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Quantification of organic acids in beer by nuclear magnetic resonance (NMR)-based methods

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ABSTRACT

The organic acids present in beer provide important information on the product's quality and history, determining organoleptic properties and being useful indicators of fermentation performance. NMR spectroscopy may be used for rapid quantification of organic acids in beer and different NMR-based methodologies are hereby compared for the six main acids found in beer (acetic, citric, lactic, malic, pyruvic and succinic). The use of partial least squares (PLS) regression enables faster quantification, compared to traditional integration methods, and the performance of PLS models built using different reference methods (capillary electrophoresis (CE), both with direct and indirect UV detection, and enzymatic essays) was investigated. The best multivariate models were obtained using CE/indirect detection and enzymatic essays as reference and their response was compared with NMR integration, either using an internal reference or an electrical reference signal (Electronic REference To access In vivo Concentrations, ERETIC). NMR integration results generally agree with those obtained by PLS, with some overestimation for malic and pyruvic acids, probably due to peak overlap and subsequent integral errors, and an apparent relative underestimation for citric acid. Overall, these results make the PLS-NMR method an interesting choice for organic acid quantification in beer.

1. Introduction

High-resolution nuclear magnetic resonance (NMR) spectroscopy has been extensively used for the characterization of complex liquid foodstuffs, presently being increasingly applied in tandem with automation and multivariate analysis methods in order to handle large sample numbers. This strategy has been applied to fruit juices [1–4], balsamic vinegar [5–7], coffee [8], olive oil [9–12], wine [13–16] and beer [17–20].

The advantages of NMR in food analysis relate to it enabling direct sample analysis and detection of large numbers of compounds in a single experiment. However, although NMR is a quantitative technique, compound quantification in complex mixtures such as foodstuffs remains a less than straightforward application. One of the approaches is the traditional method of NMR integration vs. the signal area of a reference compound, with applications in vinegars [5,6], wines [15,21], beer [17,20] and juices [4,22–24]. However, the use of internal references for quantification in complex mixtures poses potential difficulties such as (1) signal overlap and (2) chemical interactions between the reference compound and sample components, possibly leading to changes in signal area and/or shape and subsequent erroneous quantification. Some reports describe the use of the Electronic REference To access In vivo Concentrations (ERETIC) method as an alternative to the use of internal standards [25,26]. The method uses an electronic reference signal thus avoiding the potential problems arising from the use of internal standards [27,28] but, to our knowledge, this has not been applied to intact food samples.

When compound quantification by NMR is considered for routine application to large sample populations, chemometric methods are increasingly preferred to the traditional method of NMR signal integration. This alternative approach aims at correlating the NMR spectra with quantitative measurements given by a reference analytical method thus circumventing the difficulties associated with time-consuming and error-prone signal integration. This has been applied, mainly through the use of partial least squares (PLS) regression, to wine [14] and beer [17,19] and fruit juices [4].

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Table 1
Beer sample sets employed as calibration and test sets.

Beer sample groups/analytical method	No. of beer production dates	No. of bottles/date	Total no. of samples
Calibration sets			
Group A/CE with indirect detection	3	12	36
Group B/CE with direct detection	3	9	27
Group C/enzymatic essays	6	3	18
Test sets			
Group D	1	6	6
Group E	1	8	8

This paper describes a comparative study of the quantification of organic acids in beer, using different NMR-based methods. Organic acids play an important role in beer, not only contributing to flavour, colour and aroma properties, but also because they are good indicators of fermentation performance [29,30]. The quantification of these compounds in beer is usually performed by a variety of chromatographic methods [31,32] and capillary electrophoresis (CE) has also been increasingly employed [17,33–35]. NMR spectroscopy is an attractive alternative method for organic acid quantification in beer and, in fact, this has been explored before using signal integration and PLS regression, with CE with indirect detection [17] and enzymatic essays (for lactic acid only) [19] as reference methods. In this work, ^{1}H NMR is used to quantify the six main organic acids in beer (acetic, citric, lactic, malic, pyruvic and succinic acids), performing a comprehensive evaluation and comparison of the main possible analytical approaches. These are (1) PLS regression models built with different reference methods: CE/indirect detection, CE/direct detection and enzymatic assays and (2) NMR integration both vs. an internal reference compound and using the ERETIC method. These methods are hereby compared for the first time, to our knowledge, for the quantification of beer organic acids and the results should enable the best analytical choice to be made for the quantification of the main organic acids in beer.

2. Experimental

2.1. Beer samples

All beer samples were of the same brand (lager beer) and were kindly donated by UNICER, Bebidas de Portugal. All samples were selected randomly so as to mirror the actual composition characteristics of the commercial product. Several groups of samples, differing in date of production, were employed for the development of multivariate models (calibration sets) and for their testing (test sets), as shown in Table 1. The bottled samples were kept unopened at 4 °C, for a period of up to 15 days prior to analysis.

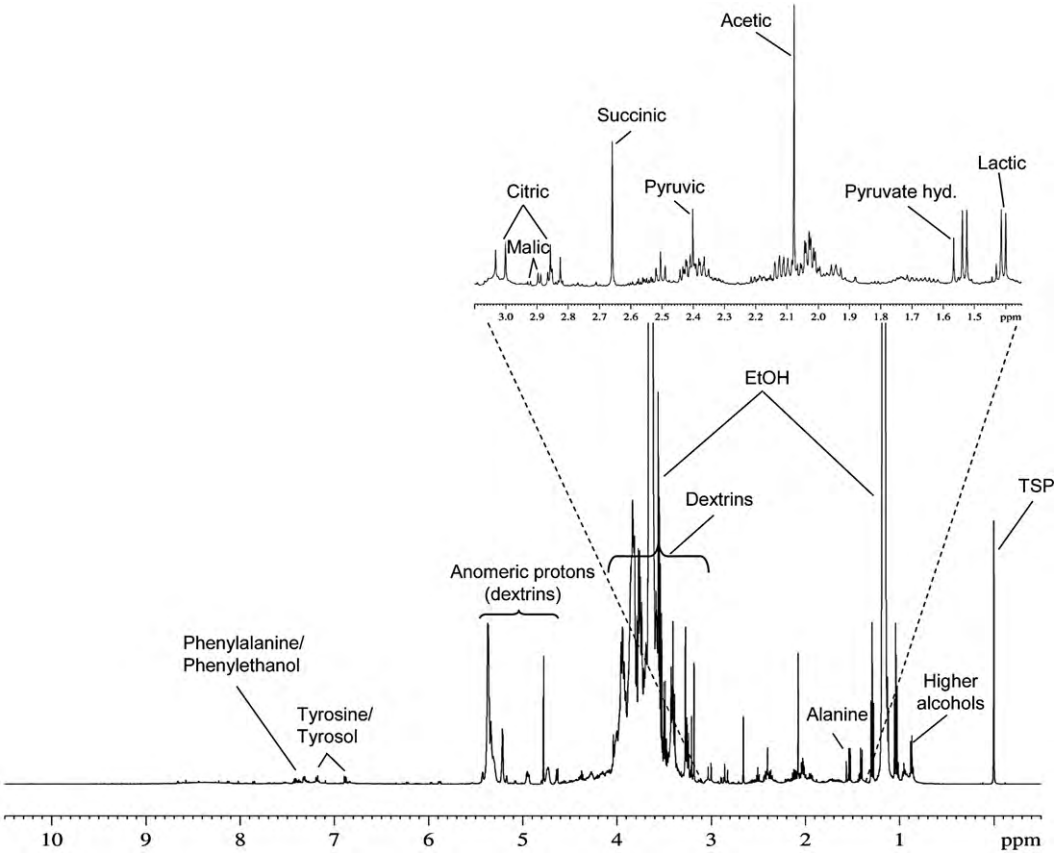

Fig. 1. 500 MHz ^{1}H NMR spectrum of beer with some assignments indicated. The inset shows the expansion of the spectral region dominated by the organic acids signals, together with higher alcohols (isobutanol, isopentanol and propanol) and amino acids.

