2	SD	X_3	SD	X_4	SD	X_5	SD	X_6	SD
Sigma–Aldrich	0.997	0.001	1.011	0.006	1.032	0.004	0.998	0.005	0.991	0.002	0.972	0.009
Carlo Erba	0.997	0.002	1.009	0.002	1.032	0.002	0.995	0.002	0.992	0.001	0.975	0.003
Acerola	1.008	0.003	0.974	0.008	0.974	0.007	1.043	0.015	0.994	0.003	1.006	0.002
Camu-camu	1.006	0.002	0.993	0.009	1.010	0.006	0.987	0.009	0.979	0.007	1.015	0.004

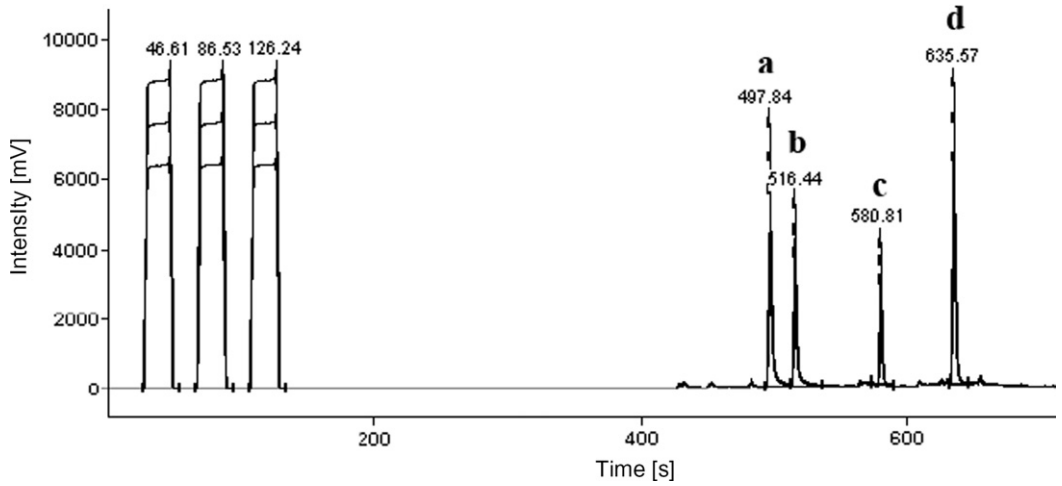

Fig. 4. GC-C-IRMS spectrum of an industrial sample. (a and b) excess of silylating agent; (c) octadecane internal standard; (d) derivatized ascorbic acid.

where δ_A: $\delta^{13}C$ for plain industrial ascorbic acid (6 atoms); δ_D: $\delta^{13}C$ for derivatized industrial ascorbic acid (18 atoms); δ_T: $\delta^{13}C$ for 4 TMS groups (12 atoms). δ_A was measured twice by EA-IRMS on both industrial samples and results were averaged: –12.57‰ and –9.96‰ (SD < 1%) were found for the Sigma–Aldrich and Carlo Erba sample, respectively. δ_D was measured three times by GC-C-IRMS on both industrial samples and results were averaged: –28.97‰ and –27.67‰ (SD < 1.5%) were found for the Sigma-Aldrich and Carlo Erba sample, respectively. δ_T was calculated for both samples as reported above and –37.17‰ (SD = 1.1%) was obtained. Taking –37.17‰ for $\delta^{13}C$ of TMS, the $\delta^{13}C$ of each plain ascorbic acid sample was calculated as follows:

$$\delta_A = \frac{18 \times \delta_D - 12 \times \delta_T}{6}$$

Table 3
$\delta^{13}C$ values for ascorbic acid from industrial and natural sources

Sample identifier	Origin of sample	Industrial (I) or natural (N) sample	$\delta^{13}C$ derivatized ascorbic acids vs PDB	$\delta^{13}C$ ascorbic acids corrected vs PDB
1	Sigma–Aldrich	I	−28.97 ± 0.26	−13.16 ± 0.77
2	Sigma–Aldrich	I	−28.96 ± 0.08	−13.13 ± 0.23
3	Sigma–Aldrich	I	−29.23 ± 0.28	−13.95 ± 0.84
4	Sigma–Aldrich	I	−29.09 ± 0.28	−13.52 ± 0.83
5	Sigma–Aldrich	I	−29.06 ± 0.29	−13.45 ± 0.77
6	Sigma–Aldrich	I	−28.81 ± 0.24	−12.71 ± 0.73
7	Sigma–Aldrich	I	−28.68 ± 0.25	−12.29 ± 0.74
8	Carlo Erba	I	−28.00 ± 0.19	−10.27 ± 0.58
9	Carlo Erba	I	−27.67 ± 0.19	−9.26 ± 0.56
10	Carlo Erba	I	−28.12 ± 0.11	−10.61 ± 0.34
11	Carlo Erba	I	−27.70 ± 0.28	−9.36 ± 0.84
12	Carlo Erba	I	−27.65 ± 0.21	−9.22 ± 0.65
13	Carlo Erba	I	−27.35 ± 0.23	−8.29 ± 0.68
14	Carlo Erba	I	−27.10 ± 0.24	−7.57 ± 0.73
15	Carlo Erba	I	−27.23 ± 0.02	−7.94 ± 0.07
16	Acerola	N	−31.36 ± 0.07	−20.34 ± 0.20
17	Acerola	N	−31.40 ± 0.13	−20.46 ± 0.39
18	Acerola	N	−31.37 ± 0.15	−20.36 ± 0.46
19	Acerola	N	−31.39 ± 0.11	−20.43 ± 0.34
20	Acerola	N	−31.39 ± 0.10	−20.44 ± 0.29
21	Camu-camu	N	−30.53 ± 0.51	−18.83 ± 0.25
22	Camu-camu	N	−31.14 ± 0.13	−19.68 ± 0.38
23	Camu-camu	N	−31.06 ± 0.15	−19.43 ± 0.44
24	Camu-camu	N	−31.34 ± 0.12	−20.28 ± 0.35
25	Camu-camu	N	−31.45 ± 0.20	−20.61 ± 0.60
26	Camu-camu	N	−31.09 ± 0.14	−19.53 ± 0.43
27	Camu-camu	N	−31.12 ± 0.10	−19.61 ± 0.30

