PROCESS NMR ASSOCIATES, LLC

Technical White Paper

Determining Plant Identity and Geographic Origin: Isotopic NMR (^{2}H SNIF-NMR and Site-Specific ^{13}C NMR) Compared with Isotope Ratio Mass Spectrometry

A Process NMR Associates white paper on method selection for botanical and geographical authentication of plant-derived foods, beverages, flavors and oils

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Abstract

Two families of measurement dominate the isotopic authentication of plant-derived materials: isotope ratio mass spectrometry (IRMS), which reports bulk or compound-averaged isotope ratios of carbon, hydrogen, oxygen, nitrogen and sulfur with per-mil precision, and isotopic nuclear magnetic resonance (NMR), which resolves the isotope ratio at individual atomic positions within a molecule. This white paper sets out the physical and biochemical basis on which both families operate, describes how each measurement is made, and assembles a catalog of published applications in which plant identity (botanical species, photosynthetic pathway, natural versus synthetic origin) and geographic origin (climate, latitude, altitude, geology, agronomy) have been determined. The site-specific natural isotope fractionation NMR method (^{2}H SNIF-NMR) applied to fermentation ethanol, and its extension to vanillin, benzaldehyde, anethole, linalool, acetic acid, glycerol and fatty acids, is treated in detail, together with the more recent isotopic ^{13}C NMR (position-specific isotope analysis) that has resolved cane, maize and agave sugars, natural and synthetic vanillin, and lipid biosynthetic history at the 1‰ level. Non-isotopic ^{1}H and ^{13}C NMR metabolite profiling is treated as a third, compositional route to botanical and regional classification. The comparison is drawn candidly: NMR is favored wherever the discriminating information is intramolecular or where the adulterant shares the bulk isotope ratio of the authentic material, and IRMS is favored wherever the question turns on the oxygen, nitrogen or sulfur isotopes, on sub-milligram or trace-level analytes, on the highest bulk precision, or on comparison with the large geographic databanks that exist only for IRMS parameters. The regulatory infrastructure (OIV, AOAC, CEN and the European Union wine isotopic databank) is summarized, the practical requirements for a mid-field NMR laboratory are examined, and the principal pitfalls of both approaches — databank dependence, climatic drift, processing artifacts, isotopic engineering of adulterants and chemometric over-interpretation — are set out.

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1. Introduction and Scope

Questions of plant identity and geographic origin arise whenever a premium is attached to a declared source. The grape sugar in a wine, the cane in a rum, the agave in a tequila, the bean in a vanilla extract, the olive grove of a protected designation, the terroir of an appellation, the Basmati field, the single-origin coffee lot and the organically certified tomato all carry a declaration that can be, and routinely is, contradicted by substitution, dilution, blending or mislabeling. The analytical response to these declarations has relied for four decades on the natural variation of stable isotope ratios, because those ratios are imprinted on the plant by its photosynthetic biochemistry and by the water and atmosphere of the place where it grew, and because the imprint survives harvesting, fermentation, distillation and refining in ways that can be modeled and, in favorable cases, calculated.

Two instrumental families read that imprint. Isotope ratio mass spectrometry converts the sample, or a chromatographically isolated component of it, into a simple gas (CO_2, N_2, H_2, CO, SO_2) and measures the isotopologue ratio of that gas against a reference with a precision of tenths of a per mil or better. The result is an average over every atom of the element in the analyte. Isotopic NMR does not convert the analyte; it observes the ^{2}H or ^{13}C nuclei directly and, because chemical shift separates the atomic positions, returns an isotope ratio for each site. The distinction is not one of precision or convenience but of information content, and it is the principal theme of this paper. A bulk $\delta^{13}C$ value that has been matched by a fraudster through blending is opaque to IRMS but transparent to site-specific ^{13}C NMR; a beet sugar added to apple juice is invisible to bulk $\delta^{13}C$, because beet and apple are both C_3 plants, but is detected by the methyl-site deuterium ratio of the fermentation ethanol. Conversely, the oxygen isotope ratio of wine water, the nitrogen isotope ratio of a fertilized crop, the $^{87}Sr/^{86}Sr$ ratio that reports bedrock geology and the compound-specific $\delta^{13}C$ of a trace flavor component in a finished food are accessible only to mass spectrometry, and the geographic databanks against which an unknown must be judged have been built almost entirely from IRMS parameters.

This paper is organized as follows. Section 2 establishes the physical and biochemical origins of isotopic variation in plants, including the intramolecular (non-statistical) distributions on which isotopic NMR depends. Sections 3 and 4 describe the two measurement families, with emphasis on the ^{2}H SNIF-NMR protocol codified by the Organisation Internationale de la Vigne et du Vin (OIV) and AOAC International, and on the isotopic ^{13}C NMR methodology developed principally at the University of Nantes. Section 5 summarizes the official-method and regulatory infrastructure. Sections 6 and 7 assemble worked examples for plant identity and for geographic origin respectively, drawn from wine, spirits, fruit juices, honey, maple syrup, vinegar, flavors, edible oils, rice, coffee, cocoa, sesame and, as a forensic extension, plant-derived narcotics. Section 8 addresses the fusion of NMR and IRMS data. Section 9 draws the head-to-head comparison and offers a decision guide. Section 10 considers implementation in a mid-field NMR laboratory such as that operated by Process NMR Associates. Section 11 is a critical assessment of the pitfalls that an expert reviewer, or an opposing expert witness, would be expected to raise. Non-isotopic ^{1}H and ^{13}C NMR profiling is included throughout because, for questions of species, variety and cultivar, isotope ratios are frequently uninformative and metabolite composition is the discriminating observable.

The reference list was restricted to publications in which NMR or IRMS was applied to, or developed for, the determination of plant origin, together with the small number of foundational papers on isotope fractionation and on measurement conventions that are needed to interpret them. Every reference was verified against CrossRef metadata before the text was written.

2. Isotopic Signatures in Plants: The Physical and Biochemical Basis

2.1 Notation, reference scales and natural abundances

Stable isotope ratios are reported as δ values, defined as δ = (R_sample/R_reference − 1), where R is the ratio of the heavy to the light isotope, and expressed in per mil (‰). The international reference points are Vienna Pee Dee Belemnite (VPDB) for $^{13}C/^{12}C$, Vienna Standard Mean Ocean Water (VSMOW) for $^{2}H/^{1}H$ and $^{18}O/^{16}O$, atmospheric N_2 (AIR) for $^{15}N/^{14}N$ and Vienna Cañon Diablo Troilite (VCDT) for $^{34}S/^{32}S$. The conventions for reporting, for two-point normalization of raw data to the international scales and for the terminology of isotope-ratio results have been codified by Coplen[1] and by Werner and Brand,[2] and those conventions are followed here. Isotopic NMR uses a different and older convention in which the site-specific ratio is expressed as an absolute abundance in parts per million (ppm), for example $(D/H)^{I}$ = 101.5 ppm, calibrated through an internal standard whose D/H ratio is itself traceable to VSMOW (D/H = 155.76 ppm). The two conventions are interconvertible, and both are used in this paper according to the practice of the literature being cited. Table 1 collects the elements, reference scales, natural abundances and typical plant ranges that are relevant to origin determination.

Table 1. Isotope systems used in plant origin determination: reference scales, natural abundances, the principal natural controls on the ratio, and the technique by which each is measured.

Isotope ratio	Reference scale	Natural abundance of heavy isotope	Principal natural controls in plants	Measurement
$^{13}C/^{12}C$	VPDB	≈ 1.1 %	Photosynthetic pathway (C_3, C_4, CAM); stomatal conductance and water stress; canopy CO_2 recycling; altitude	EA-IRMS (bulk), GC-C-IRMS and LC-IRMS (compound-specific), isotopic ^{13}C NMR (position-specific)
$^{2}H/^{1}H$	VSMOW	≈ 0.0156 % (155.76 ppm)	Meteoric water (latitude, altitude, continentality, temperature, amount); leaf-water evaporative enrichment; biochemical fractionation at each C–H site	TC/EA-IRMS (bulk, exchangeable-H correction required), GC-P-IRMS (compound-specific), ^{2}H SNIF-NMR (site-specific)
$^{18}O/^{16}O$	VSMOW	≈ 0.20 %	Meteoric water and leaf-water enrichment; ≈ +27 ‰ biochemical offset in carbohydrate oxygen; processing water	TC/EA-IRMS, CO_2–H_2O equilibration for water; not accessible by NMR at natural abundance
$^{15}N/^{14}N$	AIR	≈ 0.37 %	Fertilizer type (synthetic ≈ 0 ‰; manure and compost enriched), soil nitrogen cycling, legumes, marine influence	EA-IRMS; not practicable by NMR at natural abundance for origin work
$^{34}S/^{32}S$	VCDT	≈ 4.2 %	Bedrock and soil sulfur sources, sea-spray sulfate, atmospheric deposition	EA-IRMS
$^{87}Sr/^{86}Sr$	Radiogenic (no δ scale)	≈ 7.0 % ^{87}Sr	Age and Rb/Sr of bedrock and soil; independent of climate and biology	TIMS or MC-ICP-MS (not IRMS in the strict sense)

2.2 Carbon: photosynthetic pathway as the primary determinant

The carbon isotope composition of plant material is controlled first by the enzyme that fixes atmospheric CO_2 ($\delta^{13}C \approx -8$ ‰). In C_3 plants, which include grape, apple, orange, sugar beet, rice, wheat, olive, coffee and cocoa, ribulose-1,5-bisphosphate carboxylase discriminates strongly against ^{13}C, and the resulting biomass falls in the range of approximately −33 to −22 ‰, the position within that range being set largely by the ratio of intercellular to ambient CO_2 concentration and therefore by stomatal behavior, water availability and irradiance.[3,4] In C_4 plants, which include sugarcane, maize and sorghum, CO_2 is first fixed by phosphoenolpyruvate carboxylase, which discriminates weakly, and the biomass falls between approximately −16 and −9 ‰. Crassulacean acid metabolism (CAM) plants, which include agave, pineapple and vanilla, fix CO_2 at night through the C_4 route and re-fix it by day through the C_3 route, and their $\delta^{13}C$ values are intermediate and variable, commonly −20 to −10 ‰, depending on the balance between the two modes.[3] These pathway differences are the basis of every bulk $\delta^{13}C$ test for C_4 sugar in a C_3 product: corn syrup or cane sugar in honey, apple or orange juice, maple syrup or wine is revealed by a shift of the bulk value toward the C_4 range. They are also the basis of the first and most important limitation of bulk $\delta^{13}C$, namely that sugars from two C_3 sources (beet in grape, wheat glucose syrup in apple) cannot be distinguished, and that C_4 sources (cane in maize, or either in agave) overlap sufficiently that bulk $\delta^{13}C$ alone is unreliable.

A second-order but analytically valuable effect is the environmental modulation of $\delta^{13}C$ within a pathway. Water stress closes stomata, lowers the intercellular CO_2 concentration and drives the C_3 value toward the enriched end of its range, so that grapes from a hot dry vintage or an arid region are less negative than those from a cool humid one.[4,5] This is the reason $\delta^{13}C$ of wine ethanol carries geographic and vintage information in addition to pathway information, and it is one of the reasons the European Union databank is rebuilt every year.

2.3 Hydrogen and oxygen: meteoric water, climate and evapotranspiration

The hydrogen and oxygen of plant water derive from precipitation, whose isotopic composition varies systematically with latitude, altitude, distance from the coast, temperature and rainfall amount, and whose $\delta^{2}H$ and $\delta^{18}O$ values co-vary along the global meteoric water line $\delta^{2}H \approx 8\ \delta^{18}O + 10$.[6] The spatial pattern has been interpolated into global and regional isoscapes that provide the prior expectation for any location.[7] Within the leaf, transpiration enriches the residual water in the heavy isotopes, so that carbohydrate synthesized from leaf water carries the meteoric signal plus an evaporative enrichment that itself depends on relative humidity and temperature. Oxygen enters carbohydrate with a biochemical fractionation of approximately +27 ‰ relative to the leaf water, and the ^{18}O pattern within natural products has been systematized by Schmidt, Werner and Roßmann.[8] Hydrogen enters the carbon skeleton at specific enzymatic steps, each with its own kinetic isotope effect, so that the non-exchangeable C–H positions of a metabolite carry a pattern that reflects both the water source and the biosynthetic route.[9] This is the physical origin of the site-specific deuterium distributions exploited by SNIF-NMR: a bulk $\delta^{2}H$ value averages over positions that were fractionated differently, whereas the NMR experiment reads each position separately and thereby separates the climatic contribution from the biochemical one.

