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Composition of Beer by ^{1}H NMR Spectroscopy: Effects of Brewing Site and Date of Production

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A principal component analysis (PCA) of ^{1}H NMR spectra of beers differing in production site (A, B, C) and date is described, to obtain information about composition variability. First, lactic and pyruvic acids contents were found to vary significantly between production sites, good reproducibility between dates being found for site A but not for sites B and C beers. Second, site B beers were clearly distinguished by the predominance of linear dextrins, while A and C beers were richer in branched dextrins. Carbohydrate reproducibility between dates is poorer for site C with dextrin branching degree varying significantly. Finally, all production sites were successfully distinguished by their contents in adenosine/inosine, uridine, tyrosine/tyrosol, and 2-phenylethanol, reproducibility between dates being again poorer for site C. Interpretation of the above compositional differences is discussed in terms of the biochemistry taking place during brewing, and possible applications of the method in brewing process control are envisaged.

KEYWORDS: Beer; analysis; origin; quality control; NMR; spectroscopy

INTRODUCTION

The detailed study of beer chemical composition and its relation to quality attributes is of paramount importance to accomplish efficient quality control and improved properties of the final product. The complex chemical composition of beer depends on several factors including water quality, malt, hop, yeasts, and the precise recipe and timing of the brewing process. The quality of the final product is usually mainly assessed through its appearance (color, foam properties, and clarity), taste (sweetness, sourness, saltiness, and bitterness), flavor, and aroma (*1*). However, the relationship of these properties with beer chemical composition is not fully known, a fact which prevents a full and knowledgeable control of the chemistry/biochemistry of the product in order to achieve and control specific end properties. This necessarily implies a detailed and comprehensive chemical characterization of beer and, although several standard chemical measurements are usually carried out in the brewing industry, a more complete overall chemical fingerprint of the product is still required, in real time. Nuclear magnetic resonance (NMR) spectroscopy has the ability of simultaneously detecting several families of compounds, in a range of concentrations. Requiring only sample degassing, in the case of beer, NMR may provide extensive compositional information in just a few minutes (*2*), and use of automation or flow injection technology (*3*) has been explored in order to adapt the method for routine beer analysis. High-resolution NMR and hyphenated NMR (LC-NMR and LC-NMR/MS) have enabled a considerable database of compounds found in beer to be established, with particular emphasis on carbohydrates (*4*) and aromatic compounds (*5*).

However, the information richness characterizing the ^{1}H NMR spectra of beer results in extensive signal overlap and, hence, great difficulty in extracting fully detailed compositional information. This calls for the use of multivariate analysis to interpret NMR spectra, and this approach has been the basis of numerous NMR-based methods for classification and origin determination of foods (*6*−*13*), providing the possibility of pinpointing specific compounds responsible for the occurrence and/or intensity of a particular property of the final product. In the case of beer, the possibilities of using the site-specific natural isotope fractionation NMR (SNIF-NMR) method for origin determination have been reviewed recently (*14*). However, to detect variations in terms of specific compounds other than ethanol, high-resolution ^{1}H NMR is one of the most promising spectroscopic methods. Multivariate analysis of the ^{1}H NMR profiles of beers has been shown to detect consistent compositional changes between lagers, ales, and alcohol-free beers (*8*) and between wheat malt and barley malt beers (*3*). The latter work also discusses the possibilities of detecting organoleptically undesirable beers and distinguishing beers according to brewing

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Table 1. Listing of the 27 Beer Samples under Analysis[a]

no.	code	production site	production date	bottle	pH
1	A1a	A	Feb-02	a	4.40
2	A1b	A	Feb-02	b	4.41
3	A1c	A	Feb-02	c	4.39
4	A2a	A	Mar-02	a	4.37
5	A2b	A	Mar-02	b	4.35
6	A2c	A	Mar-02	c	4.35
7	A3a	A	Apr-02	a	4.35
8	A3b	A	Apr-02	b	4.38
9	A3c	A	Apr-02	c	4.37
10	B1a	B	Feb-02	a	4.37
11	B1b	B	Feb-02	b	4.36
12	B1c	B	Feb-02	c	4.36
13	B2a	B	Mar-02	a	4.27
14	B2b	B	Mar-02	b	4.28
15	B2c	B	Mar-02	c	4.28
16	B3a	B	May-02	a	4.32
17	B3b	B	May-02	b	4.32
18	B3c	B	May-02	c	4.30
19	C1a	C	Feb-02	a	4.22
20	C1b	C	Feb-02	b	4.21
21	C1c	C	Feb-02	c	4.24
22	C2a	C	Mar-02	a	4.35
23	C2b	C	Mar-02	b	4.32
24	C2c	C	Mar-02	c	4.34
25	C3a	C	Apr-02	a	4.54
26	C3b	C	Apr-02	b	4.53
27	C3c	C	Apr-02	c	4.53

[a] The code numbers used indicate the production site (as A, B, or C), the date of production (as 1, 2, or 3), and the bottle (as a, b, or c).

sites. The possibility of using ^{1}H NMR to quantify beer components, such as organic and amino acids, has also been addressed recently (*15*). Quantification was performed both by integration of NMR signals, making use of calibration reference solutions, and by partial least-squares (PLS) regression. These results were compared with those obtained by HPLC analysis for amino acids and capillary electrophoresis for organic acids. The NMR-based methods perform satisfactorily for quantification of organic acids and amino acids, with PLS improving the accuracy of the results and enabling the handling of overlapped signals.