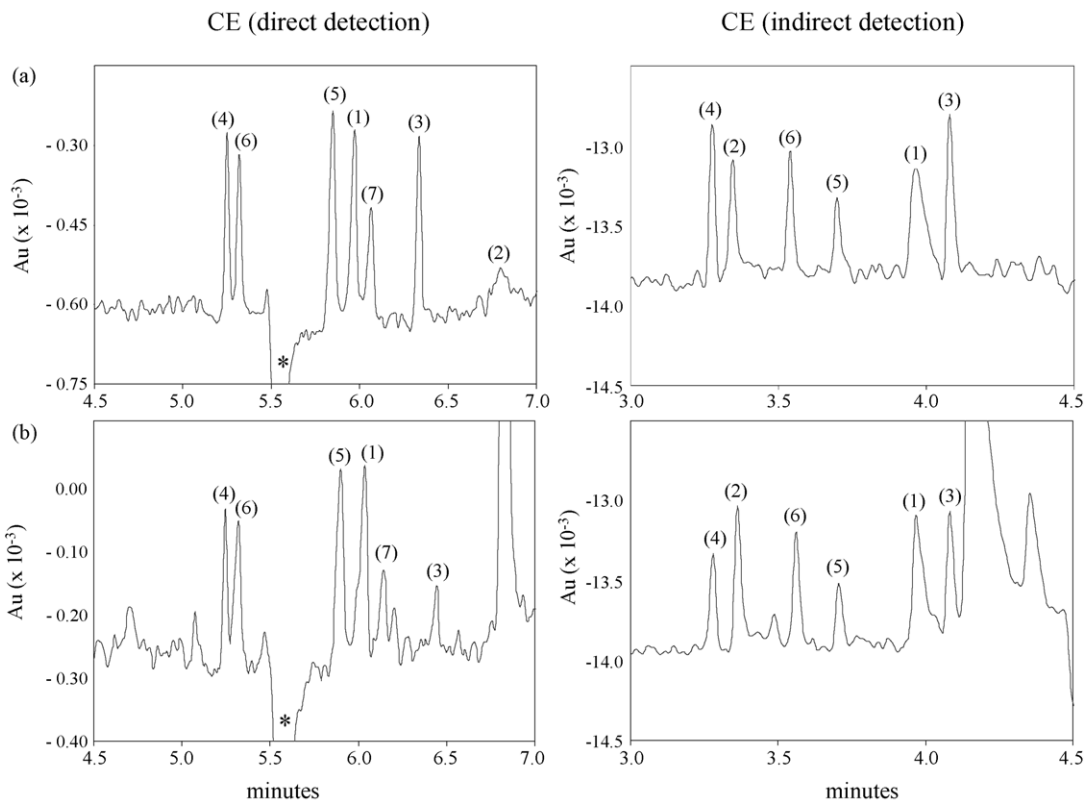

Fig. 2. CE electropherograms of (a) a standard organic acid mixture and (b) beer (diluted of 1:3) recorded with direct (left) and indirect (right) UV detection. (1) Acetic, (2) citric, (3) lactic, (4) malic, (5) pyruvic, (6) succinic and (7) glyoxylic acids (internal standard). *System peak.

Table 2
Results obtained for the PLS1-NMR regression models and reference methods applied for the quantification of organic acids in beer.

Organic acids	δ (ppm), multiplicity, assignment[a]	PLS1 models results					Reference methods results	
		LV[b]	R^2Y (%)[c]	RMSECV (%)[d]	RMSEP (%)[e]	R[f]	Linear range/(mg L^{-1})[g]	r[g]
Capillary electrophoresis (direct detection)								
Acetic	2.08, s, CH_3	3	94.0	5.5	8.8	0.97	20.1–140.1	0.97
Citric	2.84, d, CH 3.01, d, CH	–	–	–	–	–	–	–
Lactic†	1.41, d, CH_3	5	91.8	8.5	20.3	0.96	25.5–177.6	0.98
Malic†	2.89, dd, CH 2.92, dd, CH	6	72.6	6.5	16.5	0.85	20.4–141.9	0.98
Pyruvic	2.40, s, CH_3	4	92.1	4.6	10.0	0.96	15.0–105.0	0.98
Succinic†	2.66, s, CH_2	8	76.5	5.5	18.9	0.87	15.3–106.5	0.97
Capillary electrophoresis (indirect detection)								
Acetic†	2.08, s, CH_3	8	93.0	9.7	25.4	0.96	39.9–159.9	0.79
Citric	2.84, d, CH 3.01, d, CH	8	83.5	4.1	9.9	0.91	30.0–300.0	0.99
Lactic†	1.41, d, CH_3	5	78.3	17.9	32.1	0.89	25.2–250.8	0.98
Malic	2.89, dd, CH 2.92, dd, CH	8	92.2	2.8	8.9	0.96	20.1–201.0	0.99
Pyruvic	2.40, s, CH_3	8	96.0	5.1	13.2	0.98	15.0–150.0	0.96
Succinic	2.66, s, CH_2	6	91.6	2.7	4.7	0.96	15.0–150.0	0.98
Enzymatic assays								
Acetic	2.08, s, CH_3	4	88.2	5.4	10.6	0.91	20.0–180.0	0.98
Citric	2.84, d, CH 3.01, d, CH	6	92.2	2.6	7.63	0.96	55.0–274.8	0.99
Lactic†	1.41, d, CH_3	4	76.1	12.1	24.9	0.87	20.7–206.6	0.99
Malic†	2.89, dd, CH 2.92, dd, CH	6	91.2	6.8	25.2	0.96	40.6–202.9	0.98
Pyruvic	2.40, s, CH_3	6	87.5	3.9	11.4	0.94	5.0–35.0	0.98
Succinic	2.66, s, CH_2	4	82.4	8.8	16.6	0.91	30.4–121.5	0.95

[a] Assignments refer to resonances in the spectral region considered: 1.35–3.10 ppm; s, singlet; d, doublet; dd, doublet of doublets.
[b] Number of significant latent variables obtained by cross-validation.
[c] Explained accumulated variance of y data for each PLS1-NMR model.
[d] Root mean square error of cross-validation.
[e] Root mean square error of prediction.
[f] Correlation coefficients corresponding to the PLS1-NMR models.
[g] Linearity range used and correlation coefficients obtained for the calibration curves of each reference method.
† Acids for which the PLS1-NMR performance is low.

