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High-Resolution Nuclear Magnetic Resonance Spectroscopy and Multivariate Analysis for the Characterization of Beer

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Beer contains a very complex mixture of nutrients, which in this work are identified to some extent by high-field high-resolution nuclear magnetic resonance (NMR) one- and two-dimensional methods. The ^{1}H NMR spectrum of beer shows a predominance of strongly overlapped peaks arising from several carbohydrates. Minor components are clearly observed both in the aliphatic and in the aromatic regions of the spectrum. With the aid of two-dimensional methods, spectral assignment was carried out, enabling the identification of ∼30 compounds and identifying about the same number of spin systems for further assignment. The variability of the spectral profile of beers differing in type and label was studied by principal component analysis (PCA), and it was found that, although some distinction is achieved on the basis of the aliphatic and sugar compositions, clearer separation between ales and lagers is obtained by PCA of the aromatic profiles alone. The potential of this technique as a rapid and informative quality control tool is discussed.

KEYWORDS: Beer; ale; lager; nuclear magnetic resonance; principal component analysis; chemometrics

INTRODUCTION

Beer is a fermented beverage made from malted grains (usually barley), hops, yeast, and water. Fruits, herbs, and spices may also be used to give beer a particular character. The different combinations of ingredients, production processes, and storage conditions give rise to an enormous variety of beers—ales and lagers being defined as the two main types according to the conditions of their fermentation processes (*1*). There has been great interest in studying the chemical composition of beer, as this information is valuable for the assessment of beer quality and the development of new products. Besides water and ethanol, the major components of beer are carbohydrates and comprise fermentable sugars (e.g., glucose, maltose, and maltotriose), glucose oligosaccharides (dextrins), and arabinoxylans. These compounds have a marked influence on sweetness and can influence mouthfeel and sour and bitter perceptions. Carbohydrate contents in beer have been determined by chemical analysis, enzymatic analysis (*2*), and chromatography (*3*−*5*) and were found to depend significantly upon beer type (*2*, *5*). Other components play very important roles in the quality of beer; for instance, proteins and amino acids influence beer foam and haze stability, whereas phenolic compounds such as cinnamic acids, benzoic acids, catechins, and flavonols are responsible for beer flavor, physical stability, and antioxidant activity.

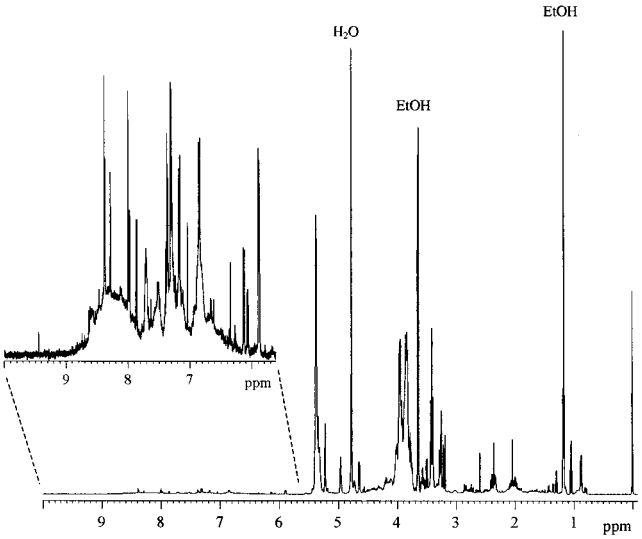

Figure 1. 500 MHz ^{1}H NMR spectrum of an ale beer, with triple suppression of water and ethanol resonances.

Liquid chromatography (LC) and mass spectrometry (MS) methods have been widely used for the determination of protein and phenolic contents in beer (*6*−*12*), sometimes along with spectroscopic methods (UV, fluorescence, and electron spin resonance), to investigate the relationship between beer phenolic

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Table 1. ^{1}H and ^{13}C Chemical Shifts, Proton Multiplicities, and J_{HH} for Assigned Metabolites in an Ale Beer[a]