For fermented and pressed products a further distinction must be made between the water that was in the fruit and the water in which fermentation, dilution or reconstitution took place. Wine water inherits the $\delta^{18}O$ of the must, which is enriched relative to local precipitation by evapotranspiration in the berry, and is

therefore a sensitive indicator both of the growing region and of any subsequent addition of tap water.[10] Fruit-juice water behaves in the same way, and the comparison of the $\delta^{18}O$ of juice water with that of the sugar-derived ethanol was introduced precisely to distinguish legitimate reconstitution of concentrate from illegitimate watering.[11] The hydrogen of fermentation ethanol is drawn partly from the sugar and partly from the fermentation water, a division that turns out to be site-specific and that is the reason the two ethanol sites report on two different things (Section 4.2).

2.4 Nitrogen, sulfur and strontium: agronomy and geology

Nitrogen isotope ratios of crops respond to the fertilizer regime. Synthetic nitrogen fertilizers are manufactured from atmospheric N_2 and carry $\delta^{15}N$ values close to 0 ‰, whereas manures, composts and other organic amendments are enriched through ammonia volatilization and microbial cycling, so that organically grown produce is on average, though not without overlap, enriched in ^{15}N relative to conventionally grown produce.[12] Sulfur isotope ratios respond to soil parent material and to marine sulfate deposition near coasts. Strontium is not fractionated biologically to any measurable degree, and the $^{87}Sr/^{86}Sr$ ratio of a plant reflects the age and rubidium content of the bedrock from which its soil derived; the ratio is therefore independent of climate and vintage and has been mapped against simplified geological maps as a food-authentication tool.[13] None of these three ratios is accessible by NMR at natural abundance with the precision required, and their use is one of the clearest cases in which IRMS (or, for strontium, multi-collector inductively coupled plasma mass spectrometry) is the only practicable route.[14,15]

2.5 Intramolecular (non-statistical) isotope distributions

The observation on which all of isotopic NMR rests is that isotopes are not distributed statistically among the atomic positions of a natural product. Rossmann, Butzenlechner and Schmidt demonstrated in 1991, by chemical degradation of glucose followed by IRMS of the fragments, that the six carbon positions of natural glucose differ by several per mil, with position C-4 markedly depleted and position C-3 depleted relative to the mean.[16] Gleixner and Schmidt subsequently traced the principal cause to the kinetic and equilibrium isotope effects of the fructose-1,6-bisphosphate aldolase reaction, which acts on the C-3/C-4 bond during triose interconversion and thereby imposes the pattern on every hexose passing through the cycle.[17] The pattern is transmitted, with further modification, to the products of glycolysis and fermentation, so that the methyl and methylene carbons of ethanol inherit the isotopic history of glucose positions C-1/C-6 and C-2/C-5 respectively.[18] The same phenomenon exists for hydrogen and was first exploited, rather than merely observed, by the Martins at Nantes in the early 1980s.[19,20] The deuterium content of the methyl site of ethanol differs from that of the methylene site by tens of ppm, and both differ from the D/H ratio of the water, in a manner that depends on the plant species, the photosynthetic pathway, the climate and the fermentation medium.[21] Site-specific patterns of similar magnitude have since been documented in fatty acids,[22] glycerol,[23] cellulose-derived glucose[24] and a wide range of aromatic and terpenoid natural products (Section 4.4).

The practical consequence is that the bulk isotope ratio measured by IRMS is a weighted mean of site-specific values that may differ from one another by more than the entire natural range of the mean. Two materials with identical bulk values can have different intramolecular patterns, and an adulterant blended to reproduce a target bulk value will, in general, not reproduce the pattern. The intramolecular pattern is therefore both a richer descriptor of origin and a more robust one against deliberate matching, and it is the property that NMR alone, among routine techniques, reads without chemical degradation of the molecule.

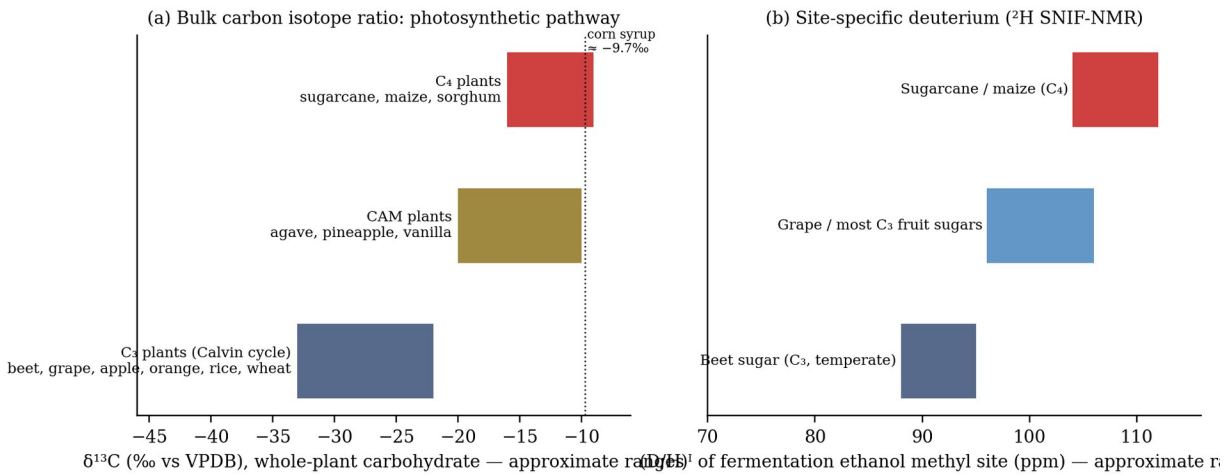

Figure 1. Schematic ranges (not measured data) of (a) bulk δ¹³C by photosynthetic pathway and (b) the methyl-site deuterium ratio (D/H)ᴵ of fermentation ethanol by sugar source. Beet and grape are both C_3 plants and overlap completely in panel (a) but separate in panel (b); cane and maize are both C_4 plants and overlap in both panels, which is the case resolved by site-specific ¹³C NMR (Section 6.2). Ranges are approximate and are drawn from the published values discussed in Sections 2.2 and 4.3; reference populations must be established for each product and vintage.

3. Isotope Ratio Mass Spectrometry

3.1 Instrumentation and sample interfaces

An isotope ratio mass spectrometer is a magnetic-sector instrument with an electron-impact source and an array of Faraday collectors positioned to register simultaneously the ion beams of the isotopologues of a light gas, for example m/z 44, 45 and 46 for CO_2 or m/z 2 and 3 for H_2. Because only a small number of simple gases are measured, and because the ratio of two beams recorded at the same instant is insensitive to source fluctuations, the precision of the ratio measurement is far better than that of any scanning mass spectrometer, typically 0.01–0.1 ‰ for a pure gas. The analytical problem is therefore not the mass spectrometer but the interface that converts the sample into that gas without fractionation and delivers it, together with a reference gas of known composition, to the source.

In elemental analyzer IRMS (EA-IRMS) a weighed sample of 0.1–2 mg is combusted in a pulse of oxygen at about 1000 °C, the combustion products are reduced over hot copper to remove excess oxygen and convert nitrogen oxides to N_2, water is removed and the N_2 and CO_2 are separated on a short gas-chromatographic column before entering the source in a helium carrier. The method delivers δ¹³C and δ¹⁵N (and, with a sulfur-configured analyzer, δ³⁴S) on bulk material and is the workhorse of geographic-origin studies. High-temperature conversion (TC/EA) pyrolyzes the sample over glassy carbon at about 1400 °C to CO and H_2, from which δ¹⁸O and δ²H are obtained; for hydrogen the exchangeable fraction bound to oxygen and nitrogen must either be removed by derivatization or equilibrated with waters of known composition, because it re-equilibrates with ambient moisture and does not report on the plant.[14] Water itself is analyzed by equilibration of a small aliquot with CO_2 (for δ¹⁸O) or with H_2 over a platinum catalyst (for δ²H), or increasingly by cavity ring-down spectroscopy, which is not a mass-spectrometric method but is now accepted for the δ¹⁸O of wine and juice water.

Compound-specific isotope analysis is achieved by inserting a chromatograph before the combustion or pyrolysis interface. Gas chromatography–combustion–IRMS (GC-C-IRMS) resolves volatile components, such as flavor molecules, fatty acid methyl esters or the ethanol and CO_2 of a beverage, and combusts each eluting peak on-line to CO_2; the technique, its sources of error and its applications were reviewed by Meier-Augenstein.[25] Liquid chromatography–IRMS (LC-IRMS) oxidizes non-volatile analytes, principally sugars and organic acids, in the aqueous eluent with persulfate and delivers the resulting CO_2 to the source; because the oxidation is chemical, organic modifiers cannot be used and the separations are restricted to purely aqueous mobile phases. Its introduction transformed honey and juice analysis because the $\delta^{13}C$ of glucose, fructose, sucrose and oligosaccharides could be compared individually with one another and with the protein internal standard.[26] For strontium, the sample is ashed, strontium is isolated by ion exchange and the $^{87}Sr/^{86}Sr$ ratio is measured by thermal-ionization or multi-collector ICP mass spectrometry.[13]

3.2 Referencing, normalization and uncertainty

IRMS results are relative measurements and are placed on the international scales through certified reference materials distributed by the International Atomic Energy Agency and the United States Geological Survey; two-point normalization against reference materials bracketing the sample range is required, and the recommended reporting conventions are those of Coplen.[1,2] Under routine conditions the combined standard uncertainty of EA-IRMS is about 0.1–0.2 ‰ for $\delta^{13}C$ and $\delta^{15}N$, 0.2–0.4 ‰ for $\delta^{18}O$ by TC/EA, and 1–3 ‰ for $\delta^{2}H$, the last being larger both because the natural abundance of deuterium is lower and because of the H_3^+ correction and the exchangeable-hydrogen problem. Compound-specific values carry additional uncertainty from chromatographic peak definition and, for derivatized analytes, from the carbon or hydrogen introduced by the derivatizing reagent. The numbers matter for the comparison with NMR, whose site-specific precision is about 1 ‰ for ^{13}C and about 0.2–0.4 ppm (equivalent to roughly 1.5–3 ‰) for ^{2}H, so that IRMS holds a five- to ten-fold advantage in bulk precision while NMR holds the entire advantage in positional resolution.

3.3 What IRMS delivers and what it cannot

IRMS delivers, on samples of a fraction of a milligram, the bulk ratio of any of five elements with the best precision available; it delivers compound-specific ratios on complex mixtures at concentrations that NMR cannot approach; and its results are directly comparable with the large reference populations that have been accumulated over three decades for wine, juice, honey, olive oil and other commodities.[15,27] It cannot, without chemical degradation of the analyte into fragments, distinguish the isotopic composition of one atomic position from another, and the degradation route, where one exists, must itself be free of fractionation and must be repeated for every position of interest.[16] Two developments have begun to close this gap from the mass-spectrometric side: the reaction of the methoxy group of vanillin or lignin to methyl iodide (the Zeisel reaction) followed by GC-IRMS gives a single-position ratio,[28] and high-resolution Orbitrap mass spectrometry of molecular fragment ions has been shown to yield position-resolved isotope ratios at natural abundance for small molecules such as serine and ethanol.[29] Both remain research techniques, and neither has yet displaced NMR as the reference method for intramolecular isotope distribution.

4. Isotopic NMR

4.1 ^{2}H SNIF-NMR: principle

Deuterium is a spin-1 nucleus present at approximately 0.0156 % natural abundance, resonating at 15.35 % of the proton frequency (46.05 MHz at 7.05 T, 61.4 MHz at 9.4 T). Its chemical-shift scale coincides with that of ^{1}H, so that the ^{2}H spectrum of a molecule shows one line for each chemically distinct hydrogen site, each line arising from the monodeuterated isotopomer in which the single deuteron occupies that site. Because the probability that two deuterons occupy the same molecule at natural abundance is negligible, the integrated intensity of each line is proportional to the number of molecules bearing a deuteron at that site, and the ratio of that intensity to the intensity of a co-dissolved internal standard of known D/H ratio and known mass yields the absolute site-specific D/H ratio. Quadrupolar relaxation is efficient, so that the deuteron T_1 values are short (tens to hundreds of milliseconds) and rapid pulsing is permitted without saturation; the same relaxation mechanism suppresses any significant nuclear Overhauser enhancement, so that ^{1}H decoupling can be applied to collapse the ^{1}H–^{2}H couplings without perturbing the intensities. The principle was established by Martin and Martin in 1981 and applied to the origin of natural alcohols and to anethole in 1982;[19,20,30] the acronym SNIF-NMR (site-specific natural isotope fractionation studied by NMR) and the associated patents date from the same period, and the phenomenon exploited is described in Section 2.5.