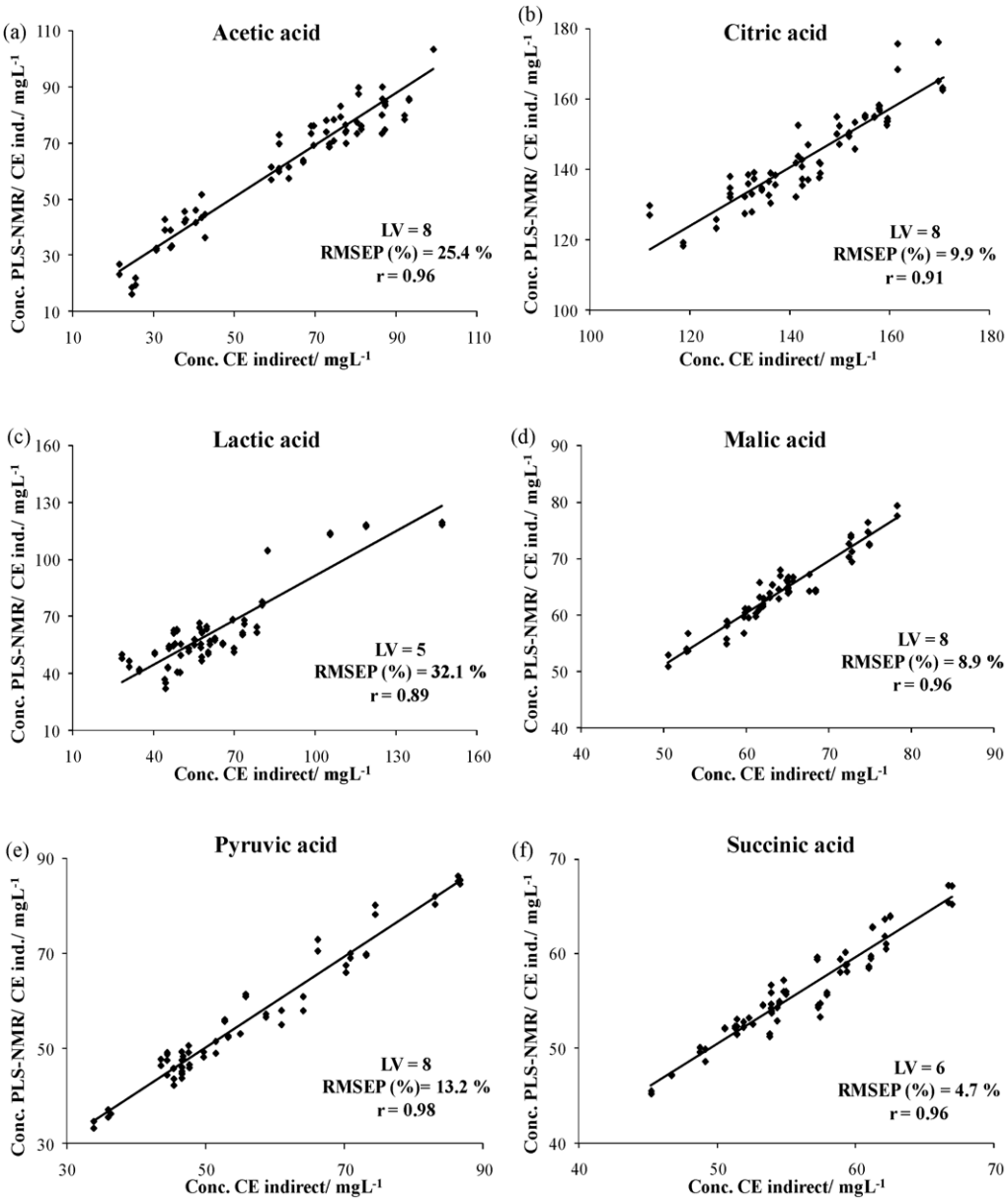

Fig. 3. PLS1-NMR prediction models using CE with indirect UV detection as the reference method for (a) acetic, (b) citric, (c) lactic, (d) malic, (e) pyruvic and (f) succinic acids. The number of latent variables (LV), RMSEP (%) and correlation coefficients (*r*) are shown for each model.

2.2. Chemicals

All chemicals employed were of analytical grade: deuterium oxide (D_2O) and 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt (TSP-d_4), glyoxilic monohydrate, malic and pyruvic acids (Aldrich), tetradecyltrimethylammonium bromide (TTAB), citric and lactic acids (Sigma), sodium dihydrogen phosphate dihydrate and disodium hydrogen phosphate (Fluka), 2,6-pyridinedicarboxylic acid (PDC), calcium chloride dihydrate, potassium dihydrogen phosphate, sodium hydroxide, hydrochloric acid and acetic acid (Merck), cethyltrimethylammonium bromide (CTAB), ethylenediaminetetraacetic acid (EDTA) (Panreac) and succinic acid (Carlo Erba).

2.3. Sample preparation

Beer samples (10 mL) were degassed in an ultrasonic bath for 10 min. For NMR analysis, samples were prepared to contain 10% D_2O and 0.025% TSP-d_4 as chemical shift and intensity reference. Samples pH was adjusted to 1.90 ± 0.03 adding 8–10 μL HCl 5% in D_2O, in order to ensure extensive protonation of carboxylic groups and reduce peak shifts.

For CE analysis, samples were diluted 3 times and filtered through a 0.22 μm PDVF membrane filter. For enzymatic analysis, the degassed beer was used without any preparation.

2.4. NMR spectroscopy

For PLS1-NMR regression studies, each spectrum was acquired on a Bruker Avance DRX 500 spectrometer, using the *noesypr1d* pulse sequence (Bruker pulse program library) with water presaturation. No ethanol presaturation was employed to avoid saturation effects in the aliphatic region of the spectrum. 128 transients were collected into 32,768 (32 K) data points, with mixing time of 100 ms, spectral width of 8013 Hz, acquisition time of 2.0 s and relaxation delay of 5 s.