The present work aims at using the established usefulness of NMR in beer analysis to address a practical problem of the brewing industry: the short-term monitoring of samples of the same type, produced in different sites and at different times. To do this, principal component analysis (PCA) in tandem with high-resolution ^{1}H NMR spectroscopy is applied to a set of beers of the same type (lager), originating from three different brewing sites (situated in three different countries), and produced on different dates. In this way, site- and/or time-related variables and their effects on beer composition may be detected and analyzed qualitatively and semiquantitatively, knowledge which may be the basis of a future finer control in the industrial environment.

MATERIALS AND METHODS

Samples. Beer lager samples of the same brand (4% v/v alcohol), originating from three different countries or production sites (A, B, C) and from different production dates (1, 2, 3), typically spaced 1−2 months, have been selected. For each production date, within each country, samples of three different bottles (a, b, c) were analyzed so that a total of 27 samples were available, as shown in **Table 1**. The unopened bottles were stored at 4 °C until their analysis, which was carried out 2−3 months after production, a period during which aging effects are believed to be negligible. Bottles were opened immediately before NMR analysis.

The compounds chosen for quantification were those involved in the PCA discussion (lactic acid, pyruvic acid, tyrosine, uridine, adenosine, and/or inosine) as well as succinic acid and histidine, as examples of other compounds suitable for quantification due to having NMR peaks free of overlap. The method followed one of those described in ref *15*, and three standard solutions were prepared to contain (a) lactic, pyruvic, and succinic acids, (b) tyrosine and histidine, and (c) adenosine/inosine and uridine, using the concentrations shown in **Table 2**. All solutions were prepared for NMR analysis in the same way as the beer samples, and their 1D ^{1}H NMR spectra were obtained under identical conditions. Quantitative analysis was performed by integration of the peak of each compound and an internal reference (TSP), in both beer spectra and standard solution spectra, using the following relationship:

$$C_{\text{analyte in beer}} = C_{\text{std}}(A_{\text{beer}}/\text{AI}_{\text{beer}})/(A_{\text{std}}/\text{AI}_{\text{std}})$$

where $C_{\text{analyte in beer}}$ is the concentration of each compound in beer, C_{std} is the concentration of each compound in the standard solutions, A_{beer} and AI_{beer} are the areas of the peaks of each compound and of the internal reference TSP in the beer, and A_{std} and AI_{std} are the areas of the peaks of each compound and TSP in the standard solutions.

NMR Spectroscopy. All beer samples were analyzed by NMR immediately after ultrasonic degassing (10 min) and following a random order, unrelated to date or site of production. Beer samples and standard solutions were prepared to contain 10% (v/v) D_2O and 0.02% (v/v) 3-(trimethylsilyl)propionate sodium salt (TSP, chemical shift and intensity reference). The ^{1}H 1D NMR spectra were recorded at 27 °C on a Bruker Avance DRX-500 spectrometer, operating at 500.13 MHz for proton spectra. A pulse sequence based on the two-dimensional NOE experiment was used, with suppression of water and ethanol signals by applying a modulated shaped pulse during the mixing time (100 ms) and 1.6 s of the relaxation delay (8.0 s). 128 transients were collected into 32768 data points with a spectral width of 8389.26 Hz and an acquisition time of 1.95 s. Three replica spectra were recorded for each sample. The free-induction decays (FIDs) were Fourier transformed (with 0.3 Hz line-broadening unless otherwise stated), and

Table 2. Concentrations for Selected Compounds and Dextrin Properties for Beers Produced at Different Sites[a]

compd (ppm chosen)	concentration of std sol/mg·L^{-1}	concentration/mg·L^{-1}		
		site A	site B	site C[b]
adenosine/inosine (8.35 ppm)	718	71 ± 9	52 ± 3	82 ± 4 (50 ± 3)
histidine (7.04 ppm)	704	49 ± 10	59 ± 5	57 ± 3 (60 ± 2)
lactic acid (1.33 ppm)	109	49 ± 5	117 ± 11	48 ± 4 (43 ± 4)
pyruvic acid (2.36 ppm)	91	120 ± 14	168 ± 7	156 ± 24 (106 ± 3)
succinic acid (2.54 ppm)	100	61 ± 3	75 ± 8	67 ± 8 (61 ± 2)
tyrosine (6.88 ppm)[c]	710	198 ± 16	229 ± 30	235 ± 26 (284 ± 2)
uridine (7.86 ppm)	721	112 ± 21	149 ± 12	134 ± 15 (139 ± 7)
Average Polymer Size and No. of Branchings for Dextrins				
average size		5.8	5.0	5.8 (4.5)
average no. of branching points		0.57	0.43	0.54 (0.35)