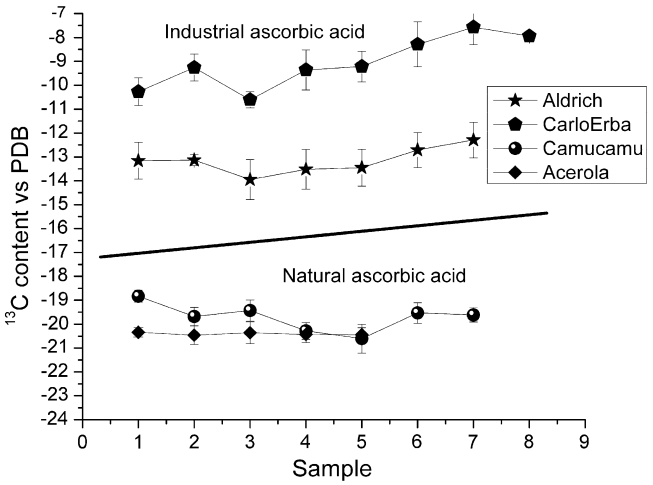

Fig. 5. $\delta^{13}C$ corrected *vs* PDB for samples plotted with error bars.

where δ_D was measured by GC-C-IRMS for each sample. In a typical GC-C-IRMS chromatogram, the peak of derivatized ascorbic acid (d in Fig. 4), was well separated from those of other compounds (the octadecane internal standard and the excess of silylating agent).

On the basis of $\delta^{13}C$ data, reported in Table 3 and plotted with error bars in Fig. 5, a discrimination between industrial and natural samples could be made. Ascorbic acids extracted from vegetal sources exhibited $\delta^{13}C$ values in the range from –20.61‰ to –19.43‰, in total agreement with a previous study (Gensler, Rossmann, & Schmidt, 1995), that reported $\delta^{13}C$ values for ascorbic acid obtained from fruit juices (ranging from –21.6‰ to –19.9‰). Industrial samples showed much higher $\delta^{13}C$ values, falling in the range from −13.95‰ to –7.57‰. These data are also in total agreement with Gensler's figures (mean value –11.3‰).

Data referring to industrial products suggests that synthetic or emisynthetic processes use C4 plant sugars as starting materials. Mass spectrometry enabled us to distinguish between natural and industrial ascorbic acid, also to discriminate between two industrial products (Sigma–Aldrich and Carlo Erba). As already mentioned, the latter discrimination could not be made by NMR alone, while, on the other hand, NMR, unlike mass analysis, could distinguish between samples from acerola and camu-camu. A complete characterization became thus possible by combining the two techniques.

4. Conclusions

A new procedure has been presented for the extraction of ascorbic acid from tropical fruits with high contents of vitamin C. This was extracted from camu-camu fruits and acerola berries, purified by HPLC and characterized by qualitative NMR and mass spectroscopy. Both SNIF-NMR and ^{13}C IRMS proved to be adequate techniques for discriminating samples obtained from industrial suppliers from those extracted from the above-mentioned natural sources.

Although time-consuming, quantitative ^{13}C NMR analysis proved also adequate to distinguish ascorbic acid extracted from camu-camu and acerola; it was however inadequate for recognizing samples from different industrial suppliers. On the other hand, EA-IRMS, performed on ascorbic acid samples and GC-C-IRMS, performed on their TMS derivatives, were able to discriminate between samples supplied by Sigma–Aldrich and those supplied by Carlo Erba, but not between acerola and camu-camu samples. In conclusion, SNIF-NMR represents a convenient method for the determination of site-specific ^{13}C relative abundances in ascorbic acid; it can also discriminate between samples of different origins after a multivariate analysis is applied to a combination of ^{13}C NMR data.

Acknowledgments

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