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^{1}H NMR in the Analysis of Apples, Apple Juice and Hard Cider

How the measurement is made and what the spectrum tells the grower, the cider maker and the consumer: a summary of PNA-WP-CIDER v3.0

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White Paper Series · PNA-WP-CIDER-SUM · Version 1.0 · 15 November 2026
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Abstract

Proton nuclear magnetic resonance (¹H NMR) spectroscopy, applied quantitatively with an internal standard, determines in one measurement most of the constituents on which the character, the classification and the label of a cider depend. This document summarizes, for a technical but not necessarily spectroscopic reader, how the measurement is made at Process NMR Associates and what it has revealed about apples, apple juice and hard cider in a survey of 96 ciders from 36 producers and of the juices of 56 apple varieties, reported in full in white paper PNA-WP-CIDER v3.0.[1] The principle of the method, the preparation of a native and a freeze-dried sample, the measurement of signal areas by integration, constrained line fitting and decomposition with pure-component spectra, and the checks that establish the reliability of the results are described first, followed by a guide to the signals of the spectrum and the information that each carries.

In the apple, the spectrum records the amount and kind of sugar, the acidity relative to the sugar, the sorbitol that will survive fermentation and pass into the cider, and minor markers of variety and of the nitrogen available to the yeast. The old varieties examined were found to be almost all sharps, with sucrose shares of 5 to 52 % and sorbitol contents that differ more than tenfold, the russets being richest. In the cider, the same spectrum gives the alcohol, which agreed with the declared value to −0.06 ± 0.71 % v/v, the residual sugar and its kind, the acids and the state of malolactic conversion, the glycerol and its bacterial conversion to 1,3-propanediol that marks natural cider, the preservatives, heat-treatment and fruit markers, and an integral measure of the polyphenols that distinguishes the European traditions. From the composition the cider is placed on any dryness scale, further scales for balance, acidity, acid profile and body are derived, and the energy and carbohydrate of a serving are calculated for the label. Multivariate analysis of the spectra confirms these distinctions but adds little that the quantitative composition does not already provide. The limitations of the method, chiefly the overlap of signals at 300 MHz, the absence of individual polyphenols and aroma compounds, and the lack of an independent reference method for most constituents, are stated, together with the points that are being revised in the next edition of the white paper.

Keywords: ¹H qNMR; cider; hard cider; apple juice; heirloom apples; internal standard; maleic acid; freeze-drying; malolactic conversion; 1,3-propanediol; natural cider; residual sugar; dryness scale; nutrition labeling; chemometrics.

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1. Introduction

Cider is chemically one of the simplest of the fermented beverages. It is made from a single fruit whose juice consists, apart from water, of three sugars, a sugar alcohol and one dominant acid, and the finished product is dominated by a short list of compounds: ethanol, the residual sugars fructose, glucose and sucrose, the polyols glycerol and sorbitol, and the organic acids malic, lactic, succinic and acetic.[2,3] Every one of these compounds gives resolved signals in the ¹H NMR spectrum of the untreated beverage, and because the area of an NMR signal is proportional to the number of nuclei that give rise to it, a single spectrum recorded with an internal standard yields their concentrations without compound-specific calibration.[4,5] No other single measurement covers the alcohol, the sugars, the acids and the polyols at once, and none records at the same time the traces that fermentation, bacterial activity, sweetening and preservation leave in the product.

Quantitative ¹H NMR (qNMR) has been applied to commercial and craft ciders at Process NMR Associates since 2013 and to the juices of dessert, heirloom and cider apples since 2015.[6–8] The complete body of data, 96 ciders from 36 producers, 87 of them measured both as received and after freeze-drying, and the juices of 56 apple varieties, was brought together and evaluated by one documented procedure in white paper PNA-WP-CIDER v3.0.[1] That document runs to some 150 pages and covers the history of the apple and of cider making, the chemistry of fermentation, the regulatory framework and an extensive chemometric study. The present summary condenses it into the two questions most often put to the laboratory: how is NMR applied to a cider or a juice, and what does the result tell the person who grows the fruit, makes the cider or drinks it?

Section 2 describes the measurement, Section 3 the signals of the spectrum and their meaning. Sections 4 and 5 report what the survey revealed about the apple and about cider, Section 6 how the composition is translated into dryness and other descriptive scales, and Section 7 how it supplies the values of a nutrition label. Section 8 considers what multivariate analysis adds, Section 9 how the method is used in practice, and Section 10 its limitations. Brand names are used as they are in the white paper; the results describe the individual cans and bottles that were analyzed and are not statements about a producer's output in general.

2. How the Measurement Is Made

2.1 The principle of quantitative ¹H NMR

In a ¹H NMR spectrum each chemically distinct group of protons in a molecule gives a signal at a characteristic frequency, the chemical shift, expressed in parts per million (ppm) of the spectrometer frequency, and the signal is split into a recognizable pattern by the neighboring protons. Under conditions in which the nuclei relax completely between successive pulses, the area of a signal is strictly proportional to the number of protons that produce it, irrespective of the compound to which they belong.[9,10] If a known amount of a reference substance is added to the sample, the concentration of any other constituent follows from the ratio of the two areas, each divided by its number of protons, and the ratio of the molar masses. This is the property that distinguishes NMR from the chromatographic methods, which must be calibrated separately for every compound with a standard of that compound.

Maleic acid was used as the internal standard throughout. Its two equivalent olefinic protons give a singlet near 6.40 ppm, in a region of the spectrum in which no constituent of cider or juice absorbs, and it is available in certified purity.[11,12] The standard solution was prepared in deuterium oxide (D_2O) by weight, and its concentration was corrected for the volume occupied by the dissolved acid, so that the amount of standard delivered to each tube is known on a gravimetric basis. For a constituent X the concentration in the cider then follows as

$$C(X) = P(MA) \times m(MA) \times [(I(X) / N(X)) / (I(MA) / 2)] \times [M(X) / M(MA)] / V(sample) \quad (1)$$

in which I is a signal area, N the number of protons it represents, M a molar mass, m(MA) the mass and P(MA) the purity of the maleic acid in the tube, and V(sample) the volume of cider or juice that the tube represents. No response factor appears in the equation, and no calibration curve is needed; the same equation serves ethanol at 50 g/L and 5-hydroxymethylfurfural at 3 mg/L.

2.2 Two samples from one cider

Two NMR samples are prepared from each cider. For the native tube, 175 µL of cider, freed of carbon dioxide, is mixed with the standard solution and D_2O. This sample contains every constituent in its original proportion, including the volatile ones, ethanol, methanol, the higher alcohols, acetic, formic and sorbic acids, which can be measured only here. Its spectrum is, however, dominated by ethanol and water: drawn on scale it consists of four signals, the ethanol triplet and quartet, the water resonance and the standard, and everything else lies two orders of magnitude lower (Section 3). The ethanol signals also cover part of the carbohydrate region and the methyl doublet of 2,3-butanediol.[1]

For the freeze-dried tube, 1000 µL of the same cider is lyophilized and the residue redissolved in D_2O with the same amount of standard. Freeze-drying removes the water and most of the ethanol and concentrates the non-volatile constituents about sixfold relative to the native tube, so that the sugars, the polyols and the non-volatile acids rise to the height of the standard and the signals of glycerol, sorbitol and 2,3-butanediol, hidden by ethanol in the native spectrum, are uncovered (Section 3). Because the volatile compounds are lost in the process, both tubes are needed. The two are tied together by the non-volatile constituents measured reliably in both, whose median ratio, the bridging factor, places the freeze-dried results on the scale of the simple native preparation.[1] Where only the native tube is available, as for nine ciders of the survey, all constituents can still be estimated from the one spectrum, at the cost of higher reporting limits for the minor ones and of a larger uncertainty for glycerol and sorbitol.

Apple juices are measured in the freeze-dried form only, 1000 µL of juice being lyophilized and redissolved with the standard. A juice contains no ethanol to hide anything, and the concentration step brings the minor acids, the amino acids and the phenolic signals within reach.[1]

2.3 Acquisition and processing

The spectra of the survey were recorded at 300 MHz on two Varian spectrometers with a simple one-pulse sequence at 27 °C, with repetition times of 14 to 24 s to allow full relaxation of the quantified signals and with suppression of the water signal in the native tubes.[1] The free induction decays were zero-filled, Fourier transformed, phased and corrected for the baseline, and every spectrum was referenced to the standard of the same tube. Spectra recorded between 2013 and 2026 in four campaigns were placed on one common chemical-shift axis, so that the same analytical window and the same pure-component reference spectra apply to all of them. The processed spectra, with the integrals and fitted lines from which each area was obtained, are kept as workspaces of the PNA NMR Processor and can be reopened and inspected.