Three practical constraints follow from the low abundance and the small magnetic moment. The receptivity of ^{2}H at natural abundance is about 10^{-6} that of ^{1}H, so that a neat or highly concentrated sample is required, in practice several milliliters of the pure analyte in a 10 mm tube; the linewidths are broad enough (several hertz) that closely spaced sites overlap and must be resolved by lineshape fitting rather than by simple integration; and the field-frequency lock, which in a conventional spectrometer uses the deuterium of the solvent, must be transferred to another nucleus, conventionally ^{19}F using hexafluorobenzene added to the sample, because the deuterium channel is occupied by the observation. Precision at the level of 0.2–0.3 ppm on a ratio near 100 ppm requires long acquisitions and averaging of replicate spectra, and the repeatability and reproducibility achievable were characterized early by Guillou, Trierweiler and Martin.[31]

4.2 The ethanol probe and the fermentation transfer model

Ethanol is the analyte on which SNIF-NMR was built and remains the one on which it is most widely applied, because it can be recovered quantitatively from wine, spirits, fermented juice, fermented honey or fermented syrup by distillation, because it is a liquid that fills a 10 mm tube without solvent, and because its two carbon-bound sites report on two different things. The ^{2}H spectrum (Figure 2) contains a line for the monodeuterated methyl group CH_2D–CH_2OH (site I), a line for the monodeuterated methylene group CH_3–CHD–OH (site II) and a line for the hydroxyl deuteron, which exchanges with water and is not used, together with the line of the internal standard N,N,N′,N′-tetramethylurea (TMU). The site-specific ratios $(D/H)^{I}$ and $(D/H)^{II}$ are obtained from the fitted intensities, the certified D/H of the TMU, the weighed masses of ethanol and TMU and the numbers of equivalent hydrogens at each site (three, two and twelve respectively); the relative distribution parameter $R = 2(D/H)^{II}/(D/H)^{I}$ is obtained directly from the intensity ratio without reference to the standard.

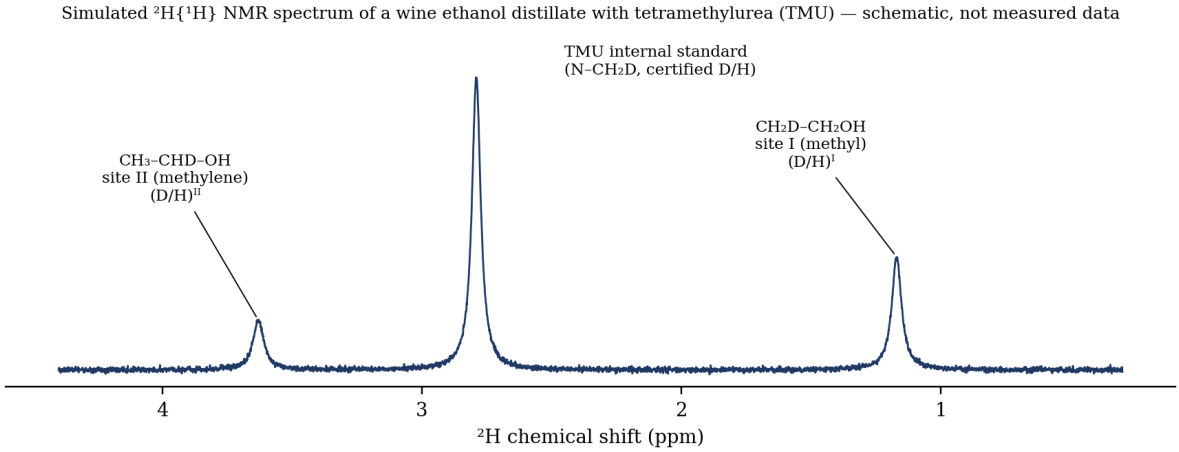

Figure 2. Simulated $^{2}H\{^{1}H\}$ NMR spectrum of a wine-ethanol distillate with TMU internal standard, drawn to illustrate the site assignments used in OIV-MA-AS311-05. Line positions follow the ^{1}H shifts of ethanol and TMU; intensities are illustrative only.

The interpretation of the two ratios rests on the deuterium-transfer study of Martin, Zhang, Naulet and Martin, in which the fermentation of glucose to ethanol was followed at natural abundance and the origin of each ethanol hydrogen was established.[21] The methyl hydrogens of ethanol derive predominantly from the C-6 and C-1 hydrogens of the hexose, with one hydrogen introduced from the medium at the enolization step, so that $(D/H)^{I}$ is governed by the sugar and therefore by the plant species, its photosynthetic pathway and, to a lesser extent, the climate under which the sugar was formed. The methylene hydrogens derive from the medium, one by protonation of the thiamine-bound intermediate at decarboxylation and one through the NADH pool, so that $(D/H)^{II}$ is governed by the fermentation water and therefore by the geographic origin of the must and by any water added to it.[32,33] The division is not perfect — a fraction of the methyl deuterium is medium-derived, and $(D/H)^{II}$ retains a sugar contribution — but it is sufficiently clean that $(D/H)^{I}$ has served for thirty-five years as the parameter for sugar origin and $(D/H)^{II}$, alone or as R, as a parameter for water origin.

Typical methyl-site values illustrate the discriminating power. Ethanol fermented from sugar beet, a C_3 crop of temperate high-latitude regions, gives $(D/H)^{I}$ near 91–93 ppm; grape ethanol from European Union wines gives approximately 98–105 ppm; ethanol from C_4 sources such as sugarcane and maize gives approximately 104–111 ppm.[27,34] The order arises because the deuterium content of C_3 carbohydrate is depleted by both photosynthetic fractionation and the depleted meteoric water of temperate regions, whereas C_4 metabolism and warmer climates give enriched sugars. The values overlap at the boundaries, are vintage- and region-dependent, and must be compared with a reference population of authentic material from the same product, region and year; in the European Union that population is the wine isotopic databank (Section 5). A sample whose $(D/H)^{I}$ falls significantly below the authentic range for its declared origin has received C_3 sugar of low deuterium content, in practice beet sugar, and the collaborative study that led to AOAC Method 995.17 established a linear relationship between the percentage of added beet sugar and the depression of $(D/H)^{I}$, with repeatability standard deviations of 0.19–0.25 ppm and reproducibility standard deviations of 0.21–0.37 ppm across laboratories.[35] The same study emphasized the complementarity of the two techniques: C_4 sugars are detected by $\delta^{13}C$ (AOAC 981.09 and 982.21) and C_3 sugars by SNIF-NMR, so that the two together cover the field of exogenous sugar sources, and their joint interpretation was set out in detail by Martin, Hanote, Lees and Martin.[36]

4.3 Measurement protocol and performance

The protocol codified in OIV-MA-AS311-05 (a Type II method, adopted originally in Commission Regulation (EEC) No 2676/90 and revised as Resolution Oeno 426-2011) is representative of all SNIF-NMR ethanol determinations and is summarized here from the published method text. Musts and concentrates are first fermented to completion, with a dilution water of known isotopic composition where the sugar concentration would otherwise inhibit the yeast. The ethanol is recovered by distillation on a Cadiot-type spinning-band column with a yield exceeding 96 %, because incomplete recovery fractionates the isotopomers, and the alcoholic strength of the distillate is determined accurately. A weighed portion of 3.2 mL of distillate is combined with a weighed 1.3 mL of certified TMU (the JRC certified reference material ERM-AE003 and its predecessors) and with hexafluorobenzene as lock substance (about 150 µL at 7.05 T or 35 µL at 9.4 T), in a 10 mm tube. The spectrometer must be fitted with a ^{2}H probe with a ^{1}H decoupling channel and a ^{19}F lock; the OIV text explicitly lists both 7.05 T (46.05 MHz) and 9.4 T (61.4 MHz) as acceptable fields. Replicate proton-decoupled spectra are acquired under conditions that are checked against a sealed certified tube (formerly CRM-123 from IRMM), the lines are fitted with Lorentzian functions, and the ratios are computed from the fitted intensities and the weighed masses. Care against evaporative loss of ethanol or TMU between weighing and measurement is stressed because such loss fractionates the sample. A complete determination, including fermentation where required, occupies one to two days per sample and several hours of spectrometer time; this throughput is one of the principal points of comparison with EA-IRMS, where the instrumental analysis of a prepared sample takes minutes.

The method has been extended without change of principle to vinegar, where the analyte is acetic acid, its methyl site is measured and the corresponding official methods are EN 16466-1 (^{2}H NMR of acetic acid), EN 16466-2 (^{13}C of acetic acid by IRMS) and EN 16466-3 (^{18}O of vinegar water), following the coupled NMR–MS approach introduced by Remaud, Guillou, Vallet and Martin and the international collaborative study of Thomas and Jamin.[37,38] It has likewise been extended to maple syrup, where the collaborative study of Martin and co-workers established detection of added beet or cane sugar and became AOAC Method 2000.19.[39]

4.4 Other SNIF-NMR probe molecules

Any molecule that can be isolated in sufficient quantity and purity, that has at least two chemically distinct carbon-bound hydrogen sites and that has an isotopic history worth reading is a candidate for ^{2}H SNIF-NMR. The flavor and fragrance field supplied the earliest and most commercially important examples because the price differential between natural and synthetic material is large and because bulk δ^{13}C can be defeated by a synthetic route from a natural-looking precursor. Vanillin is the paradigm. Natural vanillin from Vanilla planifolia, a CAM plant, has a bulk δ^{13}C near −21 to −19 ‰, whereas guaiacol-derived synthetic vanillin lies near −32 to −27 ‰ and lignin- or eugenol-derived material lies between; a fraudster who blends or who uses a precursor with a suitable bulk value can bring the mean into the natural range, and a fraudster who adds deuterium-depleted or ^{13}C-enriched material can adjust the mean deliberately. Remaud and co-workers showed that the ^{2}H pattern over the aromatic, methoxy and aldehyde sites of vanillin, and of the co-occurring p-hydroxybenzaldehyde, distinguishes the biosynthetic origin from every synthetic route and detects such sophisticated adulterations;[40] the approach became AOAC Method 2006.05, and its combination with site-specific ^{13}C is described in Section 4.5. Benzaldehyde from bitter almond, from cinnamon and from toluene oxidation was distinguished on the same basis,[41] as were anethole from anise and fennel against synthetic anethole,[30] and linalool and linalyl acetate from lavender and other essential oils

against synthetic terpenoids.[42] Glycerol from grape fermentation, from vegetable oils and from petrochemical synthesis was characterized by both ^{2}H and ^{13}C site-specific NMR,[23] which is of continuing relevance because glycerol addition to wine is a recurrent fraud. Long-chain fatty acids from plant seed oils carry a strongly non-statistical deuterium distribution along the chain that records the desaturase and elongase steps of their biosynthesis;[22] the resolution of neighboring methylene sites, which is poor in isotropic solution, has been improved by recording the ^{2}H spectra in chiral liquid-crystalline solvents, where the residual quadrupolar splittings disperse the sites. Tree-ring cellulose, converted to a glucose derivative, has been read site by site to separate climatic from biochemical hydrogen fractionation and to detect metabolic shifts driven by rising atmospheric CO_2 over the twentieth century,[24,43] a result that, while ecological rather than forensic, demonstrates that the ^{2}H isotopomer pattern of a plant carbohydrate records photorespiratory and environmental conditions with a fidelity that no bulk measurement can.

A mass-spectrometric alternative for the specific problem of exogenous sugar in juices has been proposed: compound-specific $\delta^{2}H$ of glucose and fructose by LC separation, derivatization and pyrolysis IRMS, applied to pineapple juice, gave discrimination of beet and cane sugars comparable with SNIF-NMR on smaller samples.[44] The approach is promising but has not been validated collaboratively, requires a derivatization whose hydrogen contribution must be corrected, and reports a compound average rather than a site-specific ratio; it is noted here as evidence that the bulk-versus-site distinction is not absolute where compound-specific IRMS can isolate the informative molecule.