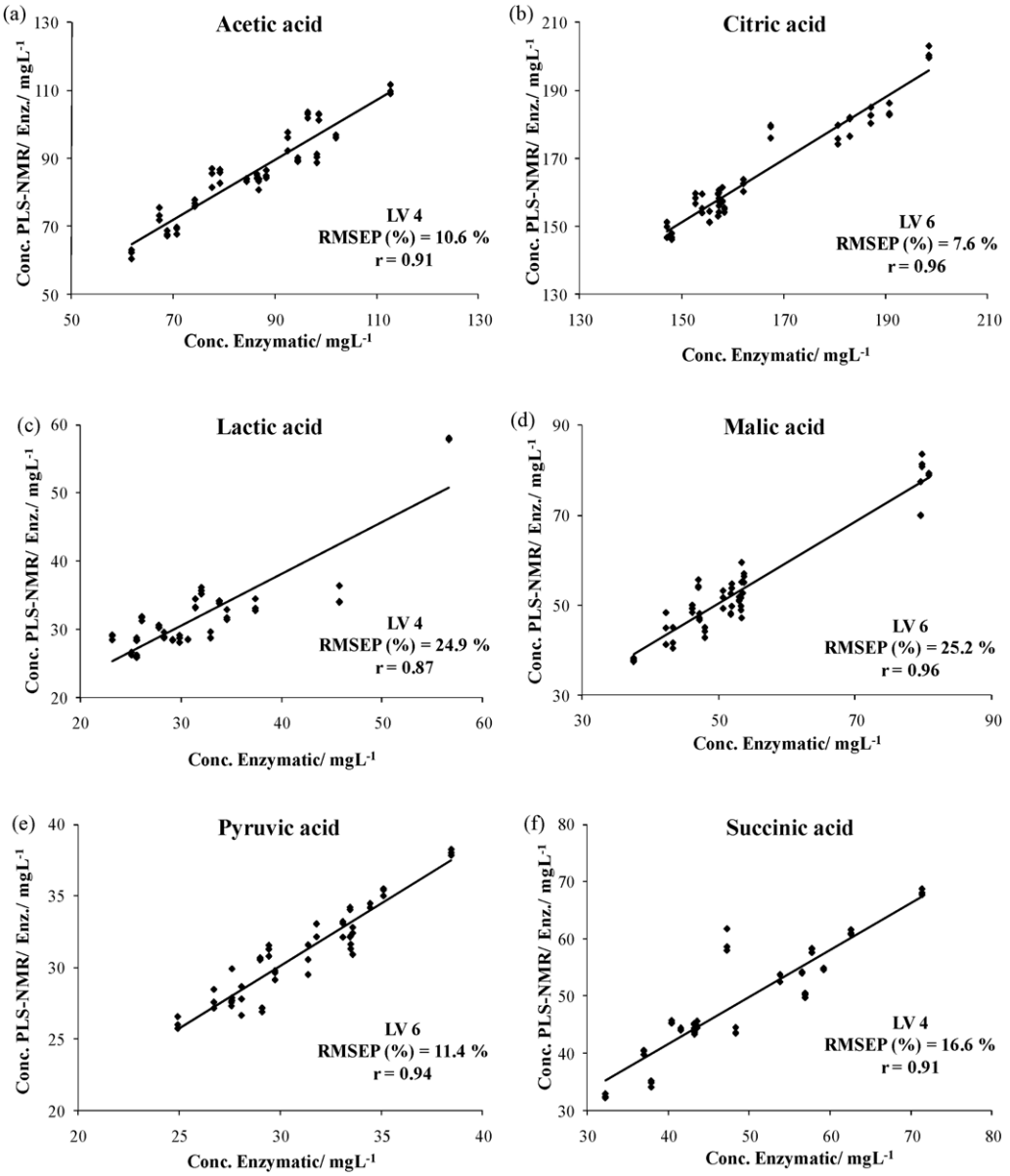

Fig. 4. PLS1-NMR prediction models using enzymatic assays as the reference method for (a) acetic, (b) citric, (c) lactic, (d) malic, (e) pyruvic and (f) succinic acids. The number of latent variables (LV), RMSEP (%) and correlation coefficients (r) are shown for each model.

For quantification by integration vs. TSP or by ERETIC, quantitative conditions (relaxation delay $\geq 5 \times T_{1\text{longest}}$) were employed, based on the T_1 relaxation times measured (3.8 s acetic, 1.1 s citric, 1.3 s lactic, 1.5 s malic, 2.9 s pyruvic and 1.7 s for succinic acids). Spectra used for integration vs. TSP were recorded on a Bruker Avance DRX 500 spectrometer, at 300 K, using the *zgpr* pulse sequence (single 90° pulse experiment with water suppression), with 128 transients collected into 32,768 (32 K) data points, spectral width of 8013 Hz, acquisition time of 2.0 s and relaxation delay of 20 s. ERETIC measurements were recorded on a Bruker Avance III 600 spectrometer, at 300 K, using the same pulse sequence, with 128 transients collected into 65,536 (64 K) data points, spectral width of 12,336 Hz, acquisition time of 2.7 s and relaxation delay of 25 s. A standard succinic acid solution (119.90 mg L^{-1}) was used to calibrate the intensity of the electronic signal of ERETIC.

2.5. Capillary electrophoresis (CE) analysis

All experiments were performed using a Beckman P/ACE MDQ CE system, equipped with a diode array detector. Standards and samples were injected using 0.3 psi (2068 Pa) pressure for 2 s. Separations were carried out in a fused silica capillary of 78 cm total length and 75 μm internal diameter, at a potential of −25 and −17 kV (reverted polarity) for direct and indirect UV detection, respectively, maintaining a constant capillary temperature of 18 °C. For indirect detection, a method previously developed for the analysis of organic acids in port wine [36] was used, employing the 196 nm wavelength, together with reference wavelength at 350 nm. The run buffer used was composed of 5 mM of PDC and CTAB 0.5 mM, acting as electroosmotic modifier, with pH adjusted to 5.60 ± 0.05, with 1 M NaOH. In order to eliminate interferences from trace metals on the determination of citric acid, 0.01 mM of EDTA was added to the buffer. For direct detection, the method reported in Ref. [37] was used, employing a wavelength of 195 nm. The run buffer was composed of 7.5 mM NaH_2PO_4 and 2.5 mM Na_2HPO_4 with TTAOH 2.5 mM and $CaCl_2$ 0.24 mM being used as electroosmotic modifier and selectively modifier, respectively, and pH adjustment to 6.4 ± 0.05, with 1 M NaOH. Glyoxilic acid was added as internal standard although it was eventually not used, due to peak overlap

with contributions from the beer matrix. Tetradecyltrimethylammonium hydroxide (TTAOH) was prepared from the bromide salt (TTAB) using a strong anion-exchange resin AG MP-1 (Bio-rad). All solutions were filtered through a 0.22 μm filter before use.

2.6. Enzymatic assays

The reagents for the enzymatic assays used were purchased from Megazyme. The assays were based on an increase/decrease in absorbance at 340 nm, caused by a change in nicotinamide-adenine dinucleotide (reduced form), NAD(H). Absorbances were measured in an ASYS UVM spectrometer reader using 96 well microplates and results were affected by an average associated error of 8%.