2.4 Measuring the signal areas

Three procedures are used, each where it is appropriate, and the standard is always measured by the same procedure as the analyte with which it is compared. Isolated signals, such as the ethanol triplet, the malic acid multiplet and the hydroxycinnamate doublet, are integrated between fixed limits above a straight baseline. Signals that overlap with a neighbor are fitted with lines whose number, spacing and relative intensity are fixed by the known multiplet structure; in this way the doublet of lactic acid is separated from the ¹³C satellite of ethanol, the doublet of 2,3-butanediol from the ethanol triplet, and the anomeric doublets of sucrose and glucose from their neighbors.[1] The crowded region between 3.18 and 4.30 ppm, in which fructose, glucose, sucrose, glycerol, sorbitol, 1,3-propanediol and residual ethanol overlap completely at 300 MHz, is decomposed by non-negative least squares into the spectra of the pure compounds recorded

under the same conditions, in the manner of targeted profiling.[13] The fit is anchored by the amounts of sucrose and glucose taken from their resolved anomeric doublets, and the equilibrium fraction of α-glucose needed to convert the α doublet into total glucose, 0.380, was measured from the reference spectrum and agrees with the accepted value.[14] The decomposition reproduced the experimental spectra with a median coefficient of determination of 0.989.[1]

2.5 How reliable are the results?

Three checks were available for the ciders. The alcohol content declared on the label is the only independent value that exists for most products, and for the 71 ciders with a declaration the qNMR result differed from the label by −0.06 % v/v on average, with a standard deviation of 0.71 % v/v; 48 ciders agreed within 0.5 % v/v and 61 within 1.0 % v/v (Figure 1a). Because labels are approved before a batch is made and carry a tolerance, this scatter is mostly a property of the products, not of the measurement. Repeated acquisitions of the same tube agreed within 1 % in the median for ethanol and the principal acids and within 1 to 2 % for the sugars and polyols (Figure 1c), and the two tubes of a typical cider agreed within a few percent, the bridging factor having a median of 1.026 times its nominal value (Figure 1b).[1] Ethanol by NMR is an established method in its own right,[15,16] and the quantitative NMR procedures for wine and beer on which the present method draws have been validated against reference methods.[17–19]

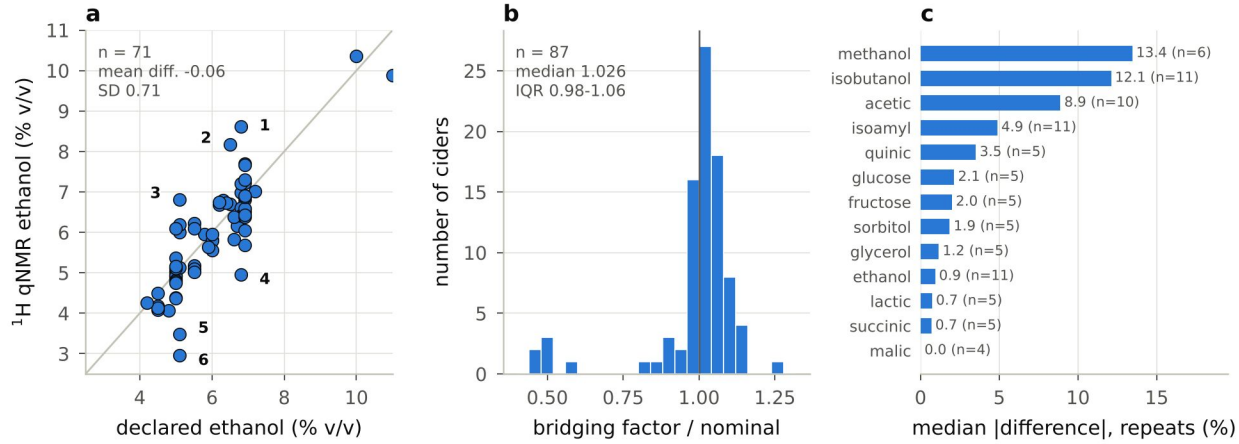

Figure 1. Performance of the measurement. (a) Ethanol by ¹H qNMR against the alcohol content declared on the label; the line is the identity. (b) Distribution of the bridging factor between the freeze-dried and the native tube relative to its nominal value. (c) Median absolute difference between repeated acquisitions of the same tube, by analyte. Reproduced from PNA-WP-CIDER v3.0.[1]

These checks establish precision and internal consistency. They do not establish trueness for constituents other than ethanol, because no values by an independent reference method were available for the sugars, acids and polyols of these samples. The point is taken up again in Section 10.

3. Reading the Spectrum

The signals used for quantification are collected in Table 1 with the information that each provides. Their assignment rests on the spectra of the pure compounds recorded under the same conditions and on the published assignments of apple juice, wine and beer.[17,18,20] Figure 2 shows the native spectrum of a typical craft cider and Figure 3 the freeze-dried spectra of two ciders of opposite character drawn on one absolute scale.

Table 1. Signals of the ¹H NMR spectrum used for quantification in cider (300 MHz, D_2O), the tube from which each value is reported, and the information it carries.[1]

Constituent and signal	δ (ppm), multiplicity	Tube	What it records
Maleic acid (internal standard)	6.40, s	both	The scale of every concentration
Ethanol, CH_3	1.17, t	native	Alcohol by volume; the extent of fermentation
Methanol, CH_3	3.34, s	native	Pectin demethylation: fruit, maceration, added fruit or pomace
Higher alcohols, CH_3	0.84–0.93, d and t	native	Yeast amino acid metabolism; strain and nitrogen supply
Acetic acid, CH_3	2.08, s	native	Volatile acidity; heterofermentative bacteria, acetic spoilage
Sorbic acid, CH_3 and H-2	1.84, d; 5.83, d	native	Added sorbate as a stabilizer
Lactic acid, CH_3	1.41, d	freeze-dried	Malolactic conversion; sour styles
Succinic acid, CH_2	2.67, s	freeze-dried	Yeast fermentation; rises with the alcohol
Malic acid, CH_2	2.80–2.99, AB of ABX	freeze-dried	The acid of the fruit; preserved or converted
Citric acid, CH_2	3.04, d	freeze-dried	Added fruit other than apple, or acidification
Quinic acid, H-6ax	1.93, dd	freeze-dried	Apple variety; cranberry and other fruit
Sucrose, H-1	5.40, d	freeze-dried	Sweetening with sugar; fruit sucrose in juice
α-Glucose, H-1	5.22, d	freeze-dried	Residual sugar; glucose is fermented first
Fructose	3.18–4.30 (library)	freeze-dried	The sweetest sugar; arrested fermentation
Glycerol, sorbitol	3.18–4.30 (library)	freeze-dried	Body; yeast metabolism (glycerol), fruit or pear (sorbitol)
1,3-Propanediol, central CH_2	1.78, quintet	both	Bacterial conversion of glycerol: natural cider
2,3-Butanediol, CH_3	1.14, d	freeze-dried	Yeast by-product
5-Hydroxymethylfurfural, CHO	9.45, s	freeze-dried	Heat: concentrate, caramel, pasteurization
Hydroxycinnamoyl esters, H-7	7.56–7.69, d	freeze-dried	Phenolic content; bittersweet fruit, European styles