4.5 Isotopic ^{13}C NMR (position-specific isotope analysis)

Carbon-13 is present at approximately 1.1 % abundance, is spin-½ and, unlike deuterium, relaxes slowly and shows a strong nuclear Overhauser enhancement under ^{1}H decoupling. A quantitative ^{13}C spectrum in which the intensity of each line is proportional to the number of ^{13}C nuclei at that site — rather than to the number of carbons at that site, which is what an ordinary quantitative ^{13}C spectrum reports — is the same experiment viewed from a different perspective: the measured molar fraction f_i of the ^{13}C at site i is compared with the statistical fraction F_i expected if ^{13}C were distributed uniformly (1/n for an n-carbon molecule), and the ratio f_i/F_i, multiplied by the bulk $^{13}C/^{12}C$ ratio, gives the site-specific ratio and hence $\delta^{13}C_i$. The difficulty is that the natural deviations from statistical distribution are of the order of a few per mil, so that the intensities must be measured with a precision of about 1 ‰, some two orders of magnitude better than conventional quantitative ^{13}C NMR achieves. Tenailleau, Lancelin, Robins and Akoka first demonstrated such precision on vanillin and used it to distinguish natural and synthetic origins,[45] and Caytan and co-workers established the conditions — inverse-gated decoupling to suppress the NOE, adiabatic pulses to remove off-resonance intensity errors across the wide ^{13}C range, relaxation delays of the order of ten times the longest T_1, careful control of decoupler heating, high signal-to-noise ratio and Lorentzian lineshape fitting — under which repeatability of 1 ‰ or better is maintained over months.[46] The subsequent development of the method, its pulse sequences and its applications have been reviewed by Jézéquel and co-workers.[47]

Two features of the method bear directly on its relationship with IRMS. First, in its original form the method requires the bulk $\delta^{13}C$ of the same sample from IRMS in order to convert the NMR molar fractions into absolute site-specific ratios, so that the two techniques are complementary rather than competing; Bayle and co-workers removed this requirement by internal referencing against a co-dissolved standard of known $\delta^{13}C$ (dimethyl sulfone), at the cost of managing radiation damping in the accompanying ^{1}H measurement.[48] Second, the method is intrinsically comparable across laboratories only if the same quantitative conditions

are met, and a pilot inter-laboratory study on vanillin across spectrometers of different field and manufacturer demonstrated agreement at the 1 ‰ level and defined the requirements.[49] The metrological foundation was completed by the National Research Council of Canada, which certified two vanillin reference materials (VANA-1 and VANB-1) for their site-specific $\delta^{13}C$ values by NMR — believed to be the first intramolecular isotopic certified reference materials — with expanded uncertainties of about 1.4 ‰,[50] and by a subsequent comparison in which the methoxy-carbon value obtained by NMR agreed with that obtained by Zeisel cleavage and GC-IRMS.[28] Site-specific ^{13}C NMR is therefore now a traceable measurement in the sense demanded by ISO/IEC 17025, which was not true of it a decade ago.

The applications relevant to plant origin are of three kinds. In ethanol, the ^{13}C ratios of the methyl and methylene carbons inherit the pattern of hexose positions C-1/C-6 and C-2/C-5, and Thomas and co-workers showed that the difference between the two sites discriminates ethanol from beet, cane, maize and agave in a way that neither the bulk $\delta^{13}C$ nor the ^{2}H pattern achieves: cane and maize, both C_4, have the same bulk value but different intramolecular patterns, and agave, a CAM plant whose bulk value overlaps both, is separated as well.[51] Gilbert and co-workers traced the pattern to the CO_2-fixation pathway and to environmental conditions and demonstrated its relationship to the intramolecular distribution in glucose, which they had earlier measured directly at natural abundance through a glucose derivative that suppresses anomerization.[18,52] The methodology was optimized and validated specifically for agave products, where the substitution of cane or maize ethanol for agave ethanol in tequila and mezcal is the fraud of interest and where the bulk parameters had been indecisive,[53] and the state of the art for ethanol-containing beverages has been reviewed by Gilbert, Thomas and Akoka.[54] In vanillin, the eight-carbon ^{13}C profile combined with the ^{2}H profile identifies the precursor — bean, guaiacol, lignin, eugenol, ferulic acid, curcumin or glucose ex-C_4 sugars fermented to vanillin — and detects blends, and a polarization-transfer (INEPT-based) acquisition reduced the sample requirement from about 250 mg to about 50 mg, opening the analysis to vanillin recovered from finished products such as vanilla sugar.[55,56] In xanthines, the site-specific ^{13}C composition of caffeine and related molecules is accessible after chemical modification to resolve the carbonyl and methyl sites, and distinguishes natural from synthetic caffeine.[57] In lipids, the challenge that long-chain fatty acid methyl esters present only about six resolved ^{13}C signals for a chain of sixteen or more carbons has been addressed by intramolecular isotopic referencing, which yields position-specific values for the resolvable sites and has been applied to plant oils.[58]

4.6 Non-isotopic NMR: metabolite profiling for botanical and geographic classification

Distinct from either isotopic method, high-field ^{1}H NMR of a juice, wine, honey, oil or extract, recorded under standardized conditions and analyzed by multivariate statistics, classifies samples on the basis of their composition — sugars, organic acids, amino acids, phenolics, lipid classes and minor components — rather than on the basis of isotope ratios. The approach has been reviewed comprehensively by Mannina, Sobolev and Viel.[59] It requires no distillation or purification, a few hundred microliters of sample, and ten to twenty minutes of instrument time, and it delivers simultaneously a targeted quantification of thirty to sixty components and an untargeted fingerprint. Against the isotopic methods it has three characteristics that determine its place in origin work. It is far more sensitive to species, variety and cultivar than isotopes are, because these are compositional attributes; it is sensitive to geographic origin only insofar as region determines composition, which for wine, olive oil and honey it does to a useful but variable degree; and it is a purely statistical classifier whose validity is bounded by the reference set on which it was trained. Its role

in the present context is therefore as the technique of choice for the species and cultivar question, as a rapid screen that precedes and targets the slower isotopic determinations, and as a source of orthogonal variables for data fusion (Section 8). The 60 MHz benchtop instruments now common in industrial laboratories reproduce the major-component part of this analysis but not the minor-component fingerprint, and they cannot perform either isotopic method at natural abundance.

5. Official Methods and Regulatory Infrastructure

The isotopic authentication of plant products is unusual among food-fraud methods in the depth of its codification. Table 2 lists the principal official methods. The European wine sector provides the most developed example: Commission Regulation (EEC) No 2676/90 adopted SNIF-NMR of wine ethanol as a Community reference method in 1990, the European Union wine isotopic databank was established at the Joint Research Centre in 1991, and the databank and the associated control regime are now governed by Article 39 of Commission Delegated Regulation (EU) 2018/273 and Articles 27–29 of Commission Implementing Regulation (EU) 2018/274, as amended by Implementing Regulation (EU) 2021/1007, with the OIV methods incorporated by reference through Regulation (EU) No 1308/2013.[27] Each year the wine-producing Member States take a prescribed number of fresh-grape samples from vineyards of documented location, variety and practice, vinify them under controlled conditions in designated laboratories, and submit the $(D/H)^{I}$, $(D/H)^{II}$, R, $\delta^{13}C$ of ethanol and $\delta^{18}O$ of water to the databank, which is managed by the European Reference Centre for Control in the Wine Sector at the JRC and is not public. Twenty-five years of that program were reviewed by Christoph, Hermann and Wachter, who describe both its success against sugaring and watering and the extension of the concept to a third-country databank for imported wines.[27] No comparable public infrastructure exists for site-specific ^{13}C NMR, although the vanillin reference materials of the National Research Council of Canada now provide the metrological anchor.[50]

Table 2. Official and standard methods for isotopic determination of plant origin. Method identifiers are as published by the issuing bodies; descriptions are paraphrased.

Method	Analyte and measurement	Technique	Principal purpose
OIV-MA-AS311-05 (Oeno 426-2011); formerly Reg. (EEC) 2676/90	Deuterium distribution in ethanol from grape musts, concentrated musts, rectified concentrated musts and wines: $(D/H)^{I}$, $(D/H)^{II}$, R	^{2}H SNIF-NMR, 7.05 T or 9.4 T, TMU internal standard (ERM-AE003), ^{19}F lock	Detection of exogenous (beet, cane) sugar; water addition; databank parameter
OIV-MA-AS312-06	$\delta^{13}C$ of wine ethanol	IRMS	Detection of C_4 sugar; databank parameter
OIV-MA-AS2-12	$\delta^{18}O$ of wine water	IRMS (CO_2 equilibration)	Water addition; geographic and vintage characterization; databank parameter
EN 16466-1 / -2 / -3; OIV-OENO 510/511-2013	Vinegar: $(D/H)_{CH_3}$ of acetic acid; $\delta^{13}C$ of acetic acid; $\delta^{18}O$ of vinegar water	^{2}H NMR; IRMS; IRMS	Synthetic acetic acid, non-wine alcohol and water addition in wine vinegar
AOAC 995.17	Beet sugar in fruit juices: $(D/H)^{I}$ of ethanol after fermentation and distillation	^{2}H SNIF-NMR	C_3 sugar addition to fruit juices

Method	Analyte and measurement	Technique	Principal purpose
AOAC 2000.19	Beet or cane sugar in maple syrup: $(D/H)^I$ of ethanol	^{2}H SNIF-NMR	Exogenous sugar in maple syrup
AOAC 2006.05	Site-specific D/H ratios in vanillin	^{2}H SNIF-NMR	Natural versus synthetic or biosynthetic vanillin
AOAC 981.09, 982.21	δ^{13}C of apple juice and orange juice (bulk)	EA-IRMS	C_4 (corn, cane) sugar addition
AOAC 2004.01	δ^{13}C of ethanol derived from fruit juices and maple syrup	IRMS on distilled ethanol	C_4 sugar in C_3 juices; C_3 sugar in pineapple (CAM)
AOAC 998.12	δ^{13}C of honey and of its protein fraction (internal standard)	EA-IRMS	C_4 plant sugars in honey; ≥ 7 % reported as adulterated
CEN ENV 12140, 12141, 12142, 13070	Fruit juices: δ^{13}C of sugars; δ^{18}O of water; δ^2H of water; δ^{13}C of pulp	EA-IRMS, equilibration IRMS	Sugar and water addition; concentrate reconstitution; pulp authenticity
Reg. (EU) 2018/273 Art. 39; Reg. (EU) 2018/274 Arts 27–29; Reg. (EU) 2021/1007	EU analytical databank of isotopic data for wine products	SNIF-NMR + IRMS (OIV methods)	Reference population for official wine control; annual authentic samples per Member State

6. Plant Identity: Worked Examples

"Plant identity" is used here in three senses that call for different measurements: the photosynthetic pathway or botanical family of the sugar, oil or flavor precursor from which a product was made; the natural or synthetic origin of a molecule that can be produced both ways; and the species, variety or cultivar of the plant. Isotopes answer the first two questions well and the third poorly; metabolite profiling answers the third well and the first two poorly. The examples below are grouped by product class and are summarized in Table 3.