2.7. Quantitative analysis by NMR signal integration

The signals with lower overlap (based on observation of both 1D and 2D spectra) were chosen for integration (acetic at 2.08 ppm, citric at 3.01 ppm, lactic at 1.41 ppm, malic at 2.89 ppm, pyruvic at 2.40 and 1.56 (hydrate) ppm and succinic at 2.66 ppm), nevertheless overlapping contributions were noted namely from proline (2.02 and 2.37 ppm), isopentanol (1.40 ppm) and some unassigned signals (e.g. at 3.01 ppm). The integration limits were chosen at the valleys on each side of the peak base and, in cases where these points were not at baseline level, baseline correction was applied. When using the TSP signal as area reference, the concentration of each compound (in mg L^{-1}), m_X, was calculated as: $m_X = (A_X/A_{TSP}) \times (m_{TSP}/(MW_{TSP}/no.H_{TSP})) \times (MW_X/no.H_X)$, where m_{TSP}: concentration of TSP, A_X and A_{TSP}: peak areas, MW_X and MW_{TSP}: molecular weights and $no.H_X$ and $no.H_{TSP}$: number of hydrogens corresponding to the peaks from compound X and TSP. For quantification by ERETIC, calibration of the electronic reference signal was carried out with a succinic acid standard solution (119.90 mg L^{-1}) and the methylene signal at 2.66 ppm. The equivalent concentration of the electronic signal, m_{ERETIC}, was determined by: $m_{ERETIC} = (A_{ERETIC}/A_{REF.}) \times m_{REF}$ where A_{ERETIC} and A_{REF} are the areas of the electronic signal and calibration peak (succinic acid), respectively. The electronic signal was then used to determine the concentration of analyte X, m_X, by computing: $m_X = (A_X/A_{ERETIC}) \times (m_{ERETIC}/no.H_X) \times (MW_X/MW_{REF})$, where A_X: peak area for the analyte, $no.H_X$: number of hydrogens corresponding to the analyte signal and MW_X and MW_{REF}: molecular weight of analyte and calibration compound (succinic acid), respectively.

2.8. Regression models

For PLS, namely PLS1, regression analysis, data matrices **X** were built using the aliphatic region from 1.35 to 3.10 ppm. Spectral bucketing was employed with variable bucket width to minimize peak shifts due to small pH differences and incomplete protonation. Capillary electrophoresis (direct and indirect UV detection) and enzymatic essays were used as reference methods (the **y** data vector) and all PLS1 studies were performed using software co-developed by the University of Aveiro and the AgroParisTech, France. In order to facilitate and improve the interpretation of the PLS1 regression models, the loadings weights profiles were coloured as a function of the correlation between each NMR data point and the organic acid content, as expressed in the **y** data vector.

3. Results and discussion

Fig. 1 shows a typical spectrum of a lager beer, dominated by resonances arising from dextrins, in the mid field region, and from organic acids and amino acids in the remaining regions. The expansion of the 1.35–3.10 ppm region clearly shows resonances from all six main organic acids to be quantified in this work: acetic, citric, lactic, malic, pyruvic and succinic. This subregion was chosen for the built up of all PLS1-NMR regression models as well as for the integration of selected peaks for each organic acid.

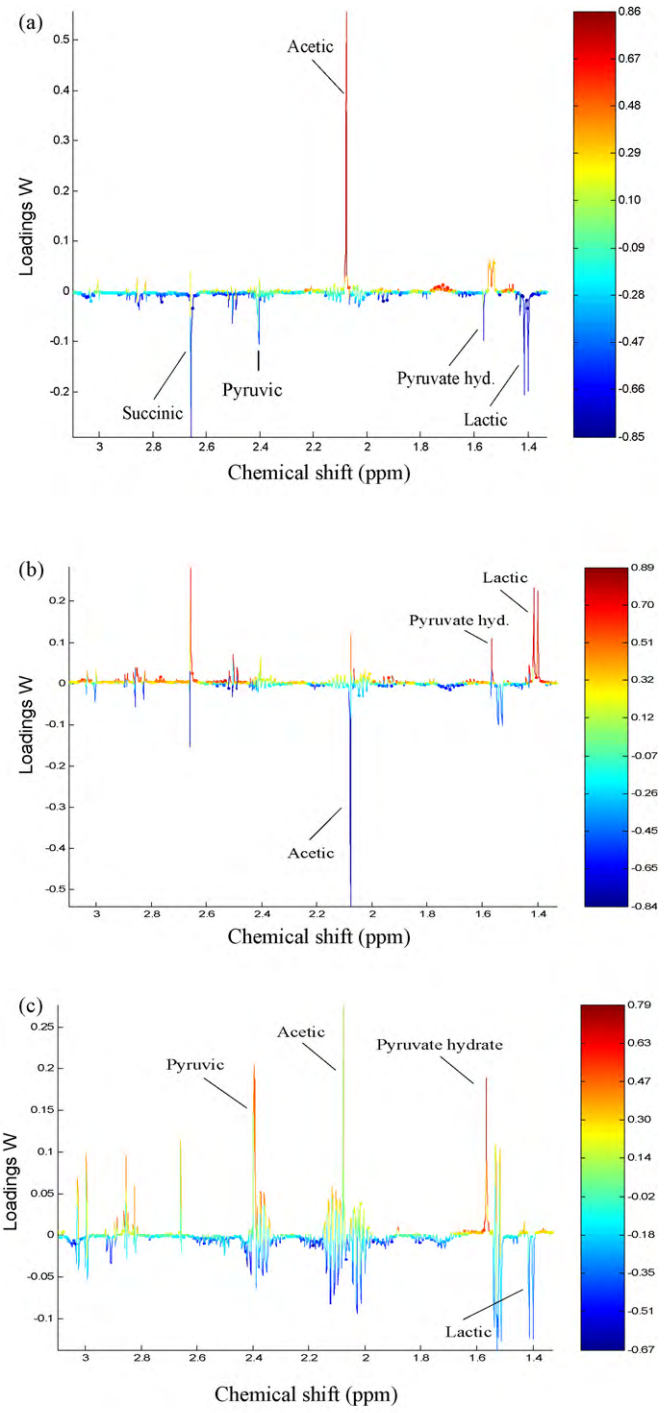

Fig. 5. Loadings weights (W) (LV1) colour plot obtained for (a) acetic acid and (b) lactic acid, by PLS1-NMR/CE direct, and (c) pyruvic acid, by PLS1-NMR/CE indirect. Colour plot reflects the correlation between each NMR signal and the organic acid content: red and blue for extreme positive and negative correlations, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