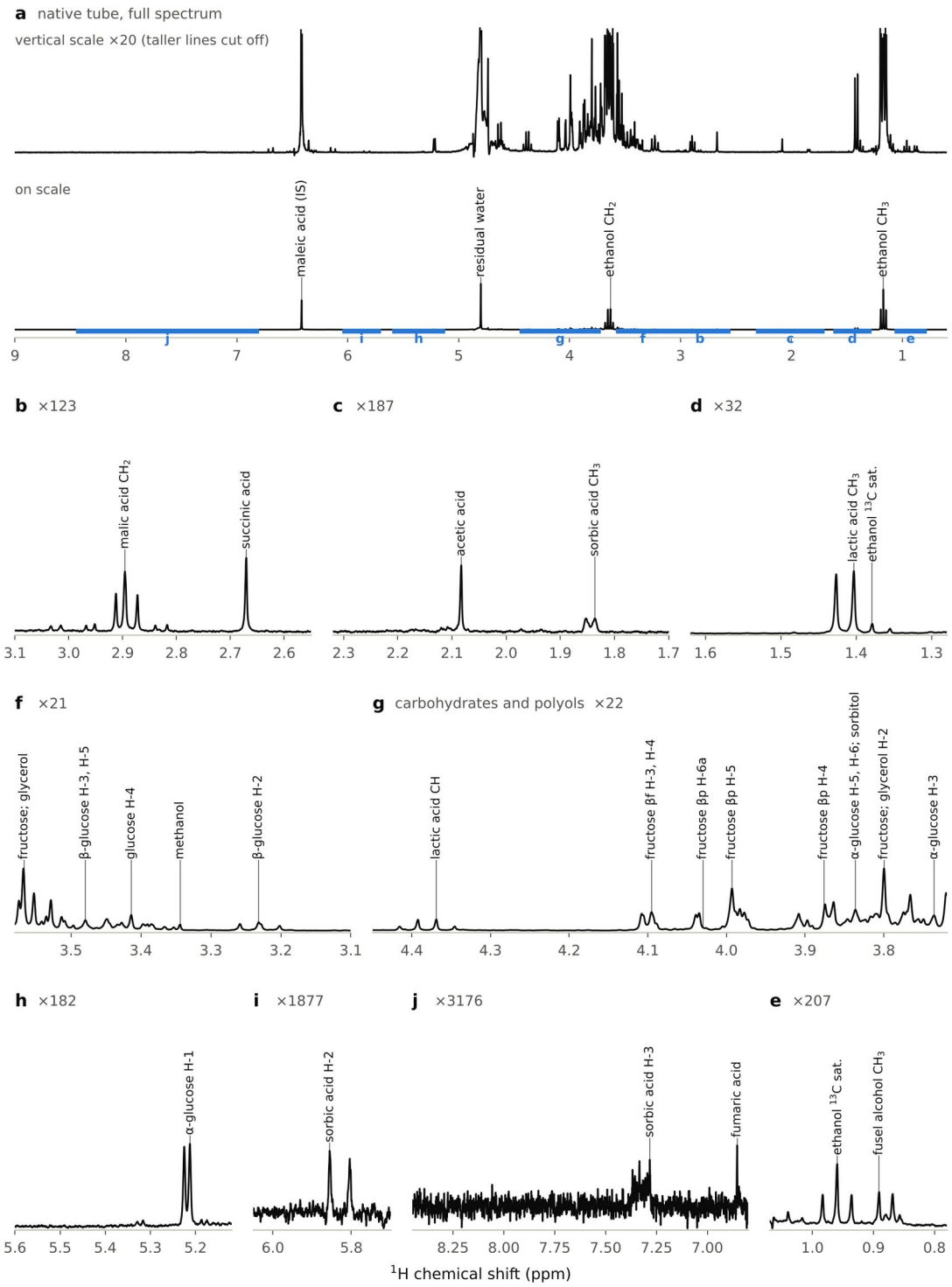

Figure 2. Assigned ¹H NMR spectrum of a cider in the native tube (Doc's Draft Original Hard Apple Cider; 175 µL of cider with maleic acid in D_2O, 300 MHz). (a) Full spectrum on scale (lower trace) and expanded twentyfold (upper trace). (b) to (j) Expansions with their magnification relative to the trace on scale. IS, internal standard; sat., ¹³C satellite. Reproduced from PNA-WP-CIDER v3.0.[1]

The magnifications marked in Figure 2 convey the dynamic range that the analysis must span. At about twenty times the scale of the ethanol signals the carbohydrate region and the anomeric doublets appear; at

one to two hundred times the organic acids, the methyl doublet of sorbic acid, the ^{13}C satellites of ethanol and the methyl signals of the higher alcohols; and only at two to three thousand times the olefinic protons of sorbic acid and a trace of fumaric acid that accompanies the standard. Freeze-drying removes this disproportion for the non-volatile constituents. In the sweet American cider of Figure 3 fructose, glucose, sucrose, sorbitol and glycerol fill the region between 3.2 and 4.3 ppm and malic acid is the only major acid; in the Basque natural cider the sugars are absent, lactic and acetic acids have replaced malic acid, and the triplet and quintet of 1,3-propanediol stand where the glycerol signals would be expected.[1]

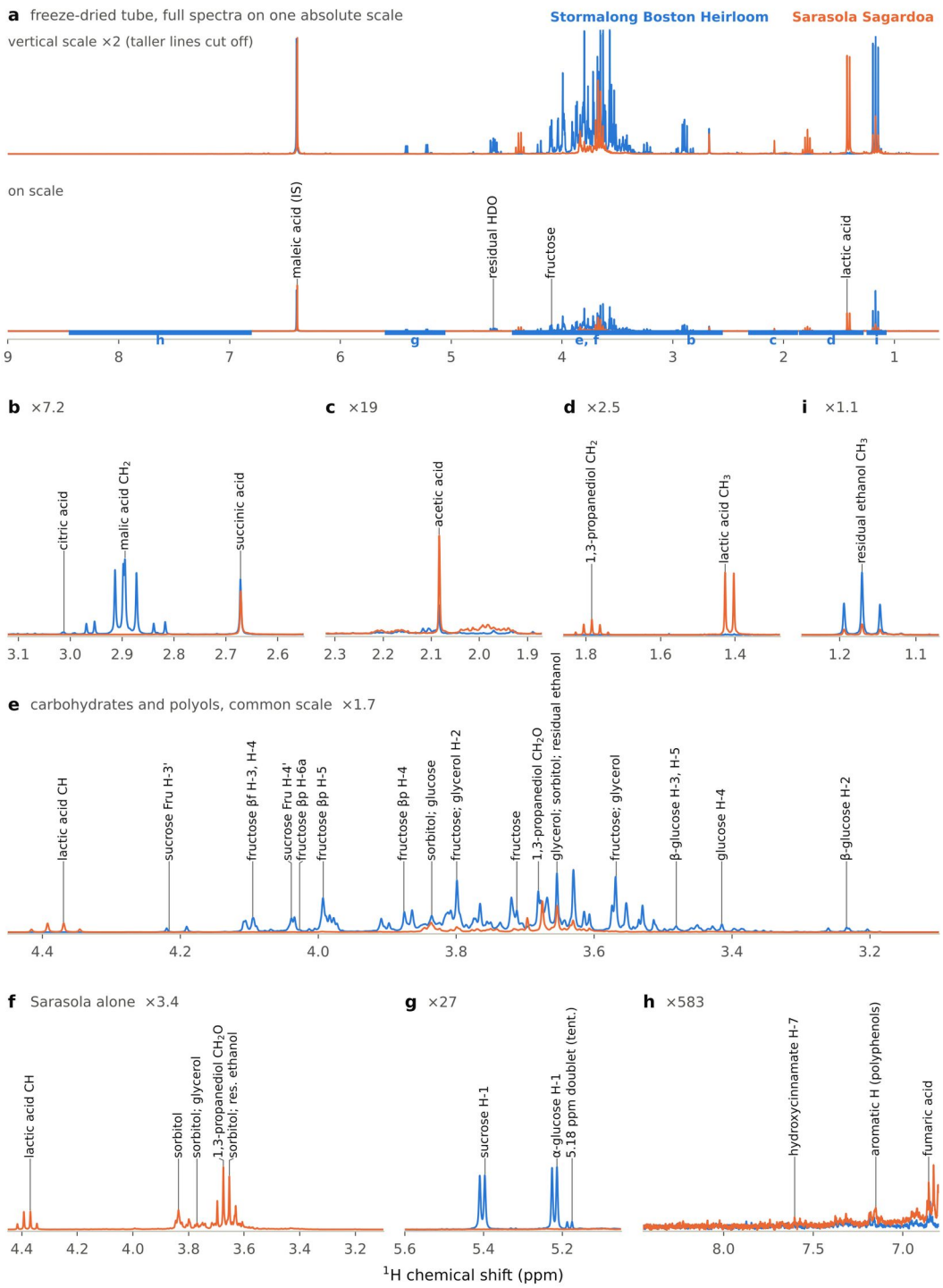

Figure 3. Assigned ¹H NMR spectra of two ciders in the freeze-dried tube (1000 µL of cider, lyophilized and redissolved in D_2O with maleic acid), on one absolute scale: Stormalong Boston Heirloom, a sweet, malate-dominant craft cider (blue), and Sarasola Sagardoa, a dry Basque natural cider after complete malolactic conversion (orange). (a) Full spectra; (b) to (i) expansions with their magnification. tent., tentative assignment. Reproduced from PNA-WP-CIDER v3.0.[1]

Three analytes are reported only when a second, independent signal confirms them: citric acid when the coupling constant of its doublet is that of citric acid, sorbic acid when the olefinic doublet of H-2 at 5.83 ppm is present, and 5-hydroxymethylfurfural when its aldehyde singlet is accompanied by the ring proton at 7.53

ppm.[1,21] The rule protects the minor markers, which carry the most interpretive weight, against chance coincidences with unassigned signals.

4. What the Spectrum Tells About the Apple

The juices of 56 varieties were analyzed: 53 old varieties from one collection, pressed in 2016 and dating from the sixteenth to the twentieth century, and, for comparison, the supermarket Granny Smith, Gala and Fuji.[1,7] Apple juice has long served as a model for the combination of NMR and multivariate statistics,[20,22] and its authentication by NMR is a routine commercial service.[23,24] What the survey adds is a quantitative description of the old varieties, which are the raw material of much of the American craft cider revival, in the terms that matter to the cider maker. The findings are summarized in Table 2 and Figure 4.

4.1 The sugars and the share of sucrose

Relative to the three sugars, the old varieties contained on average 59 % fructose, 13 % glucose and 28 % sucrose, compared with 57.7, 20.4 and 21.9 % in the survey of 175 varieties by Eisele and Drake.[25] Fructose was the most abundant sugar in all but two juices. The share of sucrose proved to be the most variable property of the set, from 5 % in Chestnut Crab to 52 % in Baldwin, with Decio, GoldRush, Ellison's Orange, Calville Blanc d'Hiver, American Beauty and Green Newtown Pippin also above 40 %. Since sucrose is hydrolyzed progressively in stored fruit, these shares are lower limits, but the ranking of the varieties is a property of the fruit.[1] The low glucose share has a practical consequence. Yeast ferments glucose in preference to fructose, so a cider whose fermentation is arrested from such fruit retains mostly fructose, the sweetest of the three sugars.

4.2 Acidity: the old varieties are sharps

For the cider maker the ratio of acid to sugar is decisive, because the sugar becomes alcohol while the acid remains. Malic acid amounted to 3.4 to 10.4 g per 100 g of sugars in the old varieties, with a median of 5.3 g. Standardized to 110 g/L of sugars, 48 of the 53 lots contain more than the 4.5 g/L of malic acid that separates sharp from sweet fruit in the Long Ashton classification, and are therefore sharps.[1,2] The modern dessert apples lay outside the range of the old varieties at both ends: Granny Smith, at 10.8 g per 100 g, was more acidic than any of them, while Gala (2.0) and Fuji (1.4) were less acidic than all of them, which is consistent with the selection for low acidity documented in the comparison of cultivated and wild apples.[26] The spectrum thus places a juice on the sharp-to-sweet axis of the cider maker directly from one measurement.