6.1 Sugar origin in juices, wines, honey and syrups

The detection of exogenous sugar is the oldest and most fully worked problem in the field, and it illustrates the division of labor between the techniques with unusual clarity. When the exogenous sugar is from a C_4 plant (corn syrup, high-fructose corn syrup, cane sugar or cane invert) and the product is from a C_3 plant (most fruit juices, maple sap, honey from the nectar of C_3 flora), bulk δ^{13}C by EA-IRMS is sufficient, cheap and rapid. Doner and White established in 1977 that the δ^{13}C of honey is uniform enough across floral sources for a fixed threshold to be useful,[60] and White and Winters converted the test into a within-sample comparison by using the honey protein, which is of bee and plant origin and cannot be adulterated, as an internal isotopic standard against which the sugar fraction is judged;[61] the resulting AOAC Method 998.12 reports a sample as containing significant C_4 sugar at or above 7 %. Bricout and Koziet applied bulk δ^{13}C to orange juice in 1987,[62] and the method for δ^{13}C of juice-derived ethanol became AOAC 2004.01. Elflein and Raezke extended the honey test with LC-IRMS, so that the δ^{13}C of glucose, fructose, sucrose and oligosaccharides could each be compared with the protein, lowering the practical detection limit for C_4 syrups and, importantly, revealing C_3 syrups (from beet, wheat or rice) through internal inconsistencies among the sugars that the bulk value could not show.[26]

When the exogenous sugar is from a C_3 plant — beet sucrose into grape must, wheat or rice glucose syrup into apple juice, beet invert into honey — bulk $\delta^{13}C$ is blind, because the added sugar carries the same pathway signature as the authentic one. This is the problem for which SNIF-NMR was adopted as an official method. Martin, Danho and Vallet mapped the $(D/H)^{I}$ and $(D/H)^{II}$ of ethanol fermented from sugars of a wide range of botanical origins and showed that beet sugar, with its strongly depleted methyl site, separates from fruit sugars;[34] the collaborative study on fruit juices established the AOAC method,[35] the maple-syrup collaborative study established AOAC 2000.19,[39] and the chaptalization of wine has been controlled by the OIV method and the EU databank since 1990.[27] A combined interpretation, in which the $\delta^{13}C$ of the ethanol, the $(D/H)^{I}$ and, where the added sugar has altered the water, the $(D/H)^{II}$ and $\delta^{18}O$ are read together, extends the coverage to mixed adulterants and to the reverse case of C_3 sugar added to a CAM product such as pineapple juice.[36] Where a wine has been legitimately enriched with concentrated or rectified concentrated grape must, the isotopic parameters remain within the grape range, which is the reason the method distinguishes permitted from prohibited enrichment. A subtlety that has attracted attention is that the ethanol isotope ratios depend on whether fermentation was allowed to go to completion; Perini and co-workers showed that stopping fermentation early, as is done for some sweet wines, shifts $\delta^{13}C$, $\delta^{18}O$ and the (D/H) ratios of the ethanol relative to complete fermentation of the same must, and that the shift must be allowed for in the reference population.[33]

Honey occupies a special position because its sugars cannot be fermented and distilled without difficulty and because its floral origin is itself a declaration. ^{1}H NMR profiling of the whole honey, or of a chloroform extract that concentrates the minor aromatic and terpenoid markers, classifies botanical origin (acacia, chestnut, eucalyptus, linden, polyfloral and so on) with good success and, in the large-scale screening model developed by Eurofins and collaborators, flags adulteration with sugar syrups through both the sugar profile and foreign marker compounds.[63–65] The isotopic and the NMR-profiling tests are complementary: the isotopic test is definitive for C_4 syrups and, with LC-IRMS, indicative for C_3 syrups, whereas the NMR profile is the only routine test for floral origin and detects syrups by composition rather than by isotopes, so that a syrup whose sugar composition and isotope ratios have both been engineered to match the honey defeats each individually but rarely both.

6.2 Agave, cane and maize ethanol: the C_4/CAM problem

The substitution of cane or maize ethanol for agave ethanol in tequila and mezcal, and the addition of C_4 sugar to agave musts beyond the permitted proportion, present a case in which both classical techniques fall short. Bulk $\delta^{13}C$ cannot separate cane from maize (both C_4, near −12 ‰) and separates agave (CAM, typically −13 to −11 ‰) from either only marginally; the ^{2}H methyl-site ratio separates the three groups partially but with overlap dependent on climate. Thomas and co-workers showed that the intramolecular ^{13}C pattern of ethanol — the difference between the methyl and methylene site values — separates beet, cane, maize and agave into distinct clusters, because the pattern records the enzymatic route of CO_2 fixation and the subsequent carbohydrate metabolism, which differ between C_4 and CAM plants and, more subtly, between the NADP-malic-enzyme and NAD-malic-enzyme subtypes of C_4 metabolism to which maize and sugarcane respectively belong.[18,51] Portaluri and co-workers subsequently optimized and validated the quantitative ^{13}C NMR methodology specifically for agave products, defining the acquisition conditions, precision and decision limits needed for authentication,[53] and the beverage applications of the method were reviewed by Gilbert, Thomas and Akoka.[54] This is the clearest current example of a plant-identity question

that IRMS in its bulk form cannot answer and site-specific NMR can; it is also a case in which the ^{2}H and ^{13}C site-specific data are obtained on the same distilled ethanol and combined.

6.3 Flavor and fragrance molecules

The natural-versus-synthetic question for a single molecule is where the intramolecular approach has its longest record. The vanillin case has been outlined in Sections 4.4 and 4.5: ^{2}H site-specific ratios distinguish bean vanillin from guaiacol, lignin and eugenol routes and detect blends,[40] ^{13}C site-specific ratios add the discrimination of the more recent biotechnological routes from ferulic acid, curcumin and glucose ex-C_4 sugars whose bulk $\delta^{13}C$ values can fall in or near the natural range,[45,55] the sample requirement has been reduced to the level at which vanillin can be recovered from finished products,[56] and certified reference materials exist.[50] The general strategy that emerged from the flavor field — to select the molecule that carries the price premium, isolate it, and read both its ^{2}H and ^{13}C site-specific patterns — is the model for any new authentication problem. Benzaldehyde (bitter almond oil, cinnamon oil, synthetic),[41] anethole (anise, star anise, fennel, synthetic)[30] and linalool with linalyl acetate (lavender, bergamot, coriander, synthetic)[42] were resolved by ^{2}H SNIF-NMR, in each case with bulk $\delta^{13}C$ by IRMS as a supporting parameter that catches the petrochemical synthetics outright and leaves the natural-versus-natural and natural-versus-biosynthetic questions to the NMR pattern. Caffeine, whose synthetic and natural forms are isotopically similar in bulk, was resolved by site-specific ^{13}C NMR after chemical modification of the xanthine ring.[57]

6.4 Vinegar acetic acid

Wine vinegar, cider vinegar and spirit vinegar differ in the botanical origin of the ethanol that was oxidized to acetic acid, and vinegar made from synthetic acetic acid or from ethanol of non-declared origin is a persistent fraud. The methyl site of acetic acid carries the (D/H) signature of the methyl site of the ethanol from which it was formed and therefore of the original sugar, and Remaud and co-workers coupled its ^{2}H NMR measurement with $\delta^{13}C$ by IRMS to authenticate natural vinegars;[37] the international collaborative study that led to the CEN methods validated ^{2}H NMR of acetic acid, ^{13}C-IRMS of acetic acid and ^{18}O-IRMS of the vinegar water together.[38] The case illustrates a general design: a single molecule is measured by NMR for the parameter that requires positional information and by IRMS for the parameters (bulk $\delta^{13}C$, $\delta^{18}O$ of the water) that do not.

6.5 Glycerol, fatty acids and oils

Glycerol added to wine to improve mouthfeel may be of fermentation, vegetable-oil or petrochemical origin, and the three were separated by the combination of ^{2}H site-specific ratios (glycerol has two distinct C–H sites) and ^{13}C site-specific ratios.[23] Vegetable oils present a different problem. The fatty acids of an oil are, for most species, so similar in composition and in bulk isotope ratio that neither bulk $\delta^{13}C$ nor bulk $\delta^{2}H$ distinguishes one seed oil from another, although both distinguish plant from animal fat and C_3 from C_4 oils (maize oil from sunflower oil, for example). The non-statistical deuterium distribution along the acyl chain, first documented by Billault and co-workers, and the corresponding ^{13}C distribution, recently made accessible for the resolvable sites by intramolecular referencing, record the desaturation and elongation steps of lipid biosynthesis and differ among plant families,[22,58] but neither has yet been reduced to a routine authentication protocol. For edible-oil origin, the practicable NMR route remains compositional ^{1}H and ^{13}C profiling (Section 6.6), and the practicable isotopic route remains bulk multi-element IRMS for geographic origin (Section 7.2).

6.6 Botanical species, variety and cultivar by NMR profiling

Where the question is which species, variety or cultivar produced a material, the stable isotope ratios are generally uninformative, because plants of the same photosynthetic pathway grown in the same place have the same isotopic input and differ only through second-order biochemical effects. Composition, by contrast, is under genetic control, and ^{1}H or ^{13}C NMR profiling with multivariate classification is the NMR technique of choice. Mannina and co-workers used high-field ^{1}H NMR of extra-virgin olive oils to characterize cultivar and region through the relative abundances of fatty acyl groups and of minor components such as squalene, sterols and terpenes;[66] Wei and co-workers classified green coffee beans by variety (arabica versus robusta) and by origin from ^{13}C NMR-based metabolomics;[67] Godelmann and co-workers, in the study that underlies the commercial wine-profiling platforms, classified 600 German wines by grape variety with high success and by region and vintage with lower but useful success from ^{1}H NMR;[68] and honey floral origin has been classified as described in Section 6.1.[64] For species identity in the strict sense — the plant taxon, as opposed to the trade variety — DNA-based methods are the reference, and Horacek and co-workers illustrated the point by combining stable isotopes for geographic origin with DNA markers for the sesame genotype in a single study.[69] The appropriate framing is that isotopes report where and how a plant grew, metabolites report what it was, and the two are best used together.

Table 3. Plant-identity questions and the technique that resolves them. "Blind" indicates that the technique returns no useful discrimination for that question in routine practice.

Question	Bulk IRMS ($\delta^{13}C$, $\delta^{2}H$, $\delta^{18}O$)	^{2}H SNIF-NMR (site-specific)	Site-specific ^{13}C NMR	^{1}H/^{13}C NMR profiling	Preferred route
C_4 sugar (corn, cane) in C_3 product (juice, honey, maple, wine)	Decisive; official methods AOAC 998.12, 2004.01, OIV-MA-AS312-06	Detects (enriched $(D/H)^{I}$) but slower and costlier	Detects; not needed	Detects only if composition is abnormal	EA-/LC-IRMS
C_3 sugar (beet, wheat, rice syrup) in C_3 product	Blind (same pathway); LC-IRMS gives indirect evidence in honey	Decisive; AOAC 995.17, 2000.19, OIV-MA-AS311-05	Decisive for beet (distinct pattern)	Detects only by composition	^{2}H SNIF-NMR
Cane vs maize vs agave ethanol (tequila, rum, spirits)	Blind between cane and maize; marginal for agave	Partial, climate-dependent overlap	Decisive	Not applicable to distilled ethanol	Site-specific ^{13}C NMR (with ^{2}H)
Natural vs petrochemical flavor (vanillin, benzaldehyde, anethole, linalool)	Decisive for petrochemical synthetics with depleted $\delta^{13}C$; defeated by blending	Decisive; AOAC 2006.05 for vanillin	Decisive; distinguishes biotechnological routes	Detects only impurity profile	Both NMR methods; IRMS as screen
Natural vs biosynthetic vanillin (ferulic acid, glucose, eugenol routes)	Ambiguous; bulk values overlap natural range	Decisive for most routes	Decisive; CRMs available	Blind	Site-specific ^{13}C + ^{2}H NMR
Synthetic vs natural acetic acid; non-wine ethanol in wine vinegar	Decisive for petrochemical; C_3 ethanol sources overlap	Decisive (methyl site); EN 16466-1	Applicable	Detects composition only	^{2}H NMR + ^{13}C/^{18}O IRMS

Question	Bulk IRMS ($\delta^{13}C$, $\delta^{2}H$, $\delta^{18}O$)	^{2}H SNIF-NMR (site-specific)	Site-specific ^{13}C NMR	$^{1}H/^{13}C$ NMR profiling	Preferred route
Glycerol origin in wine	Partial	Decisive with ^{13}C	Decisive with ^{2}H	Blind	Combined site-specific NMR
Species, variety, cultivar (olive, coffee, grape, honey flora)	Blind	Blind	Blind	Decisive within a reference set	$^{1}H/^{13}C$ NMR profiling; DNA for taxon
Seed-oil species substitution	Blind among C_3 oils; C_3 vs C_4 only	Research stage	Research stage	Decisive (fatty acyl and minor-component profile)	$^{1}H/^{13}C$ NMR profiling; GC-FID reference

7. Geographic Origin: Worked Examples

Geographic origin is, in isotopic terms, a question about the water, atmosphere, soil and management under which the plant grew, and its assignment depends on the existence of a reference population that maps isotope values onto regions. The examples below are summarized in Table 4. Two general observations should be made at the outset. The first is that IRMS has supplied nearly all of the geographic reference data that exist, because the parameters that vary most strongly with geography — $\delta^{18}O$ and $\delta^{2}H$ of water and of organic matter, $\delta^{15}N$ and $\delta^{34}S$ of tissue, $^{87}Sr/^{86}Sr$ of ash — are mass-spectrometric parameters, and because a databank is an exercise in throughput. The second is that the SNIF-NMR methylene-site ratio $(D/H)^{II}$ and the ^{1}H NMR compositional profile add real geographic discrimination but have been used, in the main, as supplements to the IRMS parameters rather than in their place.