[a] All values were obtained by NMR integration, as described in the text. [b] Values out of parentheses refer to C1 and C2 beers, and values in parentheses refer to C3 beers, which in many cases differ significantly from the former. [c] The peak at 6.88 ppm was chosen for quantitative studies since the degree of overlap with tyrosol is lower, compared to the case of the peak at 7.18 ppm.

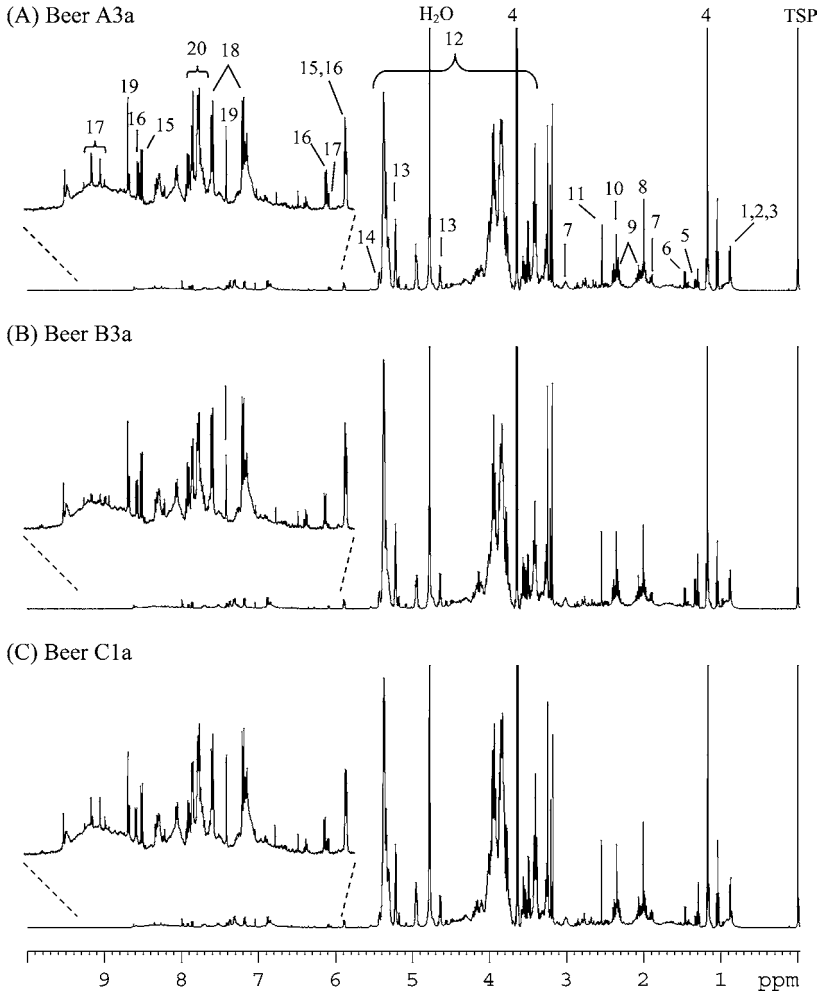

Figure 1. 500 MHz ^{1}H NMR spectra of (A) beer A3a, (B) beer B3a, and (C) beer C1a (named according to Table 1): 1, propanol; 2, isobutanol; 3, isopentanol; 4, ethanol; 5, lactate; 6, alanine; 7, γ-butyric acid (GABA); 8, acetate; 9, proline; 10, pyruvate; 11, succinate; 12, dextrins; 13, glucose; 14, maltose; 15, uridine; 16, cytidine; 17, adenosine/inosine; 18, tyrosine and/or tyrosol; 19, histidine; 20, 2-phenylethanol.

the spectra were manually phased, baseline corrected, and calibrated by the TSP signal at 0.0 ppm. The resulting spectra were converted into JCAMP format and transferred to a PC workstation for statistical analysis. The initial data matrix consisted of 27 × 3 (81) samples and 32768 variables (chemical shifts).

Principal Component Analysis (PCA). For PCA of NMR spectra, data matrixes corresponding to different spectral regions were built, excluding the segments containing water and ethanol resonances, respectively at 4.77 ppm (zeroed range: 4.69−4.90 ppm) and at 1.17 (zeroed range: 1.10−1.26 ppm) and 3.65 ppm (zeroed range 3.61−3.71 ppm), to eliminate variations in these signals suppression. The spectral regions considered were (a) aliphatic region (0.5−3.0 ppm), (b) sugar region (3.0−6.0 ppm), and (c) aromatic region (6.0−10.0 ppm). The spectra were mean-centered, and each spectral region was normalized to unit length. The calculations were performed using software codeveloped by the University of Aveiro and the "Institut National Agronomique Paris-Grignon" (*16*).