4.3 Sorbitol and the russets

Sorbitol, the form in which members of the rose family transport the products of photosynthesis, is not fermented by yeast and passes unchanged into the cider, where it adds body and a residual sweetness to a product that contains no sugar.[2,27] It varied more than tenfold among the varieties, from 0.6 g per 100 g of sugars in GoldRush to 10.6 g in Roxbury Russet, and the russets as a group contained more than twice as much as the other varieties (median 5.4 against 2.0 g per 100 g; $p = 0.010$).[1] Since sorbitol is, among the constituents of the apple, the one most closely correlated with the sweetness perceived by a trained panel,[28] the reputation of the russets for rich, full ciders has, in part, a chemical basis that the spectrum makes visible.

4.4 Minor markers of variety and of yeast nutrition

Several minor constituents are measured in the same spectrum at no additional cost. Quinic acid spanned almost thirtyfold, from 37 mg/L in Golden Russet to 1051 mg/L in Golden Noble, and was low in the russets. Citramalic acid was found in 41 juices, up to 178 mg/L in Northern Spy, and L-rhamnitol in all but one, both adding markers of variety.[1] Asparagine, the principal free amino acid of apple juice and the main source of nitrogen available to the yeast,[29] was detected in 31 of the 53 lots, at a median of 225 mg/L where

found and at 1156 mg/L in Green Newtown Pippin; Granny Smith contained 3.4 g/L. This signal gives an early indication of how fast a juice will ferment and of whether it is suited to the slow, nutrient-starved fermentations of the French tradition. The hydroxycinnamoyl esters, expressed as chlorogenic acid, reached 113 mg/L in the median juice and 376 mg/L in Winter Banana, values that are modest by the standard of bittersweet cider fruit, whose juice may contain several grams of polyphenols per liter.[30,31]

Table 2. What the ¹H NMR spectrum of an apple juice tells the grower and the cider maker, with the range found in 53 old varieties.[1]

Measured quantity	What it indicates	Finding in the survey
Sum of sugars	Potential alcohol of the cider	Varies with fruit and storage; supermarket fruit at the high end
Sucrose share of the sugars	Maturity, storage, variety	5 to 52 % (Chestnut Crab to Baldwin)
Fructose to glucose ratio	Sweetness retained by an arrested fermentation	5.0 on average, against 2.8 in the literature
Malic acid per 100 g of sugars	Sharp or sweet fruit; acidity of the cider	3.4 to 10.4 g; 48 of 53 lots are sharps
Sorbitol per 100 g of sugars	Body and sweetness of a dry cider	0.6 to 10.6 g; russets highest
Quinic, citramalic acids, L-rhamnitol	Markers of variety	Quinic acid spans thirtyfold
Asparagine, aspartic acid	Nitrogen for the yeast; speed of fermentation	Asparagine in 31 of 53 lots, up to 1.2 g/L
Hydroxycinnamates, aromatic envelope	Phenolic content, bitterness and astringency	Modest; dessert-fruit levels throughout
Ethanol, 2,3-butanediol	Fermentation begun in the fruit or juice	Found in a few lots

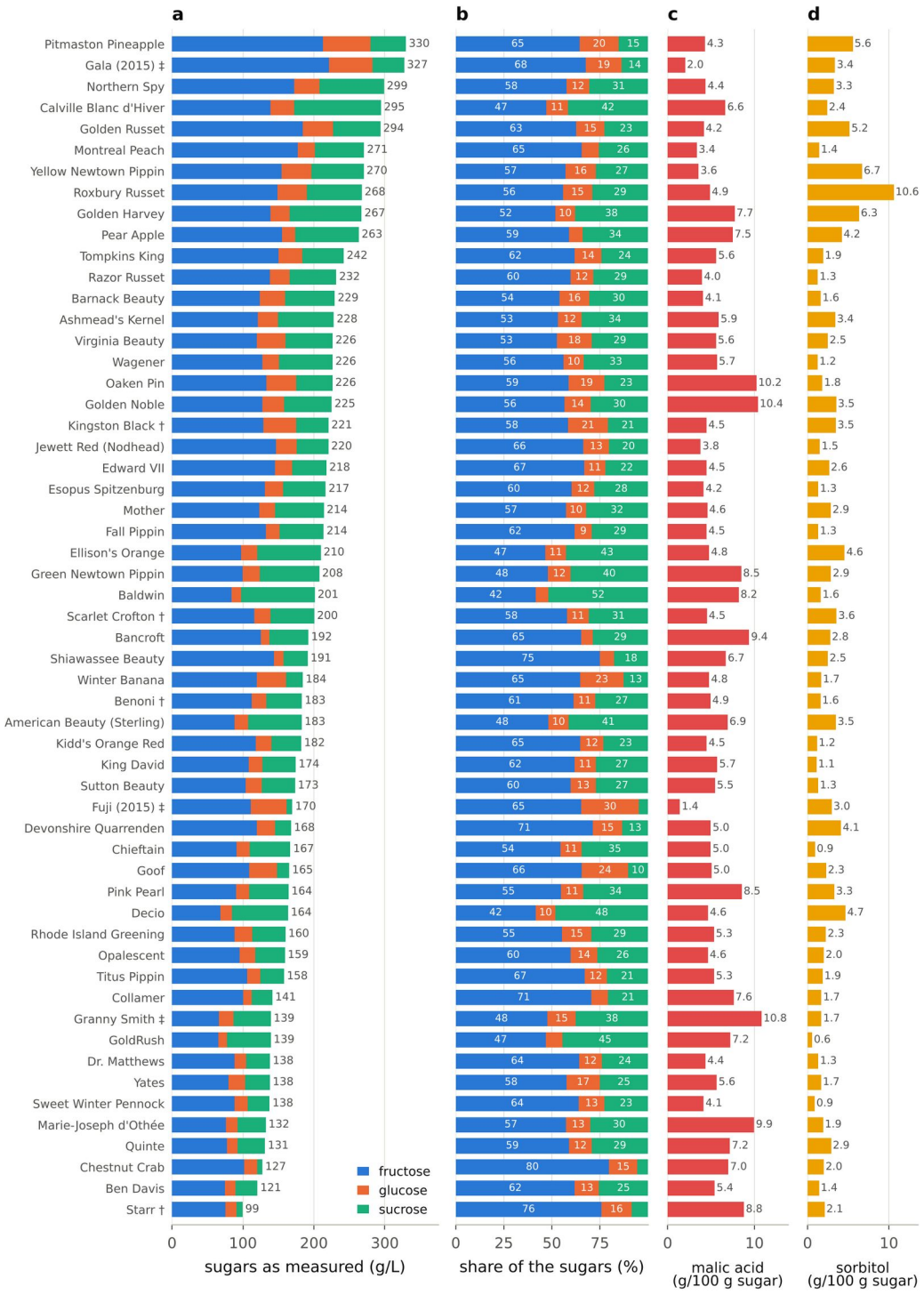

Figure 4. Sugars, malic acid and sorbitol in the 56 apple juices, ordered by the sum of the sugars. (a) Fructose, glucose and sucrose per liter of juice; the number is their sum. (b) Shares of the three sugars (% by mass). (c) Malic acid and (d) sorbitol per 100 g of sugars. † juices in which fermentation had begun; ‡ supermarket fruit. Reproduced from PNA-WP-CIDER v3.0.[1]

4.5 Implications for cider making

Taken together, the measurements characterize the old varieties of the collection as sharps with the polyphenol content of dessert fruit: fruit that gives a clean, acidic, lightly structured cider of the kind that dominates the regional craft products of the survey, and that would need bittersweet fruit in the blend, or a malolactic fermentation, to approach the traditional European styles.[3,32] Three further properties are read from the same spectrum: the low glucose share, which favors fructose in a sweet cider; the sorbitol content, which predicts the body that a completely dry cider will retain; and the asparagine content, which indicates the speed of the fermentation. In the multivariate analysis the juices formed a continuum along the sucrose share, glucose and sorbitol, and malic acid, without natural clusters, and partial least squares (PLS) regression on the spectra reproduced the sugars, sorbitol and malic acid with ratios of performance to deviation of 10 to 28, which is sufficient for screening.[1]

5. What the Spectrum Tells About Hard Cider

Each step of the cider-making process leaves a record in the composition of the product (Figure 5). The fruit determines the acids, sorbitol and polyphenols; the fermentation leaves ethanol, glycerol, succinic acid and the higher alcohols; bacteria exchange malic for lactic acid and, in some cases, glycerol for 1,3-propanediol; sweetening is recognized by the amount and the kind of sugar; and the preservative is itself observed.[1,2] The composition of all 96 ciders is displayed in Figure 6, and the properties that the spectrum reveals are collected in Table 3.