7.1 Wine and the European Union databank

The European databank contains, for every wine-growing region of every producing Member State since 1991, the $(D/H)^{I}$, $(D/H)^{II}$, R, $\delta^{13}C$ and $\delta^{18}O$ of authentic wines vinified from documented grapes, and it is used to define, for each country, region and appellation, the ranges within which an authentic wine of a given vintage must lie.[27] Roßmann and co-workers analyzed the $\delta^{18}O$ of databank wine water from Italy, France and Germany and established the relationships with climate and with the corresponding must water that make the parameter interpretable;[10] Camin and co-workers modeled the dependence of $\delta^{2}H$, $\delta^{13}C$ and $\delta^{18}O$ of Italian wines on climatic and geographic variables across the databank and showed that the isotopic values follow latitude, altitude, temperature and precipitation in the manner predicted from first principles, with year-to-year variation large enough that vintage must be part of the comparison.[5] Before the databank had matured, Martin and co-workers demonstrated the characterization of Bordeaux sub-regions by combining isotopic parameters with trace elements,[70] and Ogrinc and co-workers used IRMS and SNIF-NMR together to assign Slovenian wines to their three wine-growing regions and to distinguish vintages, with the $(D/H)^{II}$ and $\delta^{18}O$ of water carrying most of the regional information.[71] The wine case establishes the pattern that recurs throughout the field: the sugar-derived parameters ($\delta^{13}C$, $(D/H)^{I}$) discriminate pathway and, weakly, climate; the water-derived parameters ($\delta^{18}O$, $(D/H)^{II}$) discriminate region and vintage; and assignment is valid only against authentic samples of the same year.

7.2 Olive oil

Olive oil carries no water and no fermentation ethanol, so that its isotopic origin markers are the $\delta^{13}C$, $\delta^{2}H$ and $\delta^{18}O$ of the oil itself, the last two inherited from leaf water through the biosynthesis of the acyl chains and the glycerol backbone. Angerosa and co-workers first applied $\delta^{13}C$ and $\delta^{18}O$ to the regional characterization of olive oils,[72] and the large European collaborative study reported by Camin and co-workers, covering several hundred oils from eight Mediterranean countries with $\delta^{13}C$, $\delta^{2}H$, $\delta^{18}O$ and trace-element data, demonstrated that latitude, distance from the sea and climate govern the values and that classification at the level of country and, for some countries, of region is achievable with multivariate models.[73] Alonso-Salces and co-workers combined ^{1}H NMR fingerprints with $\delta^{13}C$ and $\delta^{2}H$ for a large set of Mediterranean virgin olive oils and showed that the fused data classified origin better than either data type alone, which is the point taken up in Section 8.[74] Olive oil is a case in which IRMS is unambiguously the primary isotopic technique for geographic origin, because the informative parameters are bulk $\delta^{18}O$ and $\delta^{2}H$, and in which NMR contributes through composition rather than through isotopes.

7.3 Fruit juices

The $\delta^{18}O$ of juice water reports the growing region in the same way as wine water, and Jamin and co-workers used the simultaneous $\delta^{18}O$ of the water and of the sugar-derived ethanol to separate legitimate reconstitution from dilution.[11] For the assignment of orange juices to producing countries, Rummel and co-workers combined $\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ of the juice organic matter with $^{87}Sr/^{86}Sr$ and showed that the strontium ratio, which is independent of climate, supplied discrimination between regions that the light-isotope data alone did not.[75] ^{1}H NMR profiling of juices in the SGF-profiling model classifies fruit species and detects many adulterations from composition, and its chemometric treatment was explored by Cuny and co-workers,[76] but geographic assignment of juice by NMR composition alone is weak, and the isotopic parameters remain the reference.

7.4 Rice, coffee, cocoa and sesame

For dry commodities that are neither fermented nor pressed, EA-IRMS of the bulk tissue is the natural approach and NMR has no isotopic role. Kelly and co-workers separated premium Basmati rice from North American and European long-grain rice by combining $\delta^{13}C$, $\delta^{18}O$ and trace elements, with $\delta^{18}O$ of the grain and the boron and other element concentrations carrying the geographic signal;[77] Weckerle and co-workers assigned green coffee to continent and country from the multi-element (H, C, N, O) isotope ratios of the caffeine isolated from the beans;[78] Rodrigues and co-workers added $^{87}Sr/^{86}Sr$ to $\delta^{18}O$ for green coffee and showed that the two together separated growing regions of different geology and climate;[79] and Perini and co-workers characterized cocoa beans of different continental origin by $\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and $\delta^{34}S$.[80] Sesame oil from several countries was assigned to origin by stable isotopes while the genotype was determined by DNA markers.[69] In each of these commodities the NMR contribution is compositional: ^{13}C NMR metabolomics classified green coffee by variety and origin,[67] and high-resolution magic-angle-spinning ^{1}H NMR of whole cocoa beans, compared with conventional ^{1}H NMR of extracts, classified geographic origin from the metabolite profile.[81] The compositional and the isotopic classifications rest on different causes — genotype and post-harvest processing on one side, climate and geology on the other — and are properly regarded as independent lines of evidence.

7.5 Agronomic origin and geological tracers

Two origin questions that are not geographic in the narrow sense are answered by the same infrastructure. The organic-versus-conventional question rests principally on $\delta^{15}N$, which Bateman, Kelly and Woolfe showed to be higher on average in organically fertilized crops, with sufficient overlap that the parameter is indicative rather than decisive and is best used with $\delta^{34}S$, $\delta^{18}O$ and compositional data.[12] The geological question — whether a product came from a terrain of a given bedrock — rests on $^{87}Sr/^{86}Sr$, which Voerkelius and co-workers mapped for European mineral waters against a simplified geological map and proposed as a reference for food authentication.[13] The framework for using multi-element and multi-isotope data in geographic assignment, and the requirements that such data must meet to be admissible in legal proceedings, have been reviewed by Kelly, Heaton and Hoogewerff and by Camin and co-workers respectively.[14,15]

7.6 Forensic extension: plant-derived narcotics

The assignment of geographic origin to plant-derived controlled substances is methodologically identical to food-origin work and is included because it demonstrates the approach under adversarial evidentiary conditions. Ehleringer and co-workers assigned cocaine seizures to their South American growing regions from the $\delta^{13}C$ and $\delta^{15}N$ of the cocaine together with the ratios of two trace alkaloids, achieving high classification rates against a reference collection of authenticated leaf,[82] and the approach has since been applied to heroin, cannabis and other materials. The elements of the design — a reference population of authenticated origin, multiple independent variables, an explicit classification rule with a stated error rate, and a mass-spectrometric measurement of the informative isotopes — are those that any origin determination intended for use in litigation must possess.

7.7 NMR profiling for geographic classification

Compositional ^{1}H NMR classification of geographic origin has been demonstrated for wine, where Godelmann and co-workers achieved useful regional discrimination among German growing regions,[68] for honey, where Consonni and Cagliani distinguished polyfloral and acacia honeys of different Italian regions,[63] for olive oil,[66,74] for coffee[67] and for cocoa.[81] Two cautions apply. The compositional differences that separate regions are frequently confounded with variety, cultivation practice and processing, so that a classifier trained on one season may fail on the next; and the classification is probabilistic, its stated accuracy being a property of the training set rather than of the sample. Gougeon and co-workers made the comparison explicit for wine by testing quantitative ^{1}H NMR against the established multi-technique (isotopic and elemental) approach on the same samples and concluded that NMR was a valuable screening and complementary tool but did not replace the isotopic reference methods for origin.[83] That conclusion is endorsed here.

Table 4. Geographic-origin applications by commodity, the informative parameters and the technique that measures them.

Commodity	Informative parameters	IRMS role	NMR role	Representative studies
Wine	$\delta^{18}O$ of water; $(D/H)^{II}$ and R; $\delta^{13}C$ of ethanol; trace elements	$\delta^{18}O$, $\delta^{13}C$; databank parameters	$(D/H)^{II}$ (SNIF-NMR); ^{1}H profiling for region and vintage	Refs. [5,10,27,68,70,71,84]

Commodity	Informative parameters	IRMS role	NMR role	Representative studies
Olive oil	$\delta^{18}O$, $\delta^{2}H$, $\delta^{13}C$ of oil; trace elements; ^{1}H fingerprint	Primary	Compositional profiling; data fusion	Refs. [66,72–74]
Fruit juice	$\delta^{18}O$ of water and of sugar-ethanol; $\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$; $^{87}Sr/^{86}Sr$	Primary	Compositional screening (SGF-type profiling)	Refs. [11,75,76]
Rice	$\delta^{18}O$, $\delta^{13}C$ of grain; B, Mg, other elements	Primary	None (isotopic); limited compositional	Refs. [77]
Coffee	$\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ of caffeine or bean; $^{87}Sr/^{86}Sr$	Primary	^{13}C metabolomics for variety and origin	Refs. [67,78,79]
Cocoa	$\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{34}S$ of bean	Primary	HR-MAS ^{1}H profiling	Refs. [80,81]
Sesame oil	$\delta^{2}H$, $\delta^{13}C$, $\delta^{18}O$; DNA for genotype	Primary	None reported	Refs. [69]
Honey	$\delta^{13}C$ (C_4 syrup); ^{1}H profile for floral and regional origin	Adulteration (C_4) and LC-IRMS sugar consistency	Primary for floral origin; regional classification	Refs. [26,60,61,63,65]
Organic vs conventional	$\delta^{15}N$ (with $\delta^{34}S$, $\delta^{18}O$)	Primary	Compositional support	Refs. [12]
Geological provenance	$^{87}Sr/^{86}Sr$	MC-ICP-MS / TIMS	None	Refs. [13,75,79]
Plant-derived narcotics	$\delta^{13}C$, $\delta^{15}N$; trace alkaloid ratios	Primary	None reported for origin	Refs. [82]

8. Data Fusion and Chemometric Practice

Because the isotopic and compositional variables respond to different causes, their combination should, in principle, classify origin better than either set alone, and several studies have tested the proposition directly. Monakhova and co-workers fused ^{1}H NMR spectra of wine with the SNIF-NMR, $\delta^{18}O$ and $\delta^{13}C$ parameters of the same wines and reported a synergistic improvement in the classification of geographic origin and vintage relative to the separate data sets, using both concatenation and mid-level fusion strategies.[84] The same group showed, for tomato profiles obtained by NMR, infrared spectroscopy and IRMS, that a combination of partial least squares discriminant analysis with common-components-and-specific-weights analysis improved fused-data classification.[85] Alonso-Salces and co-workers obtained the analogous result for olive oil with ^{1}H NMR fingerprints fused with $\delta^{13}C$ and $\delta^{2}H$.[74] Ogrinc and co-workers, for Slovenian wine, and Martin and co-workers, for Bordeaux, had reached the same conclusion with isotopes and elements.[70,71]

The chemometric hazards are those of any high-dimensional classification on a small sample, and they are amplified in origin work by three features of the data. The number of authentic samples per region and vintage is small relative to the number of NMR variables, so that PLS-DA and related methods will separate any labeled groups in the training set and must be judged only on independent test samples or on rigorous cross-validation with permutation testing. The classes are confounded — region with variety, vintage with climate, origin with processing — so that a classifier may be learning the confounder. And the isotopic variables are few but physically interpretable, whereas the NMR variables are many but only statistically

interpretable, so that fusion strategies which scale each block appropriately and which preserve the interpretability of the isotopic block are to be preferred over naive concatenation. A fused model should be reported with the performance of each block alone, so that the reader can see what fusion added, and with the misclassified samples identified, so that the reader can judge whether the errors are physically sensible. A classifier that cannot be explained in terms of the underlying fractionation or composition should not be offered in evidence.

9. Head-to-Head Comparison and Decision Guide

The preceding sections permit the comparison to be drawn without hedging. The two techniques are not rivals for the same measurement; they measure different things, and the question determines the choice. Table 5 sets the operational characteristics side by side, and Table 6 reduces the choice to a decision guide that is illustrated as a workflow in Figure 3.