RESULTS AND DISCUSSION

Figure 1 shows the 1D ^{1}H NMR spectra of three beers from different brewing sites (A, B, and C). The assignment of many of the signals observed has been carried out on the basis of the analysis of 2D NMR experiments and of information published elsewhere (*2*, *4*, *5*). In the aliphatic region of the spectra (0−3 ppm), peaks arise from alcohols (e.g. propanol, isobutanol, isopentanol), organic acids (e.g. citric, malic, pyruvic, acetic, succinic), amino acids (e.g. alanine, γ-aminobutyric, proline), and fatty acids. The midfield region (3−6 ppm) shows the contribution of fermentable sugars (e.g. glucose, maltose) and dextrins (glucose oligomers with different degrees of polymerization and branching). The signals in the aromatic region (6−10 ppm) (insets in **Figure 1**) show the presence of aromatic amino acids (tyrosine, phenylalanine, tryptophan), nucleosides (cytidine, uridine, adenosine/inosine), aromatic alcohols (2-phenylethanol, tyrosol, tryptophol), and polyphenolic compounds that give rise to underlying broad humps between 6.7 and 8.7 ppm (*5*). From visual inspection of the spectra shown in **Figure 1**, it is clear that the spectral profiles are very similar between different beers, although small changes may be noted in all regions in the relative proportion of some peaks.

A first PCA was performed on the whole of the 0−10 ppm range of the spectra, and as expected, it only reflected changes in the major components (dextrins), thus masking potential changes in minor components in the non-sugar regions of the spectra. Therefore, PCA was performed separately in the aliphatic (0−3 ppm), sugar (3−6 ppm), and aromatic (6−10 ppm) regions in order to detect changes, respectively, in aliphatic compounds, dextrins, and aromatic compounds. PCA was first performed on the aliphatic region of the ^{1}H NMR spectra (excluding the ethanol signal at 1.17 ppm). This should detect eventual changes in compounds such as aliphatic alcohols, organic acids, and amino acids. **Figure 2**A shows the scores scatter plot of the first two PCs where considerable sample