Figure 5. The cider-making process. Center: the sequence of operations. Left: the choices at each step that distinguish the principal styles. Right: the constituents by which each step is recorded in the ¹H NMR spectrum of the juice or of the finished cider. Reproduced from PNA-WP-CIDER v3.0.[1]

5.1 Alcohol

Among the 93 alcoholic ciders, ethanol ranged from 3.0 to 10.4 % v/v with a median of 5.9 %.[1] The boundary of 7 % v/v is of regulatory interest, because it separates cider labeled under the food rules of the Food and Drug Administration from cider labeled as wine under the rules of the Alcohol and Tobacco Tax

and Trade Bureau.[33,34] Declarations of 6.8 or 6.9 % v/v were conspicuously frequent, and 6 of the 20 ciders so declared were found at or above 7 %. Given the tolerance allowed to a label, no single product can be called mislabeled on this evidence, but the ordinary variation between batches carries a craft cider declared just below the boundary across it in a substantial fraction of cases. Methanol, which is released from the pectin of the fruit and not formed by fermentation,[35] was found in 68 ciders at 13 to 233 mg/L, the highest values belonging to ciders made with added fruit or grape skins and to a perry.

5.2 Residual sugar: how much, and of which kind

Residual sugar extended from nil to 102 g/L, with a median of 21.7 g/L, and it is the property in which ciders differ most.[1] The spectrum gives not only the amount but the kind of sugar, and the kind reveals how the sweetness was obtained. An arrested fermentation leaves the sugars of the juice enriched in fructose, because yeast consumes glucose first; sweetening with juice or concentrate restores all three sugars together with malic acid and sorbitol; and sweetening with refined sugar adds a single compound, sucrose.[2,36] Sucrose was the principal sugar in 13 ciders, ten of them mass-market products. Because sucrose is hydrolyzed slowly at the pH of cider, its presence indicates a comparatively recent addition, while its absence proves nothing. High ratios of fructose to glucose, found in several semi-sweet craft ciders, are the mark of a fermentation that stopped, or was stopped, after the yeast had consumed nearly all of the glucose.

5.3 Acids and malolactic conversion

Malic and lactic acids are complementary, and their relation is the clearest record of the microbiology of a cider. In the malolactic fermentation lactic acid bacteria decarboxylate L-malic acid to L-lactic acid, which lowers the acidity and replaces a sharp acid with a softer one.[2,37] By this criterion 36 of the 96 ciders were lactate-dominant, but the distribution over the market was uneven: one of the nineteen mass-market ciders, five of the seven traditional European ciders, all four home-made ciders and 25 of the 63 regional craft ciders.[1] Malolactic conversion is thus avoided almost without exception by the large producers and occurs, by intention or not, in two fifths of the craft products. Lactic acid reached 9.2 g/L in one sour cider, far more than the malic acid of an apple juice can supply, so that in such ciders lactic acid must also have been formed from sugar. Acetic acid followed lactic acid: its median was 145 mg/L, but the twelve ciders with 1 g/L or more were all lactate-dominant sour or farmhouse-style products. Succinic acid, a product of the yeast, rose with the alcohol content and lay between 0.2 and 0.8 g/L in nearly all ciders.

The spectrum also records lot-to-lot differences that a label does not. In the survey, one lot each of two craft products that are otherwise malate-dominant contained no malic acid and about 3 g/L of lactic acid, whereas the other lots of the same products contained 4.3 to 4.7 g/L of malic acid; these two lots had undergone a complete malolactic fermentation that the others had not, and the acidity perceived by the consumer differs accordingly between cans carrying the same label. In another case two cans of the same batch of one product differed in sugar and alcohol, the swollen can having refermented in the package.[1]

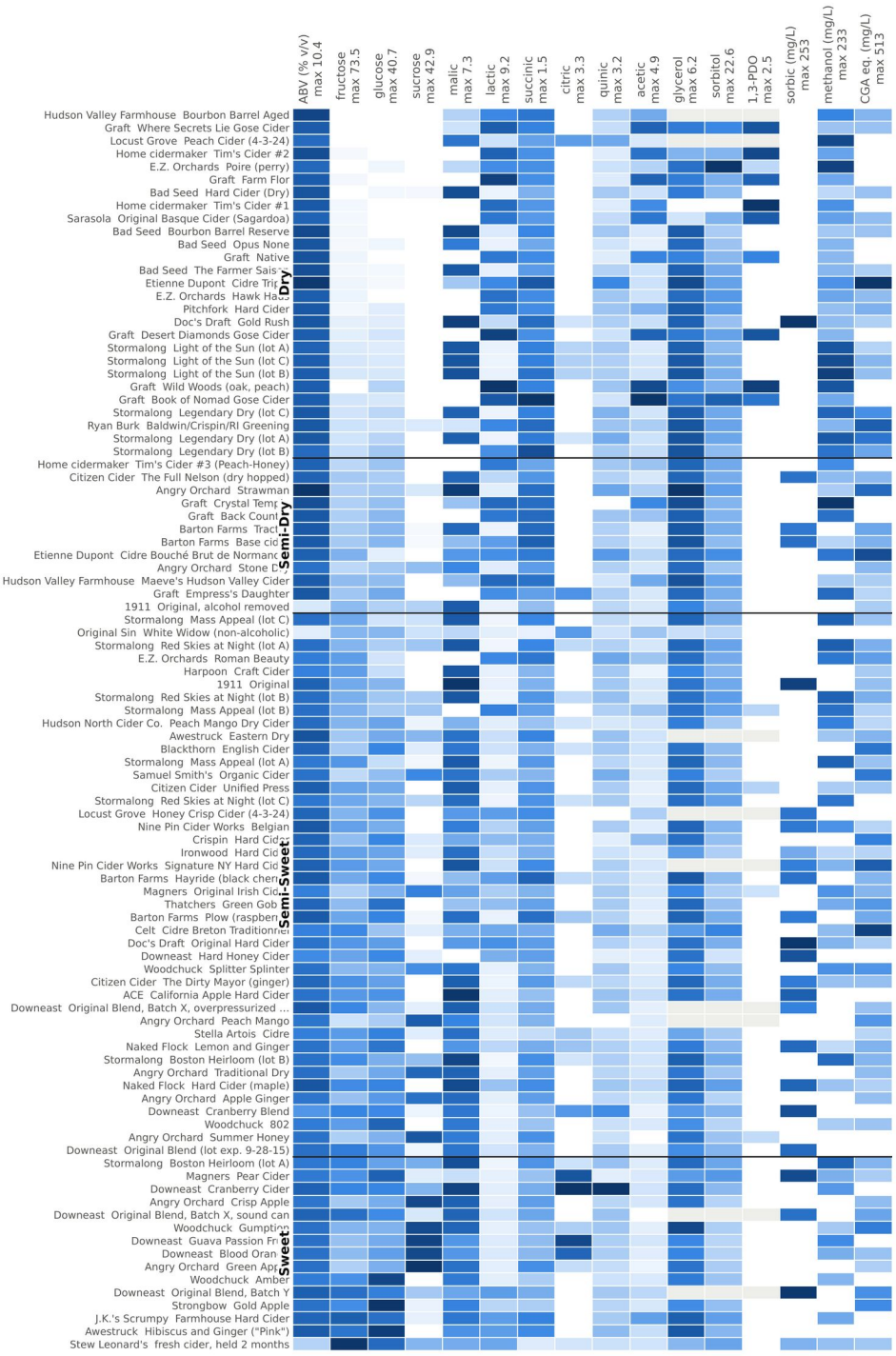

Figure 6. Composition of the 96 ciders by ¹H qNMR, ordered by residual sugar and grouped by dryness class (American Cider Association). Each column is scaled to its own maximum, given in the heading (g/L unless stated otherwise); the color scale follows the square root of the scaled value. Gray cells: not determined (no freeze-dried tube). Reproduced from PNA-WP-CIDER v3.0.[1]

5.4 Glycerol, 1,3-propanediol and the signature of natural cider

Glycerol is formed by the yeast in proportion to the ethanol, and its mass ratio to ethanol had a median of 0.069 in the survey, the range expected for an alcoholic fermentation.[1,38] The ciders that fall far below this relation are the instructive ones. Certain heterofermentative lactic acid bacteria, among them *Lactobacillus collinoides*, now *Secundilactobacillus collinoides*, convert glycerol by way of 3-hydroxypropionaldehyde to 1,3-propanediol;[39,40] the aldehyde can lose water to give acrolein, which reacts with the polyphenols to give the bitterness most feared in Basque natural cider.[41,42] 1,3-Propanediol itself is tasteless, stable and non-volatile, and it remains in the cider as a permanent record of the conversion. In the spectrum its central methylene group gives a quintet at 1.78 ppm, in a region where nothing else in cider absorbs, so that the spectrum shows at a glance whether, and to what extent, the glycerol of a cider has been metabolized by bacteria (Figure 7a).

Nine ciders contained 0.9 to 2.5 g/L of 1,3-propanediol: the one Basque natural cider of the survey, two home-made ciders and six craft ciders made by spontaneous or mixed fermentation.[1] All nine were lactate-dominant and contained more than 1.5 g/L of acetic acid, and acetic acid rose with 1,3-propanediol across the whole collection (correlation coefficient 0.83; Figure 7d), as the coupling of the two reactions in the metabolism of the bacteria requires.[40] In the Basque cider, Sarasola Sagardoa, only 0.2 g/L of glycerol remained against the 3.4 g/L that a fermentation to 5.9 % v/v would be expected to produce, and 1.6 g/L of 1,3-propanediol had taken its place. A balance in moles showed that glycerol and 1,3-propanediol together accounted for 62 % of the expected glycerol in that cider (Figure 7c), the remainder having gone to other products of the pathway. The combination of exhausted malic acid, high lactic and acetic acids and conversion of glycerol is the chemical signature of natural cider, and the survey found it in American ciders as well as in the Basque one.