9.1 Where NMR holds the advantage

NMR is the technique of choice whenever the discriminating information is intramolecular. That condition is met in four recurring situations. The first is the addition of a C_3 material to a C_3 product — beet sugar to grape, apple or orange; wheat or rice syrup to honey; C_3 ethanol to wine vinegar — where the bulk carbon isotope ratio is identical by construction and the methyl-site deuterium ratio is not; this is the situation for which SNIF-NMR became an official method and it remains the one in which IRMS, other than through the indirect internal-consistency arguments of LC-IRMS, has no answer.[26,35] The second is the discrimination of materials that share a photosynthetic pathway and a similar climate — cane from maize, and both from agave — where the site-specific ^{13}C pattern of ethanol separates what neither bulk $\delta^{13}C$ nor bulk $\delta^{2}H$ can.[51,53] The third is the detection of an adulterant that has been engineered, by choice of precursor or by blending, to reproduce the bulk isotope ratio of the authentic material; the intramolecular pattern is not reproduced, and vanillin is the documented case.[40,55] The fourth is the resolution of natural from biosynthetic origin for a molecule made from a natural precursor by a biotechnological route, where bulk values fall within the natural range and only the pattern distinguishes the enzyme chemistry.[55,57]

Three further advantages are operational rather than informational. The ^{2}H and ^{13}C site-specific patterns are read on the same isolated molecule, so that a single distillate of ethanol or a single recrystallized vanillin supports both determinations and the bulk $\delta^{13}C$, giving a multi-parameter fingerprint from one preparation. The measurement is non-destructive and involves no combustion, pyrolysis or derivatization, so that the sample remains available for re-analysis or for adversarial re-testing, which is a material consideration in litigation. And the internal-referencing and certified-reference-material developments of the past decade have made the site-specific ^{13}C measurement traceable in its own right, no longer dependent on a supporting IRMS determination.[48,50]

9.2 Where IRMS is the better choice

IRMS is the technique of choice whenever the informative isotope is oxygen, nitrogen or sulfur, whenever the sample is small or the analyte dilute, whenever the highest bulk precision is needed, and whenever the assignment must be made against an existing reference population. The first condition is absolute: the $\delta^{18}O$ of wine, juice and vinegar water, the $\delta^{18}O$ and $\delta^{2}H$ of olive oil, the $\delta^{15}N$ that reports fertilizer regime and the $\delta^{34}S$ that reports soil sulfur are not measurable by NMR at natural abundance with the required precision,

and these are precisely the parameters that carry the strongest geographic and agronomic information.[10,12,73] The second condition is quantitative: EA-IRMS requires a fraction of a milligram of tissue, GC-C-IRMS resolves a flavor component at the microgram level in a complex matrix, whereas SNIF-NMR requires milliliters of neat analyte and site-specific ^{13}C NMR requires, even with polarization transfer, tens of milligrams of pure compound.[25,56] The third condition matters for climate-related geographic discrimination within a pathway, where the effects are of the order of a few per mil in $\delta^{13}C$ and the ten-fold precision advantage of IRMS (0.1 ‰ against about 1 ‰) is decisive. The fourth condition is institutional: the European wine databank, the honey, juice and olive-oil reference populations and the isoscapes are built from IRMS parameters, and an unknown can be placed only in the coordinate system in which the references were measured.[7,15,27] To these should be added throughput and cost. A prepared sample occupies an EA-IRMS for minutes and a SNIF-NMR spectrometer for hours; the capital cost of a 9.4 T spectrometer with a ^{2}H probe and ^{19}F lock exceeds that of an EA-IRMS system; and for the routine screening of C_4 sugar in honey or juice the isotopic test by IRMS costs a small fraction of the NMR determination and answers the question completely.

It must also be stated plainly that for the ordinary case of geographic origin — is this wine from the region it claims, is this oil from Italy or Tunisia, is this rice Basmati — IRMS with a suitable reference population is the established, legally tested route, and NMR contributes as a supplement. The one geographic parameter that SNIF-NMR contributes directly, $(D/H)^{II}$, reports the same fermentation water that $\delta^{18}O$ reports and is generally the less sensitive of the two.[5,71]

Table 5. Operational comparison of IRMS and isotopic NMR for plant-origin determination.

Characteristic	IRMS (EA, TC/EA, GC-C, LC)	^{2}H SNIF-NMR	Site-specific ^{13}C NMR	$^{1}H/^{13}C$ NMR profiling
Isotopes accessible	^{13}C, ^{2}H, ^{18}O, ^{15}N, ^{34}S (plus Sr by ICP-MS)	^{2}H only	^{13}C only (^{15}N in special cases)	None (compositional)
Information level	Bulk or compound average	Site-specific	Site-specific	Molecular composition
Precision (typical)	0.1–0.2 ‰ (C, N); 0.2–0.4 ‰ (O); 1–3 ‰ (H)	0.2–0.4 ppm on ≈ 100 ppm (≈ 2–4 ‰)	≈ 1 ‰ per site	Not applicable; 1–5 % on concentrations
Sample requirement	0.1–2 mg bulk; µg–ng compound-specific	≈ 3 mL neat ethanol (from ≈ 100–300 mL wine); grams of pure flavor molecule	≈ 50–250 mg pure compound	≈ 0.5 mL liquid or extract
Sample preparation	Weighing (bulk); extraction, derivatization (compound-specific)	Fermentation, quantitative distillation, weighing with TMU	Isolation, purification, weighing with internal reference	Dilution, buffering
Instrument time per sample	Minutes	Hours (replicate spectra)	Hours to a day (long T_1 delays)	10–20 min
Destructive	Yes (combustion/pyrolysis)	No	No	No
Traceability	IAEA/USGS CRMs; two-point normalization	ERM-AE003 TMU; sealed check tubes	VANA-1/VANB-1 vanillin CRMs; internal referencing	Internal standard; ERETIC

Characteristic	IRMS (EA, TC/EA, GC-C, LC)	^{2}H SNIF-NMR	Site-specific ^{13}C NMR	^{1}H/^{13}C NMR profiling
Official methods	AOAC 981.09, 982.21, 998.12, 2004.01; OIV-MA-AS312-06, AS2-12; CEN ENV 12140–12142, 13070; EN 16466-2/-3	OIV-MA-AS311-05; AOAC 995.17, 2000.19, 2006.05; EN 16466-1	None yet (published validated protocols)	None (commercial platforms; ISO 17025 in-house)
Reference populations	Extensive (EU wine databank; juice, honey, oil, meat collections; isoscapes)	EU wine databank ($(D/H)^{I}$, $(D/H)^{II}$); flavor-house collections	Research collections (ethanol, vanillin, caffeine)	Commercial and in-house spectral databases
Field strength required	Not applicable	≥ 7.05 T with ^{2}H{^{1}H} probe and ^{19}F lock	≥ 9.4 T in published work; cryoprobe advantageous	≥ 9.4 T for minor components; 1.4 T benchtop for major
Capital cost (order)	Moderate	High	High	High (high-field) or low (benchtop)
Best suited to	Geographic origin; C_4/C_3 adulteration; O, N, S questions; trace analytes; screening	C_3-into-C_3 sugar; natural vs synthetic flavors; water addition (with δ^{18}O)	Same-pathway discrimination; engineered adulterants; biosynthetic route	Species, variety, cultivar; compositional adulteration; screening

Table 6. Decision guide. The first technique named is the one that should be run first; those that follow are added when the first is indecisive or when legal weight requires corroboration.

Question	Run first	Add if needed	Not useful
Is C_4 sugar (corn, cane) present in a C_3 product?	EA-IRMS δ^{13}C (AOAC 998.12, 2004.01) or LC-IRMS	^{2}H SNIF-NMR to quantify mixed C_3/C_4 additions	NMR profiling as primary
Is beet (or other C_3) sugar present in a C_3 juice, wine, must or syrup?	^{2}H SNIF-NMR $(D/H)^{I}$ (OIV-MA-AS311-05, AOAC 995.17)	δ^{13}C and δ^{18}O IRMS; LC-IRMS sugar consistency for honey	Bulk δ^{13}C alone
Is this tequila/mezcal/rum ethanol from agave, cane or maize?	Site-specific ^{13}C NMR of ethanol	^{2}H SNIF-NMR on the same distillate; bulk δ^{13}C	Bulk δ^{13}C alone; ^{1}H profiling
Is this vanillin/benzaldehyde/anethole/linalool natural, synthetic or biosynthetic?	^{2}H SNIF-NMR (AOAC 2006.05 for vanillin) with bulk δ^{13}C	Site-specific ^{13}C NMR for biosynthetic routes and blends	Bulk δ^{13}C alone against a sophisticated adulterant
Has water been added to wine or juice?	δ^{18}O of water by IRMS or CRDS against the databank	$(D/H)^{II}$ and R by SNIF-NMR; δ^{18}O of sugar-ethanol	NMR profiling
Is this wine/oil/juice/rice/coffee from the declared region?	Multi-isotope IRMS (δ^{18}O, δ^{2}H, δ^{13}C, δ^{15}N, δ^{34}S) ± ^{87}Sr/^{86}Sr against an authentic same-year reference set	$(D/H)^{II}$; ^{1}H NMR profiling; trace elements; data fusion	NMR profiling as sole evidence
Was this crop organically fertilized?	EA-IRMS δ^{15}N with δ^{34}S	Compositional data	Any NMR isotopic method
Which species, variety or cultivar is this?	^{1}H/^{13}C NMR profiling against a reference set; DNA for taxon	GC or HPLC compositional confirmation	All isotopic methods

Question	Run first	Add if needed	Not useful
Is this seed oil the declared species?	$^{1}H/^{13}C$ NMR compositional profiling (with GC-FID)	Bulk $\delta^{13}C$ for C_3/C_4 oils	SNIF-NMR (research stage)
Is this glycerol in wine of fermentation origin?	^{2}H and ^{13}C site-specific NMR	Bulk $\delta^{13}C$	NMR profiling

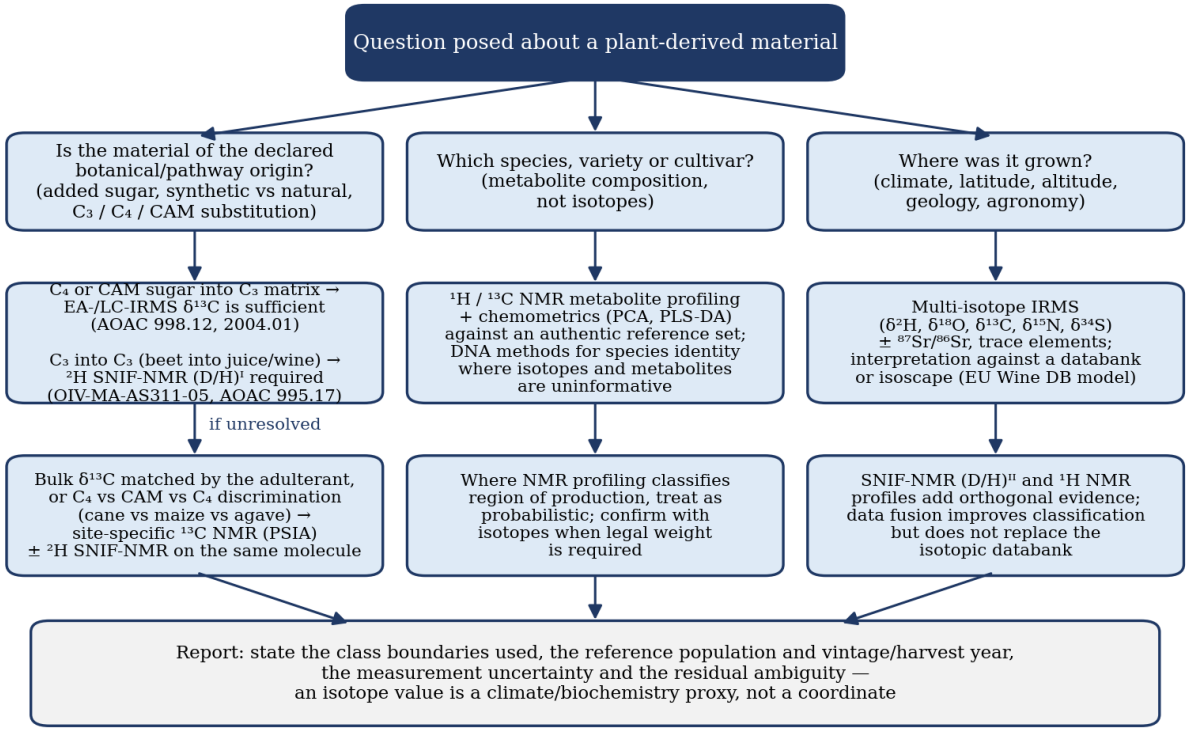

Figure 3. Decision workflow for selecting between IRMS, isotopic NMR and NMR profiling according to the origin question posed. Method identifiers refer to Table 2.