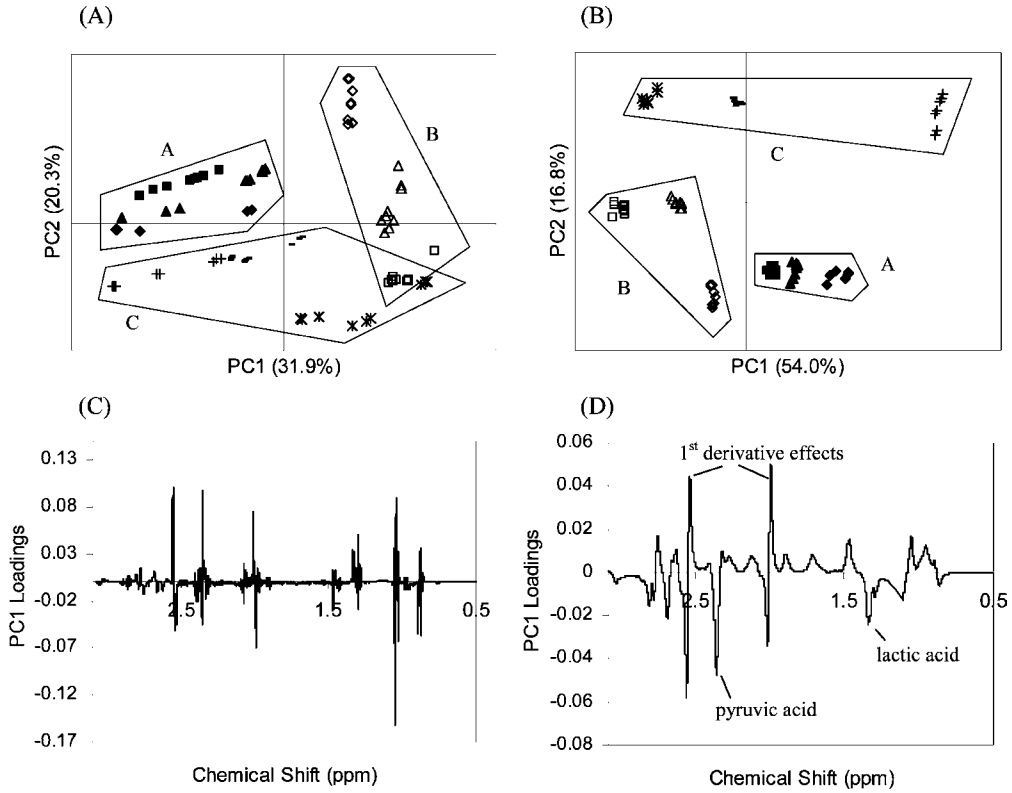

Figure 2. PCA of aliphatic NMR spectral regions (0.5–3.1 ppm): (A) scores scatter plot of PC1 vs PC2 for spectra processed with LB 0.3 Hz; (B) scores scatter plot of PC1 vs PC2 for spectra processed with LB 10 Hz; (C) PC1 loadings profile corresponding to part A; (D) PC1 loadings profile corresponding to part B. Site A beers: (◆) A1; (■) A2; (▲) A3. Site B beers: (◇) B1; (□) B2; (△) B3. Site C beers: (∗) C1; (–) C2; (+) C3. Grouping shapes were drawn manually in the scores scatter plots to aid the eye. Peaks arising from lactic acid, pyruvic acid, and first derivative artifacts are indicated in part D.

dispersion may be observed. However, beers of site A (full symbols) are clearly separated from all remaining ones, whereas some overlap occurs between beers from sites B and C. Within each site, significant variability is observed for different production dates, particularly for sites B and C. Apparently, this would suggest the occurrence of large compositional variations, within each site, between production dates and even between different bottles produced on the same date (identical symbols in **Figure 2**). However, the many first-derivative-like effects observed in the PC1 loadings profile (**Figure 2C**) indicate the occurrence of small shifts of the NMR peaks, caused mainly by the small differences in samples pH (range is 4.21−4.54) (**Table 1**). This artifact compromises the classification ability and stability of the multivariate models and may be the cause of the apparent variability observed in **Figure 2A**. The addition of a buffer solution to the samples was ruled out since it would increase the invasiveness of the method. To minimize small peak shifts, the PCA analysis was repeated using the same spectra processed with an enhanced line-broadening factor. Alternatively, bucket analysis, consisting of segmenting each spectrum into narrow regions of predefined chemical shift and integrating all of the intensity in each region, may be applied (*3*, *17*). Spectral line-broadening is achieved by multiplying the free-induction decay (FID) by an exponential function which results in the broadening of each peak of a few hertz. This does have the effect of masking small peak shifts, but it also carries a risk of information loss. Therefore, several line-broadening factors were studied, and the adequate factor was carefully selected for each spectral region. For the aliphatic region, the best results were obtained using a line-broadening factor of 10 Hz, for which an increase of the amount of variability contained in the first two principal components is observed, from 52% (for 0.3 Hz) to 70% (for 10 Hz). **Figure 2B** shows how reproducibility within each production date is much improved, thus demonstrating the absence of compositional differences between beer bottles. Site separation also improves slightly, compared to the spectra processed with 0.3 Hz line-broadening (**Figure 2A**), with no sites overlapping despite the relatively large sample dispersion still observed for beers of sites B and C, for different production dates.

The samples separation observed in the scores plots may be interpreted on the basis of the loadings profile of each principal component. Care is required, however, not to overinterpret the complex loadings information, and thus, all compositional changes suggested on the basis of the loadings profile were checked by subsequent validation, such as (i) visual inspection of the spectra and, (ii) in some cases, quantitation for particular compounds by peak integration in beer samples and in standard solutions (**Table 2**), following a method proposed recently (*15*) and as described in the Experimental Section. Beers from sites A and B are clearly distinguished along PC1 (**Figure 2B**). Through inspection of the PC1 loadings profile obtained with 10 Hz line-broadening (**Figure 2D**), it is clear that the major features are negative peaks for pyruvic and lactic acids and two major first derivative effects. The contribution of the latter for the PCA results was observed to be negligible, since identical results (not shown) were obtained by considering zeroed segments in place of the first derivative peaks. On the basis of the results, it is suggested that beers from site B (negative PC1) have higher amounts of lactic and pyruvic acids (**Figure 2D**), compared to beers from site A. This is confirmed for both acids by the quantitation results (**Table 2**). In addition, beers produced at site C are separated along PC2 from all remaining ones. PC2 loadings (not shown) and inspection of spectra indicate that these