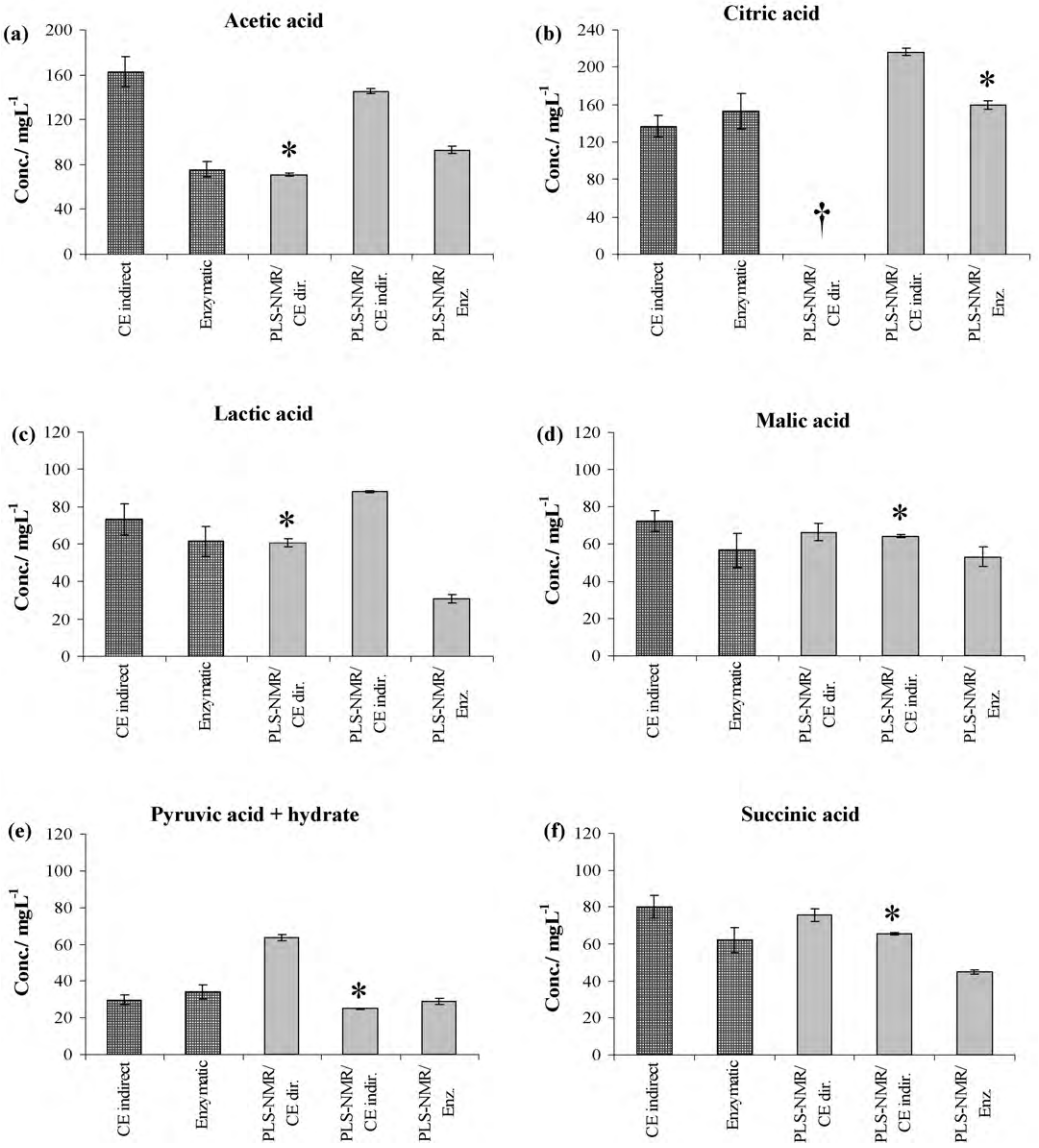

Fig. 6. Column plots of organic acid concentrations obtained by the direct application of the reference methods CE/indirect detection and enzymatic assays (darker bars) and the PLS1-NMR models developed (PLS1-NMR/CE direct, PLS1-NMR/CE indirect and PLS1-NMR/Enzymatic). †Model not developed due to difficulties in citric acid detection by CE indirect detection. *PLS1-NMR model showing best performance with basis on the parameters shown in Table 2.

3.1. Quantification of organic acids in beer by PLS1-NMR regression models

PLS1-NMR regression models were built using CE (with either direct or indirect detection) and enzymatic essays as reference analytical methods, however, prior to the discussion of the specific PLS1 regression models obtained, the response of each reference method is hereby discussed. Fig. 2 shows the CE electropherograms obtained for a standard mixture of the six organic acids (Fig. 2a) and a beer sample (Fig. 2b), using both direct and indirect UV detection (respectively, left and right in Fig. 2). All assignments were confirmed using standard solutions of each acid. It is clear that the mode of detection significantly determines the quality of the electropherograms, particularly affecting citric acid detection (peak 2). In fact, a broad peak is obtained for citric acid in direct detection (Fig. 2a, left) whereas, if indirect detection is employed, the same acid shows up clearly at a different separation time (the difference in migration times between the two electropherograms is due to differences in buffer pH and ionic strength). Indirect detection seems to suit most organic acids in the standard mixture, with only some broadening being noted for peak 1 (acetic acid). Consistently, the CE calibration curves obtained for direct UV detection (Table 2, right columns) give correlation coefficients $r > 0.97$ for all acids, except for citric acid, which cannot be quantified. For indirect UV detection, calibration curves are characterized by $r > 0.96$ with the exception of acetic acid ($r = 0.79$), reflecting the peak broadening noted before (Fig. 2a). When beer samples are considered (Fig. 2b), additional (unidentified) peaks arising from the beer matrix hinder citric acid detection further (peak 2) and overlap significantly with peak 7 (reference compound glyoxylic acid) in direct mode, while affecting peaks 1 and 3 (acetic and lactic acids) in indirect mode. Furthermore, some peaks are clearly broadened in direct detection mode: peaks 1 (acetic acid) and 6 (succinic acid), the latter affecting the neighbouring peak 4 (malic acid). In addition to CE analysis, enzymatic assays were used to quantify all six organic acids and good linearity ($r > 0.95$) (Table 2, right columns) is also obtained for the corresponding calibration curves.