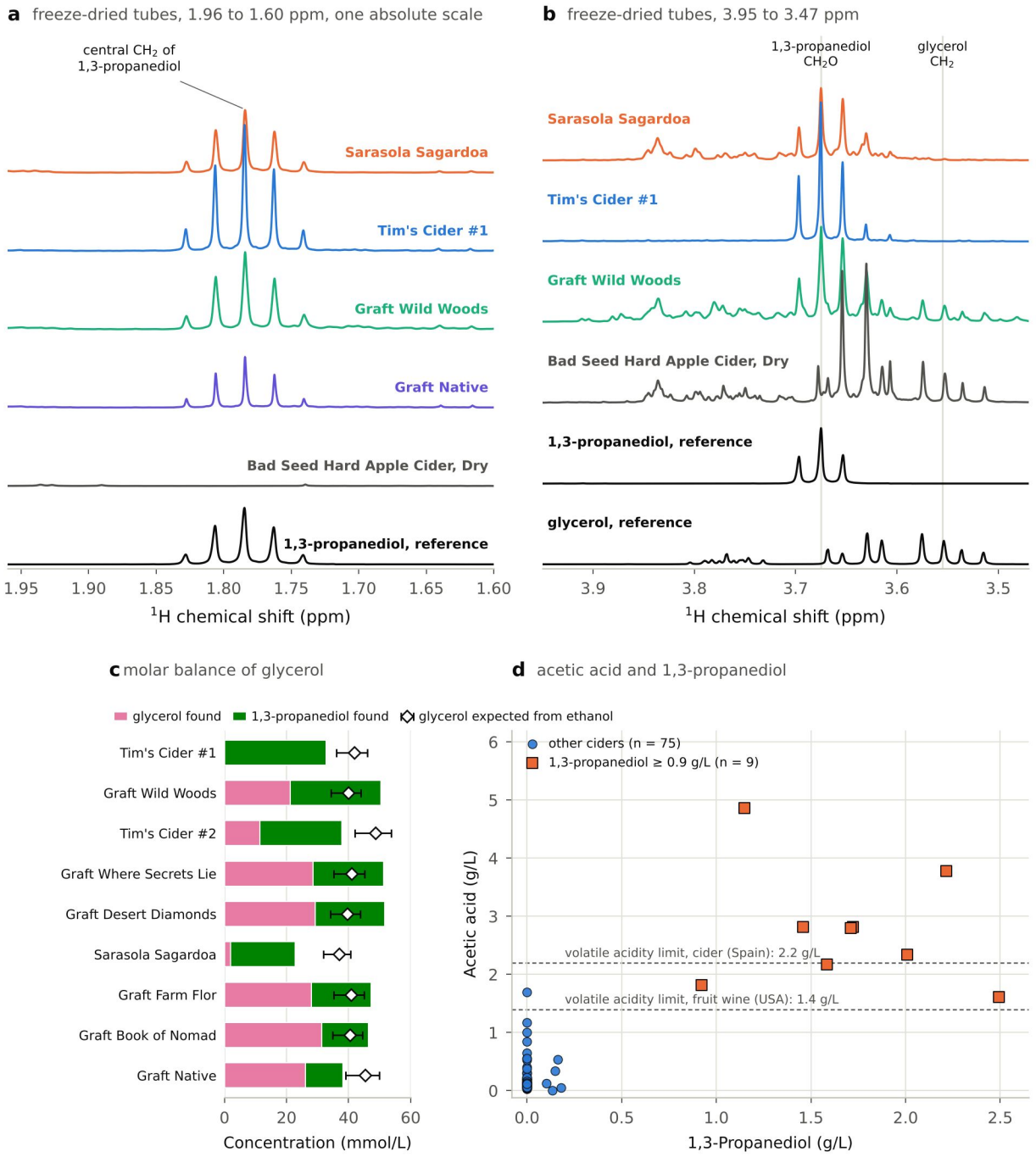

Figure 7. Natural cider and the bacterial conversion of glycerol. (a) The quintet of 1,3-propanediol at 1.78 ppm in the freeze-dried tubes of four ciders, compared with a dry cider that contains none and with the reference compound. (b) The region of the glycerol and 1,3-propanediol methylene signals. (c) Molar balance of glycerol in the nine ciders that contain 1,3-propanediol: glycerol and 1,3-propanediol found, and glycerol expected from the ethanol content. (d) Acetic acid against 1,3-propanediol in 84 ciders; the broken lines mark regulatory limits for volatile acidity. Reproduced from PNA-WP-CIDER v3.0.[1]

5.5 Preservation, heat treatment and added fruit

Several minor constituents report on what was done to the cider after fermentation. Sorbic acid, from added potassium sorbate, was confirmed in 24 ciders at 44 to 253 mg/L; twenty-one of them were regional craft ciders and twenty of the 24 were semi-sweet or sweet.[1] This is the expected pattern: a large producer stabilizes a sweetened cider by pasteurization or sterile filtration, a small one by adding sorbate. 5-Hydroxymethylfurfural, formed when fructose is heated, was detected in ten ciders at 1.4 to 6.2 mg/L and indicates the use of concentrate, of a cooked sweetener or of pasteurization. Citric acid above 0.8 g/L occurred only in products with declared fruit other than apple, such as cranberry, blood orange, guava and passion fruit, and in a pear cider, and quinic acid stood out in the two cranberry ciders, as the composition of cranberry juice predicts. Sorbitol, at a median of 3.7 g/L, rose to 22.6 g/L in the perry, as the composition of pears demands, and a content well above 6 g/L in an apple cider is consistent with pear or another sorbitol-rich fruit among the ingredients.[1]

5.6 Polyphenols and the European traditions

Individual polyphenols cannot be determined at 300 MHz in a matrix of this kind, but two integral measures are available: the doublet of the hydroxycinnamoyl esters, expressed as chlorogenic acid, and the integral of the whole aromatic region. Both single out the traditional European ciders, whose hydroxycinnamate content averaged about 290 mg/L, several times that of the American craft and mass-market ciders, and whose aromatic intensity was twice as high.[1] This is the spectroscopic expression of the bittersweet apple,[30,31] and it is the property in which most American ciders, made from dessert and heirloom fruit, differ from the European tradition.

Table 3. What the ¹H NMR spectrum of a cider reveals, the constituents on which each property rests, and the findings of the survey of 96 ciders.[1]

Property	Constituents	Finding in the survey
Strength	Ethanol	3.0 to 10.4 % v/v; label agreement −0.06 ± 0.71 % v/v
Sweetness and how it was obtained	Fructose, glucose, sucrose; fructose/glucose ratio	0 to 102 g/L; sucrose principal sugar in 13 ciders
Malolactic conversion	Malic and lactic acids	36 of 96 lactate-dominant; 1 of 19 mass-market
Volatile acidity, sour styles	Acetic acid	Median 145 mg/L; 12 ciders at 1 g/L or more
Bacterial glycerol conversion (natural cider)	1,3-Propanediol, glycerol, acetic acid	9 ciders, 0.9 to 2.5 g/L of 1,3-propanediol
Body of a dry cider	Glycerol, sorbitol	Polyols 1.8 to 25.8 g/L (median 7.3)
Stabilization	Sorbic acid	24 ciders, 44 to 253 mg/L
Heat treatment or concentrate	5-Hydroxymethylfurfural	10 ciders, 1.4 to 6.2 mg/L
Added fruit or pear	Citric and quinic acids, sorbitol	Citric above 0.8 g/L only with other fruit
Fruit and pectin	Methanol	68 ciders, 13 to 233 mg/L
Bittersweet fruit, European style	Hydroxycinnamates, aromatic envelope	Several times higher in European ciders
Refermentation, batch differences	Sugars against ethanol; acids between lots	One refermented can; two lots with unexpected conversion

6. From Composition to the Consumer: Dryness and Other Scales

No term on a cider label is used less consistently than dry. The American Cider Association classifies by residual sugar alone, dry below 9 g/L, semi-dry to 18 g/L, semi-sweet to 45 g/L and sweet above;[43] the Beer Judge Certification Program uses five classes with other boundaries;[44] and the orchard-based scale of the

New York Cider Association classifies by the ratio of sugar to acid, recognizing that acidity suppresses the perception of sweetness.[45,46] European rules again use residual sugar but with different boundaries, the Spanish standard calling a cider *extra-seca* up to 20 g/L.[47] A cider with 25 g/L of sugar is therefore semi-sweet in the United States, *seca* in Spain and *brut* in France, a difference of vocabulary that only a statement of the measured sugar content resolves. Because the spectrum gives sugar and acid together, any of these scales can be applied to the same result.