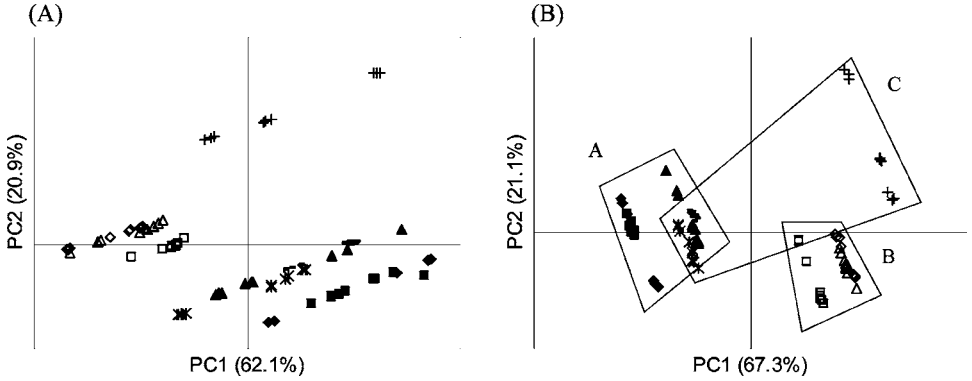

Figure 3. PCA of sugar NMR spectral regions (3.1–5.8 ppm): (A) scores scatter plot of PC1 vs PC2 for spectra processed with LB 0.3 Hz; (B) scores scatter plot of PC1 vs PC2 for spectra processed with LB 10 Hz. Site A beers: (◆) A1; (■) A2; (▲) A3. Site B beers: (◇) B1; (□) B2; (△) B3. Site C beers: (∗) C1; (–) C2; (+) C3. Grouping shapes were drawn manually in the scores scatter plots to aid the eye.

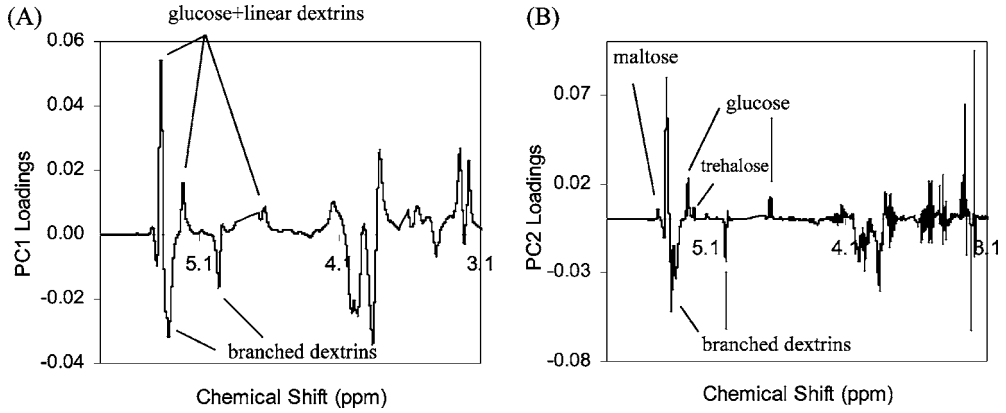

Figure 4. PCA of sugar NMR spectral regions (3.1–5.8 ppm): (A) PC1 loadings profile for spectra processed with LB 10 Hz; (B) PC2 loadings profile for spectra processed with LB 0.3 Hz. The main peaks responsible for variations in positive and negative PC1 and PC2 are indicated and assigned (glc, glucose; mal, maltose; tre, trehalose).

beers are significantly poorer in lactic acid than beers from B, the effect being only slight relative to site A beers (**Table 2**). Pyruvic acid is an intermediate product in the biosynthesis of ethanol by yeast, and it is known that beers with elevated levels of pyruvate may have been produced with yeast that has undergone autolysis (destruction of a cell after its death) during fermentation. It is possible, therefore, that higher contents of pyruvic acid and its formal reduction product, lactic acid, may be indicative of poorer yeast quality or of the action of older generations of yeast, the effect prevailing in site B, for the particular dates investigated, followed by site A and, finally and more closely, by site C.

The large sample dispersion seen within production dates at site C (**Figure 2B**) should originate from the same compositional differences that cause the separation of sites A and B along PC1; that is, samples C1 and C2 are expected to be richer in lactic and pyruvic acids than samples C3. This is confirmed by the concentrations shown in **Table 2**, the effect being more marked for pyruvic acid. This is also consistent with the relatively higher pH values of C3 beers. These results suggest that variations within a brewing site could reflect variable yeast quality between production dates or, alternatively, given that most brewers periodically replace their yeast with fresh slopes, the yeast used to produce C3 could be younger in generation number than that used to produce C1 or C2.

A similar analysis of the midfield spectral regions (excluding the ethanol peak at 3.65 ppm) should provide information on the carbohydrate composition of the samples and eventual differences between samples. **Figure 3**A shows the scores scatter plot of the first two PCs, obtained by analysis of the spectra processed with 0.3 Hz line-broadening. The beers produced in site B (open symbols) are clearly differentiated from all remaining ones in terms of their carbohydrate composition, being located on the negative PC1 axis. Beers produced in sites A and C are grouped together, except for beers C3, and show a considerable large dispersion along PC1. Interpretation of the PC1 loadings corresponding to **Figure 3A** (not shown) was again hindered by first-derivative-like effects; therefore, PCA analysis was carried out on the spectra processed with an enhanced line-broadening of 10 Hz. This was seen not to change the scores scatter plot significantly (the variability accounted by both principal components remains almost the same for both line-broadening values) although a slight improvement in sample dispersion is noted (**Figure 3B**), which facilitates the loadings analysis. The corresponding PC1 loadings (**Figure 4A**) clearly show that the origin of the sample separation observed is the degree of branching in the predominating dextrins. Indeed, glucose and linear dextrins (peaks at 4.64 (H1β red), 5.22 (H1α red), and 5.37 (H1α, 1 → 4) ppm in **Figure 4A**) prevail in site B beers and in C3 beers (positive PC1), whereas branched dextrins (peaks at 4.96 (H1α, 1 → 6) and 5.30−5.35 (H1α, 1 → 4) ppm) predominate in all other beers. To confirm this, selected signals in the anomeric regions of the ^{1}H NMR spectra were integrated to give estimates of the average number of branching points per molecule. This involves the calculation of the average number of glucose rings per molecule, which is given by the ratio of the summed areas of H1 protons in both α(1−4) and α(1−6) glycosidic linkages (signals at 5.30−5.40 and 4.9−5.0 ppm, respectively) and the summed areas for the reducing H1 protons (5.22 and 4.64 ppm for the α and β