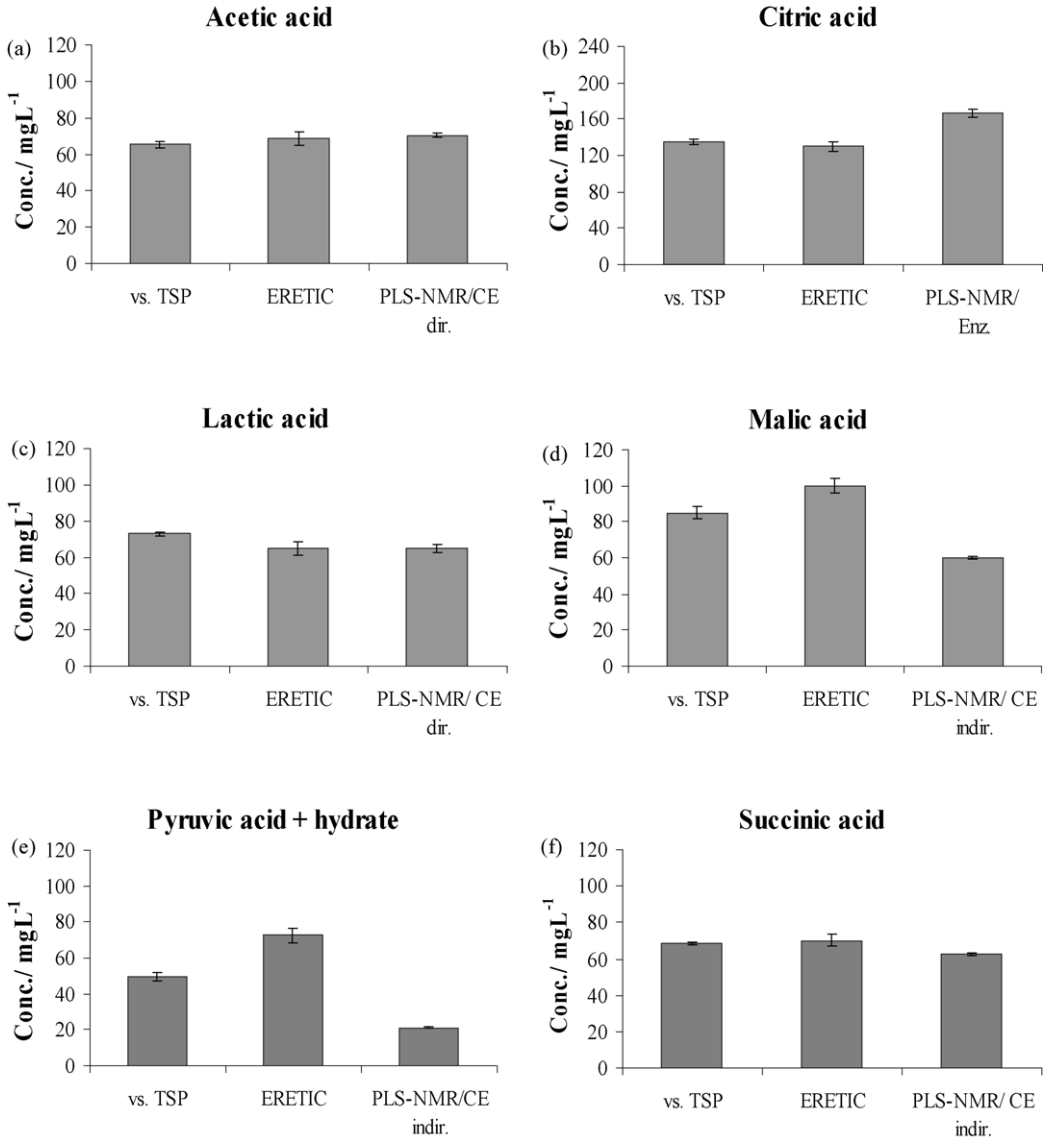

Fig. 7. Column plots of organic acid concentrations obtained by NMR integration vs. TSP, the ERETIC method and the PLS1-NMR model showing best performance (* in Fig. 6).

The PLS1-NMR regression models developed for beer were found to correspond to numbers of Latent Variables (LVs) ranging from 3 to 8 (Table 2). The models using CE/direct detection as the reference method were characterized by accumulated R^2Y ranges from 72.6 to 94% for all acids (excluding citric acid), with lower values (<80%) for malic and succinic acids (probably reflecting the broadening of the corresponding CE peaks, as noted above). Hence, the prediction power of these PLS1 regression models is relatively low for these acids, as expressed by the high values (>15%) of the root mean square error of prediction (RMSEP), and a similar result is also noted for lactic acid. The latter observation probably reflects the fact that the lower intensity of the lactic acid peak in beer (peak 3 in Fig. 2) leaves it rather close to the noise level and, therefore, more prone to quantification error. The regression models obtained for PLS1-NMR with CE/indirect detection (Fig. 3) exhibit reasonable correlation coefficients (>0.89) for all acids, however, a lower accumulative R^2Y value (78.3%) is found for lactic acid, with RMSEP > 25% noted for both lactic and acetic acids. These findings could reflect the peak broadening and overlap effects noted previously for these two acids, in indirect detection mode (Fig. 2). Finally, for the PLS1-NMR model obtained using enzymatic method as reference (Fig. 4, Table 2), good general prediction powers are found, again with the exception of lactic acid (with low accumulative R^2Y and high % RMSEP) and malic acid (with RMSEP > 25%). In the case of malic acid, a cluster of samples is seen at higher concentrations and, if this is removed, the model is indeed significantly improved (RMSEP 14.7%). However, lager beers may contain malic acid in a wide range of concentrations [17,33], thus justifying a model built on the concentration range shown in Fig. 4 (30–90 mg L^{-1}). It is possible, however, that the model obtained is improved if a larger universe of samples is considered. The organic acids identified with the symbol † in Table 2 are those for which lower PLS1-NMR predictive power is found, thus showing that the PLS1-NMR regression models based on CE indirect detection and enzymatic methods are the most suitable (for 4 acids out of the total 6), in spite of none having a satisfactory predictive power for lactic acid.

In order to facilitate the interpretation of the variability contained in each PLS-NMR regression model, Fig. 5 shows examples of LV1 loadings weights profiles obtained for some of the PLS1-NMR regression models. The profiles are coloured as a function of the correlation between each NMR data point and the **y** vector data and they show that maximum correlations (in red) are indeed obtained for the peaks arising from the organic acid under study in each case: acetic and lactic acid by PLS1-NMR with CE/direct detec-

tion (Fig. 5a and b) and pyruvic acid by PLS1-NMR with CE/indirect detection (Fig. 5c). However, in all cases, additional peaks arising from some of the remaining acids are noted with non-zero correlations. These observations should reflect concomitant variations within the different organic acids e.g. in beer with higher acetic acid content, succinic, pyruvic and lactic acids seem to tend to lower concentrations (Fig. 5a).

In order to assess the applicability of the PLS1-NMR regression models compared to those of the reference methods (CE/indirect detection and enzymatic) applied directly, the organic acid concentrations determined by each method were compared for one same beer set, test set D (Table 1, Fig. 6). Considering the best performance PLS1-NMR regression models (noted with * in Fig. 6), as defined according to the model parameters in Table 2, it is clear that the PLS1-NMR results approach those obtained by the reference methods (CE/indirect and enzymatic essay), except for acetic acid for which a large positive deviation is noted for the results obtained with CE/indirect detection. This is probably due to the peak broadening and overlap problems found in the quantification of this acid by this analytical method, as noted before (Fig. 2).

3.2. Comparison of PLS1-NMR regression and NMR integration methods

This part of the work was carried out on a second test set of beer samples (test set E, Table 1) for which quantification by NMR integration vs. TSP and by ERETIC was performed, as well as by the PLS1-NMR regression method selected with basis on its performance (* in Fig. 6).

Comparing the results obtained by integration vs. TSP and by ERETIC (Fig. 7), it becomes clear that the agreement is satisfactory (<10%), with the exception of malic and pyruvic acids (Fig. 7d and e) for which ERETIC gives relatively higher concentrations. In the case of pyruvic acid, this is justifiable by an underlying resonance from proline, possibly made more significant at the higher field at which ERETIC measurements were carried out. It is possible that a similar effect occurs for the less intense resonances arising from malic acid.

Comparison of the results obtained through peak integration with those obtained by the selected PLS1-NMR regression models shows that good agreement exists for acetic, lactic and succinic acids (Fig. 7). Again, in the case of malic and pyruvic acids (Fig. 7d and e), the apparent overestimation given by the integrals is consistent with the suggestion advanced above that underlying resonances affect the resonances of these acids. In the case of citric acid, the reason for the discrepancy between integration and the PLS1 method is less clear and an overestimation by PLS1-NMR may not be ruled out.