On the scale of the American Cider Association, 27 of the 96 ciders were dry, 12 semi-dry, 42 semi-sweet and 15 sweet, and the word dry on a label did not predict the class: products with dry in their name contained from 0.2 to 14 g/L or more of sugar.[1] The orchard-based scale separated the dry ciders usefully but had no resolving power for the sweeter commercial ones. For that reason seven further scales were derived from the same spectrum, for balance, acidity, acid profile, body, aromatic character, alcohol tier and energy. The balance index, a sweetness equivalent that credits fructose, glycerol and sorbitol with their relative sweetness, divided by the acidity, resolves the whole market in five classes, from bone dry to sweet, and the acid profile, malic, mixed, lactic or lactic with acetic, is the most direct indication of style that the spectrum offers. Figure 8 shows six ciders of contrasting style placed on these scales. The boundaries are proposals derived from the survey and would need sensory benchmarking before they were adopted for communication with consumers.

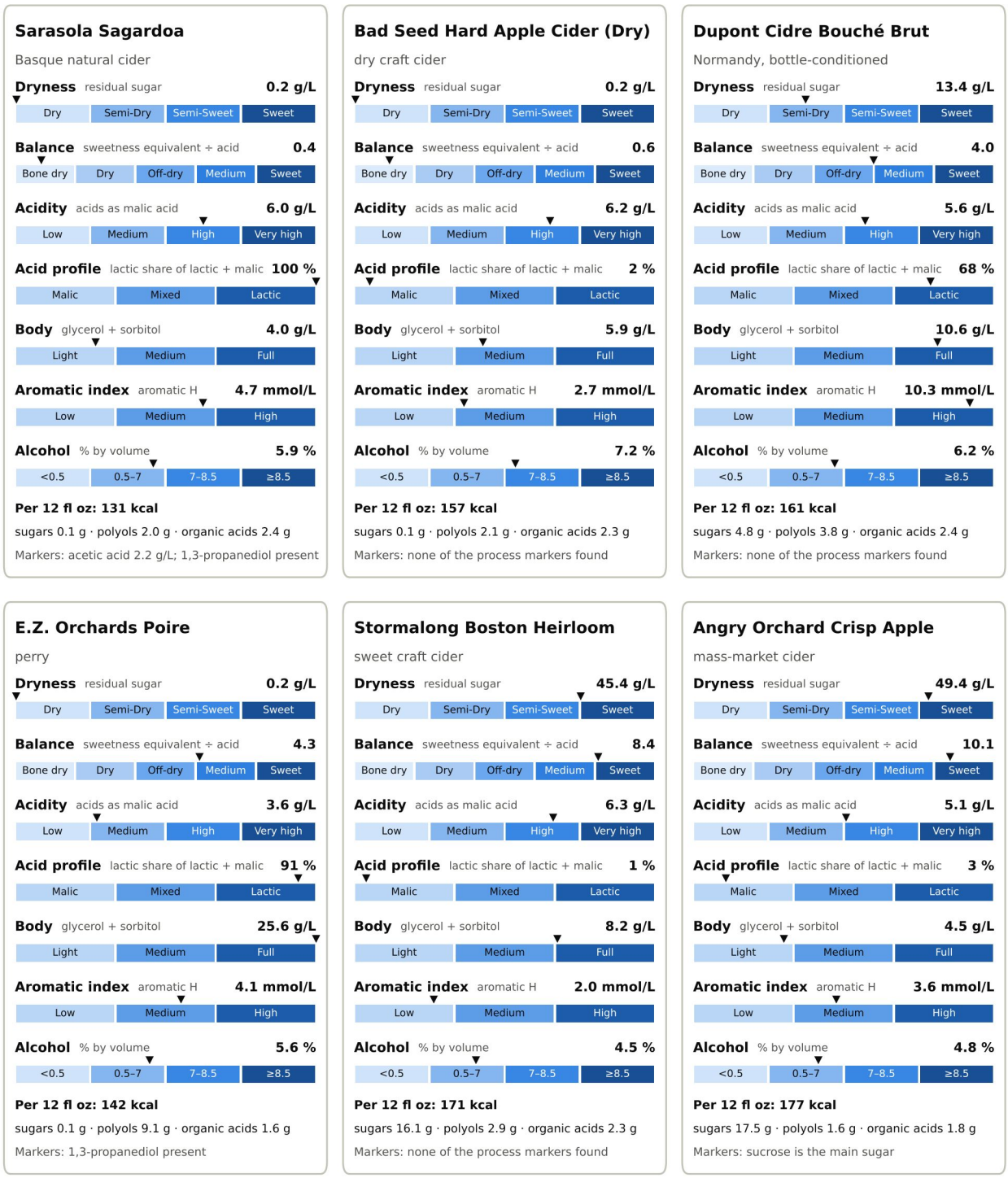

Figure 8. Profile cards of six ciders of contrasting style, calculated from the ¹H qNMR composition: placement on the dryness scale of the American Cider Association and on six of the further scales proposed in the white paper (the triangle marks the measured value within its class), with energy, sugars, polyols and organic acids per 12 fl oz and the process markers found. Reproduced from PNA-WP-CIDER v3.0.[1]

7. Nutrition Labeling from the Spectrum

The energy of a beverage is calculated from its composition with conversion factors: 7.07 kcal/g for ethanol, 4 kcal/g for the sugars, reduced factors for the sugar alcohols, 2.6 kcal/g in the case of sorbitol, and conventionally 3 kcal/g for the organic acids.[33,48] ¹H qNMR supplies every term of this calculation from one measurement. The energy of a 12 fl oz serving of the alcoholic ciders of the survey ranged from 115 to 247 kcal. The polyols and organic acids, which a calculation from alcohol and sugar alone omits, contributed about a tenth of the energy of a dry cider and about 5 g of carbohydrate per serving, which is more than some labels declare in total.[1] Of the eight ciders whose labels stated an energy value, one was exceeded by more than the 20 % that the tolerances admit.[34]

Complete nutrition panels can therefore be produced from a single spectrum (Figure 9), and the same composition supplies the entries of the Alcohol Facts statement proposed in 2025 for wines, spirits and malt beverages, namely the alcohol content, the fluid ounces of pure alcohol, the calories and the carbohydrate of a serving.[49] The values that NMR cannot supply, sodium and the other minerals, protein, fat and dietary fiber, are close to zero in cider or are better taken from other measurements, and are marked as such on the panels.

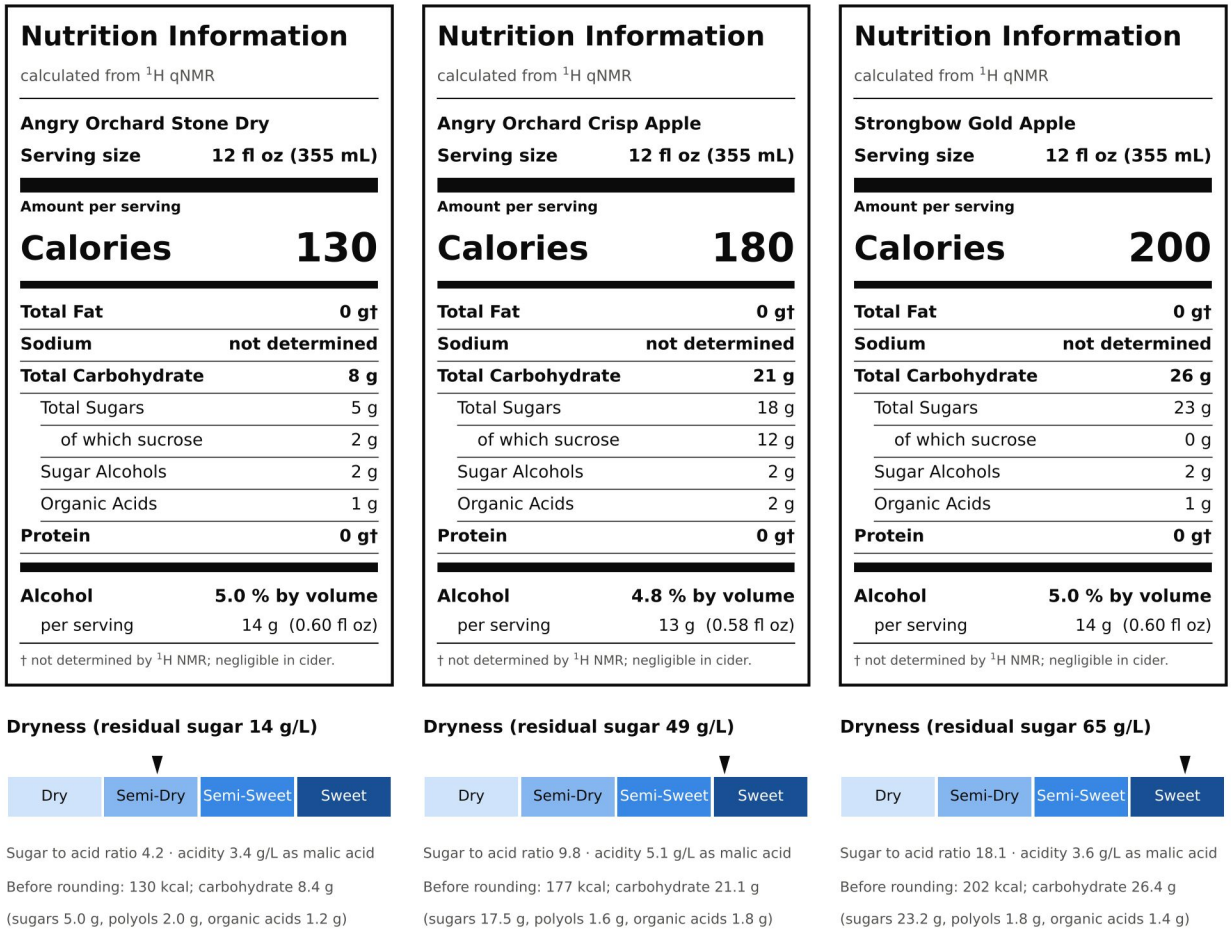

Figure 9. Nutrition panels calculated from the ¹H qNMR composition for three ciders (serving of 12 fl oz; values rounded according to the food labeling regulations), each with the placement of its residual sugar on the dryness scale of the American Cider Association. The panels are illustrations derived from the measurements and are not the labels of the producers. † not determined by ¹H NMR. Reproduced from PNA-WP-CIDER v3.0.[1]