10. Implementation Considerations for a Mid-Field NMR Laboratory

The requirements for performing the isotopic NMR methods in-house differ sharply from those for the compositional profiling that a food-analysis NMR laboratory already performs, and they are set out here with reference to the instrument base of Process NMR Associates, which comprises 7.05 T (300 MHz ^{1}H) superconducting systems and 60 MHz benchtop systems.

10.1 ^{2}H SNIF-NMR

The OIV method explicitly admits a 7.05 T spectrometer, at which ^{2}H resonates at 46.05 MHz, and the original Nantes work was performed at 9.4 T; a 300 MHz system is therefore within the envelope of the official method, with the penalty that the lower sensitivity requires more replicate spectra and larger lock-substance additions (the OIV text specifies about 150 µL of hexafluorobenzene at 7.05 T against 35 µL at 9.4 T) to reach the same precision. The hardware requirements beyond the magnet are specific: a 10 mm ^{2}H observe probe with a ^{1}H decoupling channel; a field-frequency lock operating on ^{19}F rather than on ^{2}H, which on most modern consoles requires either a dedicated lock configuration or the use of a broadband

channel as an external lock; a spinning-band (Cadiot) distillation column and the associated gravimetric and densitometric apparatus for quantitative ethanol recovery and alcoholic-strength determination; certified TMU (ERM-AE003) and sealed check tubes; and lineshape-fitting software. The consumable cost per sample is modest, the labor cost is one to two days, and the spectrometer occupancy is several hours. A laboratory contemplating the method should recognize that its value lies in the C_3-into-C_3 sugar question and in flavor authentication, that the reference populations for wine and juice are held by official and commercial laboratories in Europe, and that a domestic laboratory would need to establish its own reference ranges for the products it intends to test.

10.2 Site-specific ^{13}C NMR

All published position-specific ^{13}C work has been performed at 9.4 T or above, usually with a cryogenically cooled ^{13}C-optimized probe, because the precision target of 1 ‰ demands a signal-to-noise ratio of several hundred on every site and because the relaxation delays required for quantitative conditions (about ten times the longest T_1, which for the quaternary and carbonyl carbons of vanillin approaches several seconds) make each spectrum long.[46,49] At 7.05 T the sensitivity is lower by a factor of roughly $(9.4/7.05)^{3/2} \approx 1.5$ for the same probe technology, so that about 2.4 times the acquisition time is needed for the same precision, and the smaller chemical-shift dispersion in hertz increases the overlap of the six resolvable signals of a fatty acid methyl ester or the eight signals of vanillin. The method is feasible at 7.05 T in principle for ethanol, whose two signals are widely separated and whose T_1 values are short, and the agave validation of Portaluri and co-workers provides the protocol from which such an implementation would begin;[53] for vanillin, caffeine and lipids a higher field would be required. An implementation would also need either an IRMS partner for the bulk $\delta^{13}C$ of each sample or the internal-referencing protocol,[48] and the vanillin reference materials of the NRC for validation.[50]

10.3 The benchtop instruments

At 1.4 T the ^{2}H resonance lies near 9 MHz and the receptivity of natural-abundance deuterium is such that no site-specific measurement is possible; the same is true of ^{13}C at 15 MHz for the 1 ‰ precision required. Benchtop NMR contributes to origin work only through ^{1}H compositional profiling of major components — ethanol, sugars, organic acids, fatty acyl classes — and through the qNMR determinations that precede the isotopic methods, for example the alcoholic strength of a distillate or the sugar content of a must.

10.4 A hybrid service model

For a laboratory whose strength is high-resolution compositional NMR, the practicable model for origin work is a hybrid one in which ^{1}H and ^{13}C profiling is performed in-house as the screening and species-identity tier, bulk multi-element IRMS is subcontracted to a specialist laboratory for the geographic and C_4-sugar tier at a cost per sample that no NMR laboratory can match, and SNIF-NMR or site-specific ^{13}C NMR is either subcontracted to one of the few laboratories that operate them routinely or developed in-house for the specific product classes — spirits, flavors, vinegars — in which the intramolecular question arises repeatedly and commands a fee commensurate with a day of instrument time. Under ISO/IEC 17025 the subcontracted determinations must be placed with accredited laboratories and identified as such in the report, and the in-house compositional classifications must be supported by documented reference sets with stated classification performance. The expert-witness setting adds a further requirement: the isotopic parameters offered in evidence should be those for which official methods and public reference ranges exist, and the compositional classifications should be presented as supporting rather than determinative.

11. Critical Assessment and Pitfalls

The strengths of both technique families have been stated; their failure modes deserve equal attention, because it is on these that an expert reviewer or an opposing expert will concentrate.

Databank dependence. Neither an isotope ratio nor an NMR profile identifies an origin; it identifies a position in a distribution, and the assignment is a comparison with authentic samples. Where the authentic population is small, unrepresentative, or from a different year, the comparison is unreliable, and the European wine databank exists precisely because annual re-sampling was found necessary.[5,27] A laboratory that reports an origin without stating the reference population, its size and its year has not reported an origin.

Climatic drift and the isoscape problem. The hydrogen and oxygen isotope ratios that carry geographic information are climate proxies, and the same value is found in different regions of similar climate; a Mediterranean wine and a Californian wine may be isotopically indistinguishable, and rising temperatures shift the ranges from decade to decade. Assignment is therefore probabilistic and is only as good as the exclusion of alternative regions, which requires their inclusion in the reference set.[7,14]

Processing artifacts. Must concentration, dealcoholization, stopping of fermentation, dilution of concentrates with tap water before fermentation for SNIF-NMR, and incomplete distillation all shift the isotopic parameters, and each has been documented as a source of false positives or of masked adulteration.[33] The OIV method's insistence on distillation yields above 96 % and on correction for the isotopic composition of any dilution water is a response to these effects and must be observed.

Isotopic engineering. An adulterant can be selected, blended or synthesized to reproduce the bulk isotope ratio of the authentic material, and the vanillin literature documents the escalation from crude synthetics to precursor-matched and deliberately enriched materials.[40,55] Site-specific measurement raises the cost of such engineering because the entire pattern must be matched, but it does not make it impossible, and the appropriate posture is to combine ^{2}H, ^{13}C and compositional evidence rather than to rely on one.

Exchangeable hydrogen and sample handling. Hydrogen bound to oxygen and nitrogen exchanges with ambient water and reports nothing about the plant; bulk $\delta^{2}H$ by TC/EA requires either removal or equilibration of that fraction, and SNIF-NMR simply excludes the hydroxyl site. Evaporative loss of ethanol or of the TMU standard between weighing and measurement fractionates the sample and is a recognized source of error.

Precision limits of the intramolecular methods. The site-specific ^{13}C precision of about 1 ‰ is adequate for pathway and route discrimination, where the differences are several per mil, but not for the within-pathway climatic effects that IRMS resolves at 0.1 ‰; the ^{2}H site-specific precision of 0.2–0.4 ppm corresponds to about 2–4 ‰ and limits the detection of small additions. Claims of discrimination based on differences smaller than the reproducibility should be rejected.[31,49]

Chemometric over-interpretation. A PLS-DA model that separates the training classes has demonstrated nothing until it has been tested on samples it did not see, and a classification accuracy quoted without cross-validation, permutation testing and an independent test set is not a result. Confounding of region with variety, vintage and processing must be examined explicitly.[83,84]

Species identity. Isotopes do not identify a plant taxon, and NMR profiles identify it only within the trained reference set; a claim of species identity should rest on DNA or on a validated compositional marker, with isotopes supplying the origin.[69]

Legal admissibility. In proceedings, the weight given to an isotopic origin determination depends on the existence of an official method, a documented reference population, a stated uncertainty and a stated error rate for the classification rule, and on the availability of the sample for re-testing; the requirements have been set out by Camin and co-workers, and they favor the official IRMS and SNIF-NMR parameters over research-stage measurements.[15]

12. Conclusions

Isotope ratio mass spectrometry and isotopic NMR are complementary readers of the same natural record. IRMS reports, with the best available precision and on the smallest samples, the average isotope ratio of five elements, and it alone reaches the oxygen, nitrogen, sulfur and strontium parameters on which geographic and agronomic assignment principally rests; the reference populations and official methods that make origin determination legally usable were built on it. Isotopic NMR reports the isotope ratio at each position within a molecule, and it alone, among routine techniques, reads the intramolecular pattern that distinguishes sugars, flavors and acids of the same photosynthetic pathway, that survives blending to a target bulk value, and that records the enzymatic route of biosynthesis. The historical division of labor — SNIF-NMR for beet sugar and for natural flavors, IRMS for C_4 sugar, water and geography — has been extended by site-specific ^{13}C NMR to the cane–maize–agave problem and to the biotechnological vanillin routes, and has been made metrologically sound by internal referencing and certified reference materials.

For plant identity in the sense of pathway and route, NMR is favored; for plant identity in the sense of species and cultivar, compositional NMR profiling and DNA are favored and isotopes are uninformative; for geographic origin, IRMS against an authentic same-year reference population is the established route, with SNIF-NMR and NMR profiling as supplements that improve classification when fused. A laboratory equipped for high-resolution compositional NMR can enter this field through profiling and through subcontracted IRMS, and can add SNIF-NMR at 7.05 T within the envelope of the official method if the product classes justify the investment; site-specific ^{13}C NMR beyond ethanol requires a higher field. In every case the result should be reported with its reference population, its uncertainty and its residual ambiguity, because an isotope value is a proxy for climate and biochemistry, not a coordinate.

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Summary

This white paper compared isotope ratio mass spectrometry with isotopic NMR — ^{2}H SNIF-NMR and site-specific ^{13}C NMR — as means of determining the plant identity and geographic origin of foods, beverages, flavors and oils, and placed non-isotopic ^{1}H/^{13}C NMR profiling alongside them as the route to species and cultivar. The physical basis was traced to photosynthetic pathway (δ^{13}C), meteoric water and evapotranspiration (δ^{2}H, δ^{18}O), agronomy and geology (δ^{15}N, δ^{34}S, ^{87}Sr/^{86}Sr) and to the non-statistical intramolecular distributions imposed by enzymatic isotope effects, which are the property that NMR reads and IRMS averages.

NMR was shown to be the technique of choice for C_3 sugar added to C_3 products (beet in wine, juice, maple syrup, honey), for cane–maize–agave discrimination in spirits, for natural-versus-synthetic and natural-versus-biosynthetic flavor molecules (vanillin, benzaldehyde, anethole, linalool, caffeine), for the origin of acetic acid and glycerol, and for any adulterant engineered to reproduce a bulk isotope ratio. IRMS was shown to be the technique of choice for C_4 sugar in C_3 products, for water addition, for geographic origin of wine, olive oil, juice, rice, coffee, cocoa and sesame, for organic-versus-conventional status and geological provenance, for trace and compound-specific analytes, and for every question that turns on oxygen, nitrogen or sulfur; it also underlies the reference populations, including the European Union wine databank, against which any origin claim is judged. Data fusion of NMR and IRMS parameters improves classification but does not remove the need for a same-year authentic reference set.

For a mid-field laboratory the practicable path is compositional profiling in-house, subcontracted multi-element IRMS, and SNIF-NMR at 7.05 T — which the OIV method admits — for those product classes in which the intramolecular question recurs; site-specific ^{13}C NMR beyond ethanol requires 9.4 T or higher. The principal pitfalls — databank dependence, climatic drift, processing artifacts, isotopic engineering, exchangeable hydrogen, precision limits, chemometric over-interpretation, the species-identity gap and legal admissibility — were set out so that results can be reported with the reference population, uncertainty and residual ambiguity that a court or a reviewer will require. All references were verified against CrossRef before drafting; Figures 1–3 are schematic and contain no measured PNA data, which will be added in a subsequent version.