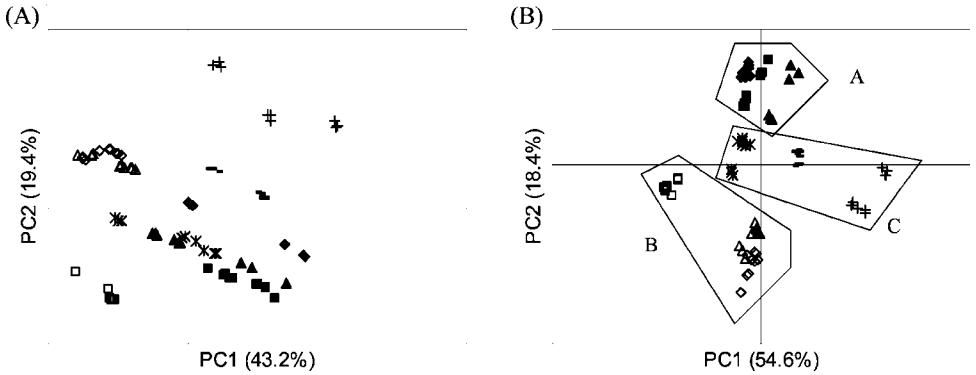

Figure 5. PCA of aromatic NMR spectral regions (5.8–10.4 ppm): (A) scores scatter plot of PC1 vs PC2 for spectra processed with LB 0.3 Hz; (B) scores scatter plot of PC1 vs PC2 for spectra processed with LB 5 Hz. Site A beers: (◆) A1; (■) A2; (▲) A3. Site B beers: (◇) B1; (□) B2; (△) B3. Site C beers: (∗) C1; (–) C2; (+) C3. Grouping shapes were drawn manually in the scores scatter plots to aid the eye.

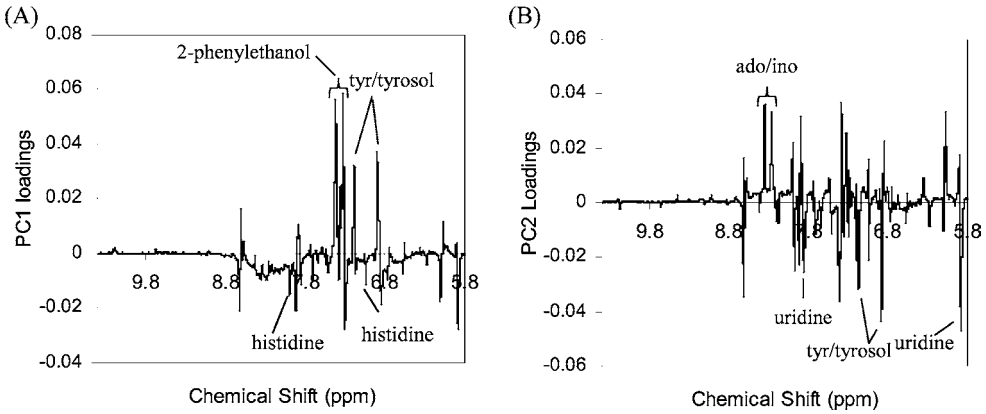

Figure 6. PCA of aromatic NMR spectral regions (5.8–10.4 ppm): (A) PC1 loadings profile for spectra processed with LB 5 Hz; (B) PC2 loadings profile for spectra processed with LB 5 Hz. The main peaks responsible for variations in positive and negative PC1 and PC2 are indicated and assigned: tyr, tyrosine; ado, adenosine; ino, inosine.