8. What Multivariate Analysis Adds

Beyond the targeted analysis, the spectrum can be treated as a pattern and analyzed with the tools of chemometrics: principal component analysis, clustering, non-linear maps, supervised classifiers and PLS regression.[50,51] The white paper examined what this approach adds, and the answer is more modest than is often supposed. For data that are referenced to an internal standard, the spectra are best kept on the absolute scale that the standard provides, mean-centered and Pareto-scaled; whether uniform bins or optimized buckets are used made no difference.[1,52] The ciders formed a continuum governed by residual sugar, its kind and the acid profile, without natural clusters.

Classes defined by the composition, such as dryness, dominant acid and the presence of sucrose, were recognized from the spectra almost without error, which confirms the information content of the bucket table but is no more than the targeted analysis already provides. The distinction between mass-market and craft ciders, which is not defined by the composition, was recognized with a balanced accuracy above 0.8, also within a single measurement campaign, in cross-validation that never tested a model on a producer it had been trained on. Declared adjuncts were recognized by their individual markers, citric acid for added fruit and so on, and not by a common model. PLS regression on the spectra reproduced alcohol, sugars, the principal acids and energy with ratios of performance to deviation above 7 and estimated the total polyols from the native spectrum alone within about 0.6 g/L.[1] Such models are useful where a fast screen is wanted, but they inherit their accuracy from the targeted analysis on which they are trained, and with fewer than one hundred samples their validation must be strict, with nested cross-validation and permutation tests, before they are relied upon.[53–55]

9. Using the Method in Practice

The measurement serves different purposes at different points of the chain from orchard to consumer. For the grower and the cider maker at the press, a spectrum of the juice gives the sugar, the sucrose share, the malic acid and the sharp-or-sweet character of the fruit, the sorbitol that will carry into the cider and the asparagine that will feed the yeast, from which the potential alcohol, the acidity of the finished cider and the likely speed of the fermentation can be judged. During fermentation the same measurement follows the consumption of glucose and fructose, the formation of ethanol, glycerol and succinic acid, and the onset of malolactic conversion, as NMR studies of apple-juice fermentation have demonstrated.[56,57]

For the finished product, the analysis supplies the alcohol for the label and for the classification at 7 % v/v, the residual sugar for any dryness scale, the energy and carbohydrate for a nutrition panel, and a check on the stability of a sweetened cider through its sorbate content and the presence of fermentable sugar. For troubleshooting, the spectrum shows refermentation in the package, unexpected malolactic conversion, excessive volatile acidity and the bacterial conversion of glycerol that precedes acrolein bitterness. For questions of authenticity and style, it records whether a cider was sweetened with juice or with sugar, whether fruit other than apple was used, whether it was heat-treated, and whether it carries the phenolic signature of bittersweet fruit; established NMR procedures for juice authentication rest on the same principles.[23,24] Comparable procedures are in routine use for wine and beer,[17,18] and benchtop instruments operating at 60 to 80 MHz have been shown to quantify the principal constituents of fermented beverages, which brings the measurement of alcohol and the major sugars and acids within reach of a production site.[58]

10. Limitations

The method has limits that a user should know. At 300 MHz the carbohydrate region is crowded, and glycerol and sorbitol are obtained from the decomposition of overlapping signals rather than from resolved ones, so that they carry a larger uncertainty than the sugars with anomeric doublets or the acids. Individual polyphenols, the aroma esters and most volatile aroma compounds, and sulfur dioxide are not measured; the

phenolic measures are integrals, and the hydroxycinnamates of most American ciders lie close to their detection limit. Without the freeze-dried tube, the minor non-volatile constituents are lost below higher reporting limits and the polyols become less certain. Sucrose continues to hydrolyze slowly in the acidified sample, so that all sucrose values are lower limits.[1]

The more fundamental limitation concerns trueness. The agreement with the declared alcohol, the repeatability and the consistency of the two tubes demonstrate that the measurement is precise and internally consistent, but for the sugars, acids and polyols of these samples no values by an independent reference method, such as enzymatic assays or HPLC, were available, and the accuracy of those constituents rests on the gravimetric standard and on the published validations of comparable procedures rather than on a direct comparison.[19,59] A set of such comparisons, together with spike recoveries and a formal estimate of uncertainty, is the step required before the method is offered within an accredited scope.

Finally, the transfer of the method into a standalone analyzer program has identified several points that will be revised in version 3.1 of the white paper. Among them are the separation of small amounts of lactic acid from the ¹³C satellite of ethanol, the position and tube from which 2,3-butanediol is taken, a more realistic detection limit for the window integrals of the hydroxycinnamates, and the probable reassignment of a weak signal at 1.58 ppm, reported tentatively as alanine, to the hydrate of pyruvic acid, which remains to be confirmed by spiking. None of these points affects the principal constituents or the conclusions summarized here, but the affected minor values should be read as provisional.

11. Conclusions

A single ¹H NMR measurement with an internal standard, supplemented by a second measurement of the freeze-dried sample, determines the alcohol, the three sugars, the principal organic acids, glycerol, sorbitol and a series of minor constituents and markers of a cider, and the sugars, acids, sorbitol and markers of variety and nutrition of an apple juice. Applied to 56 apple juices, it characterized the old varieties of a heirloom collection as sharps with widely differing sucrose and sorbitol contents, and it placed the modern dessert apples outside their range of acidity. Applied to 96 ciders, it showed that ciders differ chiefly in two independent respects, the amount and kind of residual sugar, which is a decision of the producer, and the microbiological history recorded in the acids and polyols, which the fermentation decides. It identified malolactic conversion, the bacterial conversion of glycerol to 1,3-propanediol that marks natural cider, sweetening with sugar, stabilization with sorbate, heat treatment and added fruit, and it supplied the values from which any dryness designation and any nutrition label can be derived.

What the spectrum offers the cider maker is therefore more than a list of concentrations: the composition of the fruit and of the product, the record of the fermentation, the designation of dryness in the vocabulary of any market, and the nutrition values of the label, all from one sample. Its limits lie in the overlap of signals at moderate field, in what NMR does not see, and in the absence so far of an independent check of trueness for the minor constituents, which is the next step in the development of the method.[1]

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Summary

Scope. This document summarizes white paper PNA-WP-CIDER v3.0: how quantitative ¹H NMR is applied to apple juice and hard cider and what it revealed about 56 apple juices and 96 ciders from 36 producers measured at 300 MHz between 2013 and 2026.

Method. Signal areas are proportional to the number of protons, so concentrations follow from comparison with a gravimetric maleic acid standard without compound-specific calibration. Each cider is measured as received (volatile constituents) and after freeze-drying (non-volatile constituents), the two tubes being tied by a bridging factor. Areas are obtained by integration, constrained line fitting and decomposition of the carbohydrate region with pure-component spectra. Alcohol agreed with the label to −0.06 ± 0.71 % v/v (n = 71); repeatability was 1 to 2 % for the principal constituents.

Apples. The old varieties contained 59 % fructose, 13 % glucose and 28 % sucrose on average, with sucrose shares of 5 to 52 %; 48 of 53 are sharps by their malic acid; sorbitol varies more than tenfold and is highest in the russets; asparagine, quinic and citramalic acids and L-rhamnitol add markers of nitrogen supply and variety.

Ciders. Alcohol 3.0 to 10.4 % v/v; residual sugar 0 to 102 g/L, its kind revealing arrested fermentation or sweetening with juice or sugar; malolactic conversion in 36 ciders, almost never in mass-market products; 1,3-propanediol from bacterial glycerol conversion in nine, the signature of natural cider; sorbic acid in 24, 5-hydroxymethylfurfural in ten, citric acid as a marker of added fruit; polyphenol signals highest in the European traditions.

Scales and labels. 27 ciders are dry, 12 semi-dry, 42 semi-sweet and 15 sweet on the scale of the American Cider Association, and the word dry on a label does not predict the class; further scales for balance, acidity, acid profile and body are derived from the same spectrum. Energy per 12 fl oz is 115 to 247 kcal, and complete nutrition panels are calculated from one spectrum.

Chemometrics and limits. Multivariate analysis confirms the distinctions of the targeted analysis, separates mass-market from craft ciders with a balanced accuracy above 0.8 and predicts the principal constituents by PLS regression, but adds little that the composition does not provide. The limitations are the overlap of signals at 300 MHz, the absence of individual polyphenols and aroma compounds, and the lack, so far, of an independent reference method for the sugars, acids and polyols.