4. Conclusion

In this work, different NMR-based methodologies have been used to quantify the six main organic acids found in beer (acetic, citric, lactic, malic, pyruvic and succinic) and their performance compared. PLS1-NMR regression models were built using different reference analytical methods: CE, both with direct and indirect UV detection, and enzymatic essays. The regression models found to perform better were obtained using CE indirect detection and enzymatic essays and their predictive power was compared with the results obtained through NMR integration methods, either using an internal reference or the ERETIC method. Results show that NMR integration methods are in good agreement with the PLS1-NMR models, except for malic and pyruvic, for which integration overestimates concentrations (probably due to additional underlying resonances), and for citric acid, for which an apparent overestimation by PLS1-NMR is observed. Overall, the less time consuming method of PLS1-NMR is found to be suitable for organic acid quantification in beer, as long as the best combination of organic acid and particular PLS1-NMR model (differing in the reference method used for NMR calibration) is employed: PLS1-NMR/CE direct for acetic and lactic acids, PLS1-NMR/CE indirect for malic, pyruvic and succinic acids and PLS-NMR/enzymatic for citric acid.

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References

[1] P.S. Belton, I.J. Colquhoun, E.K. Kemsley, I. Delgadillo, P. Roma, M.J. Dennis, M. Sharman, E. Holmes, J.K. Nicholson, M. Spraul, Food Chem. 61 (1998) 207.
[2] G. Le Gall, M. Puaud, I.J. Colquhoun, J. Agric. Food Chem. 49 (2001) 580.
[3] M. Cuny, E. Vigneau, G. le Gall, I. Colquhoun, M. Lees, D.N. Rutledge, Anal. Bioanal. Chem. 390 (2008) 419.
[4] M. Spraul, B. Schutz, E. Humpfer, M. Mortter, H. Schafer, S. Koswig, P. Rinke, Magn. Reson. Chem. 47 (2009) 130.
[5] R. Consonni, A. Gatti, J. Agric. Food Chem. 52 (2004) 3446.
[6] A. Caligiani, D. Acquotti, G. Palla, V. Bocchi, Anal. Chim. Acta 585 (2007) 110.
[7] R. Consonni, L.R. Cagliani, F. Benevelli, M. Spraul, E. Humpfer, M. Stocchero, Anal. Chim. Acta 611 (2008) 31.
[8] A.J. Charlton, W.H.H. Farrington, P. Brereton, J. Agric. Food Chem. 50 (2002) 3098.
[9] L. Mannina, M. Patumi, N. Proietti, D. Bassi, A.L. Segre, J. Agric. Food Chem. 49 (2001) 2687.
[10] L. Mannina, G. Dugo, F. Salvo, L. Cicero, G. Ansanelli, C. Calcagni, A.L. Segre, J. Agric. Food Chem. 51 (2003) 120.
[11] M. D'Imperio, L. Mannina, D. Capitani, O. Bidet, E. Rossi, F.M. Bucarelli, G.B. Quaglia, A. Segre, Food Chem. 105 (2007) 1256.
[12] R.M. Alonso-Salces, K. Héberger, M.V. Holland, J.M. Moreno-Rojas, C. Mariani, G. Bellan, F. Reniero, C. Guillou, Food Chem. 118 (2010) 956.
[13] M.A. Brescia, V. Caldarola, A. De Giglio, D. Benedetti, F.P. Fanizzi, A. Sacco, Anal. Chim. Acta 458 (2002) 177.
[14] F.H. Larsen, F. van den Berg, S.B. Engelsen, J. Chemom. 20 (2006) 198.
[15] L. Viggiani, M.A.C. Morelli, J. Agric. Food Chem. 56 (2008) 8273.
[16] M. Anastasiadi, A. Zira, P. Magiatis, S.A. Haroutounian, A.L. Skaltsounis, E. Mikros, J. Agric. Food Chem. 57 (2009) 11067.
[17] L.I. Nord, P. Vaag, J. Duus, Anal. Chem. 76 (2004) 4790.
[18] I.F. Duarte, A. Barros, C. Almeida, M. Spraul, A.M. Gil, J. Agric. Food Chem. 52 (2004) 1031.
[19] D.W. Lachenmeier, W. Franck, E. Humpfer, H. Schafer, S. Keller, M. Mortter, M. Spraul, Eur. Food Res. Technol. 220 (2005) 215.
[20] C. Almeida, I.F. Duarte, A. Barros, J. Rodrigues, M. Spraul, A.M. Gil, J. Agric. Food Chem. 54 (2006) 700.
[21] E. Lopez-Rituerto, S. Cabredo, M. Lopez, A. Avenoza, J.H. Busto, J.M. Peregrina, J. Agric. Food Chem. 57 (2009) 2112.
[22] I. Berregi, J.I. Santos, G. del Campo, J.I. Miranda, J.M. Aizpurua, Anal. Chim. Acta 486 (2003) 269.
[23] G. del Campo, I. Berregi, R. Caracena, J.I. Santos, Anal. Chim. Acta 556 (2006) 462.
[24] I. Berregi, G. del Campo, R. Caracena, J.I. Miranda, Talanta 72 (2007) 1049.
[25] A. Cazor, C. Deborde, A. Moing, D. Rolin, H. This, J. Agric. Food Chem. 54 (2006) 4681.
[26] C. Deborde, M. Maucourt, P. Baldet, S. Bernillon, B. Biais, G. Talon, C. Ferrand, D. Jacob, H. Ferry-Dumazet, A. de Daruvar, D. Rolin, A. Moing, Metabolomics 5 (2009) 183.
[27] V. Rizzo, V. Pinciroli, J. Pharm. Biomed. Anal. 38 (2005) 851.
[28] S. Akoka, M. Trierweiler, Instrum. Sci. Technol. 30 (2002) 21.
[29] C.J. Belke, A.J. Irwin, J. Am. Soc. Brew. Chem. 50 (1992) 26.
[30] L. Montanari, G. Perretti, F. Natella, A. Guidi, P. Fantozzi, Lebensm. Wiss. Technol. 32 (1999) 535.
[31] Z. Yan, Z. Xingde, N. Weijun, Mikrochim. Acta 127 (1997) 189.

[32] T. Perez-Ruiz, C. Martinez-Lozano, V. Tomas, J. Martin, J. Chromatogr. A 1026 (2004) 57.
[33] C.W. Klampfl, J. Agric. Food Chem. 47 (1999) 987.
[34] S. Cortacero-Ramírez, A. Segura-Carretero, C. Cruces-Blanco, M.L. Romero-Romero, A. Fernandez-Gutierrez, Anal. Bioanal. Chem. 380 (2004) 831.
[35] G.L. Erny, J.E.A. Rodrigues, A.M. Gil, A.S. Barros, V.I. Esteves, Chromatographia 70 (2009) 1737.
[36] V.I. Esteves, S.S.F. Lima, D.L.D. Lima, A.C. Duarte, Anal. Chim. Acta 513 (2004) 163.
[37] I. Mato, S. Suarez-Luque, J.F. Huidobro, Food Chem. 102 (2007) 104.