anomers, respectively). The average number of branching points per molecule is estimated by the ratio of the integral of the peak for H1 protons in α(1−6) linkages (4.9−5.0 ppm) and that of the peak for H1 protons in α(1−4) linkages (5.3−5.4 ppm), multiplied by the number of total linkages. The results thus obtained (**Table 2**) show that average carbohydrate size and number of branching points are, respectively, five monomers and 0.4 for beers of site B (and C3) and 6 monomers and 0.5−0.6 for beers of sites A and C (except C3 beers). This confirms that most beers from sites A and C have more highly branched oligosaccharides, with the exception of beer C3, which has a composition similar to site B beers, i.e., enriched in linear carbohydrates. The degree of branching of beer polysaccharides is presumably due to enzyme activity during malting and mashing, reflecting the activities of α- and *β*-amylases as well as of other minor enzymes. It is possible, therefore, that the variations detected here are a reflection of fine variations in the conditions of the malting and mashing processes, primarily from site to site and, in the case of C, within production dates. Indeed, C3 beers are distinctly different from C1 and C2 beers, with C3 composition being characterized in detail through the inspection of the PC2 loadings (**Figure 4B**). Even for the results obtained with lower line-broadening, PC2 loadings clearly show positive peaks for glucose (4.64 and 5.22 ppm), trehalose (5.18 ppm), and, possibly, maltose (small peak at 5.43 ppm) (**Figure 4B**). This suggests that C3 beers are richer in these compounds, compared to all remaining beers. Conversely, the observed negative peaks confirm a lesser dextrin branching degree in C3 beers, compared to the remaining beers from C and similarly to site B beers. The presence of detectable levels of the fermentable sugars glucose and maltose in beer C3 indicates that fermentation has not been fully attenuated. This might be indicative of either premature cooling to complete the flocculation of the yeast crop or premature flocculation of the yeast during fermentation (*1*).

Finally, to account for possible changes in aromatic compounds, analysis proceeded on the aromatic regions (5.8−10.4 ppm) of the spectra. The scores scatter plot of PC1 vs PC2 obtained for the aromatic regions of the spectra (processed with 0.3 Hz LB) shows considerable sample dispersion (**Figure 5**A). Again, this large dispersion seems to originate from significant pH-induced shifts for many organic acids, thus causing large first-derivative-like effects on the loadings profile (not shown). **Figure 5B** shows the PC1 vs PC2 scores scatter plot obtained for the spectra processed with 5 Hz line-broadening. Sample separation is significantly improved, with all three groups of beers being separated according to site: beers from site A in positive PC2, beers from site B on negative PC1 and PC2, and beers from site C characterized by PC2 close to zero.

Considering that the main sample separation occurs along the PC2 axis, it may be seen and confirmed by spectral integration (**Table 2**) that beers from site A are enriched in adenosine and/or inosine (positive PC2 peaks at 8.35 and 8.26 ppm) and poorer in uridine (negative PC2 at 5.88 and 7.90 ppm) and tyrosine and/or tyrosol (negative PC2 at 7.18 and 6.88 ppm), compared to beers from site B (**Figure 6**B). This is confirmed by the values quantified (**Table 2**). PC1 loadings (**Figure 6A**) suggest that variations along the PC1 axis may reflect small differences in the amounts of compounds such as 2-phenylethanol, tyrosine and/or tyrosol, and histidine. This is the case

of C3 beers suggested to be richer in 2-phenylethanol and tyrosine and/or tyrosol compared to beers C1 and C2. The alcohol 2-phenylethanol was not quantified, but the calculations based on the 6.88 ppm peak of tyrosine (**Table 2**), which may, nevertheless, have a small contribution from tyrosol, confirm this suggestion. While it is not completely clear as to whether the hydroxyphenyl residue detected is from tyrosol or tyrosine, the detection of tyrosol and phenylethanol in final beer is presumably due to amino acid degradation. Certain amino acids undergo Strecker degradation to yield their corresponding aldehydes during beer production, and there is some evidence to suggest that aldehydes present during fermentation can be enzymatically reduced during fermentation to their corresponding alcohols (*1*).

In conclusion, the use of ^{1}H NMR spectroscopy in tandem with PCA has been shown to be a potentially promising method to detect consistent compositional differences in beers produced at different sites and at different times. In relation to aliphatic composition, lactic and pyruvic acids contents were found to vary significantly between production sites, good reproducibility between dates being found for site A but not for sites B and C beers. It is suggested that these variations may reflect yeast quality and/or yeast generation number. Regarding carbohydrate composition, linear dextrins were found to predominate in site B beers whereas A and C beers were richer in branched dextrins, carbohydrate reproducibility being poorer for site C beers. This may be correlated to the fine conditions during malting and mashing and the resulting enzymatic activities. Finally, all production sites could be successfully distinguished mainly by their contents in adenosine/inosine, uridine, tyrosine/tyrosol, and 2-phenylethanol, reproducibility between dates being again poorer for site C. It is suggested that the latter compounds may be indicative of Strecker degradation of amino acids. While it is not clear at this stage whether and how these aspects affect beer quality, it is shown that the factors probed may provide important clues as to yeast quality or the performance of malting and/or mashing. These are the first and promising indications that the PCA/NMR method may be a useful method to monitor and control the beer production process.

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