compound	assignment	δ ^{1}H	multiplicity	J (Hz)	δ ^{13}C	compound	assignment	δ ^{1}H	multiplicity	J (Hz)	δ ^{13}C
acetaldehyde	CH_3	2.23	d	3.0	32.22	lactic acid	CH_3	1.35	d	7.0	22.48
	CHO	9.67					α-CH	4.19	q	7.0	70.87
acetaldehyde		1.32				malic acid	CH_2	2.63	dd	7.8; 16.2	42.78
hydrate		5.24					CH_2	2.82	dd	4.4; 16.1	
acetic acid/acetates	CH_3	2.04	s		23.82		α-CH	4.38			71.20
adenosine/inosine	C4′H	4.27				maltose/	β-C2H	3.26	dd		76.77
	C3′H	4.41				maltotriose/	α/β-C4H	3.41	dd		72.14
	C2′H	4.79				dextrins	β-C1H	4.64	d	8.0	98.50
	C1′H	6.07	d				α-C1H	5.22	d	3.7	94.58
	C4H	8.28	s				C1H glyc bond	5.38	d	3.9	102.35
	C2H	8.38	s			proline	γ-CH_2	2.00	m		26.60
alanine	CH_3	1.51	d	7.2			β-CH_2	2.06	m		31.70
	α-CH	3.77	q				β-CH_2	2.33	m		31.70
citric acid	$^1/_2$-CH_2	2.72	AB	15.7	46.50		δ-CH_2	3.32	m		48.90
	$^1/_2$-CH_2	2.84	AB	15.7			δ-CH_2	3.41	m		48.90
cytosine	C4H	6.13	d	7.9	98.36		α-CH	4.11	dd		64.06
	C3H	7.97	d	7.9	145.72	propanol	CH_3	0.88	t	7.5	13.7
ethanol	CH_3	1.17	t	7.2	19.56		CH_2	1.53	m		26.9
	CH_2	3.64	q	7.2	60.19		CH_2OH	3.54	t	5.8	62.46
ethyl ester	CH_3	1.24	t		12.92	pyruvic acid/	CH_3	2.35	s		29.12
(e.g., ethyl acetate)	O–CH_2	4.12			60.34	diacetyl					
formic acid	CH	8.37	s		143.80	succinic acid	CH_2	2.59	s		32.79
GABA	β-CH_2	1.92			26.28	tryptophan	C5H/C6H	7.15			
	α-CH_2	2.39	t		32.28		C5H/C6H	7.24			
	γ-CH_2	3.02			41.83		C7H	7.50			114.49
gallic acid[b]	C2H, C6H	7.03	s		119.00		C4H	7.69			
histidine[b]	C4H	7.03	s		119.00	tyrosine	C3H, C5H	6.85	d	8.5	118.16
	C2H	7.99	s		140.62		C2H, C6H	7.17	d	8.5	133.08
isobutanol	CH_3	0.87	d	6.6		uridine	C4′H	4.11			
	CH	1.73					C3′H	4.21			
	CH_2OH	3.36	d	6.5			C2′H	4.38			
isopentanol	CH_3	0.88	d	6.6	24.60		C5H	5.88	d	8.2	105.00
	CH	1.42			43.00		C1′H	5.89			90.51
	CH_2	1.64			26.98		C6H	7.85	d	8.2	144.56
	CH_2OH	3.63									
	Possible Assignments										
lipid		0.95				polyphenol		4.12–4.45			
		1.53						7.87–8.49			
		1.70				polyphenol		6.81			118.23
lipid		0.89						7.11			133.55
		1.30									
		1.58				polyphenol		6.84			
		2.33						7.51			
polyphenol		3.81–4.01									
		8.24–8.56									

[a] s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; m, multiplet; AB, multiplet corresponding to an AB spin system. [b] Histidine peaks and gallic acid peaks may overlap at 7.03 ppm.

composition and its antioxidant properties (*13*−*15*). The presence of many other minor components, such as medium-chain fatty acids (*16*), vitamins (*17*, *18*), volatiles (*19*), bitter acids (*20*, *21*), and biogenic amines (*22*, *23*), has also been reported in beer.

The analytical techniques mentioned above often involve some kind of pretreatment of the beer sample to concentrate the desired group of compounds. Moreover, the choice of method and protocol followed depends on the specific family/type of compounds under study, making the process of overall characterization of the beer very time-consuming. Nuclear magnetic resonance (NMR) spectroscopy enables a more rapid and non-invasive characterization of foods, potentially giving information about a very wide range of different metabolites. Some of the applications of high-resolution NMR in the detailed characterization of foods comprise studies of fruit juices (*24*−*26*), coffee (*27*), and wine (*28*, *29*). To our knowledge, the direct characterization of beer by NMR has not been carried out, although the technique has already been applied to specific problems such as the identification of hop bitter acids by LC-NMR (*21*) and of oligosaccharides in beer extracts (*30*).

In this work, high-resolution NMR spectroscopy was used to characterize the overall composition of beer, aiming at identifying a wide range of compounds from the major carbohydrates to minor amino acids, aromatic compounds, and others. A second aim was to explore the potential of NMR in tandem with chemometrics to enable a rapid identification of the nature and origin of the beer sample. This approach has already proved to be of value for the detection and interpretation of spectral changes, as shown previously for apple juices (*31*) and grape extracts (*32*).

MATERIALS AND METHODS

Samples. Seventeen beer samples differing in type (ale or lager) and label were obtained from Brewing Research International, Nutfield, Surrey, U.K. Samples were degassed in an ultrasonic bath and prepared to contain 10% D_2O and 0.02% sodium 3-(trimethylsilyl)propionate-d_4 (TSP) as chemical shift reference. The samples' pH values were found to fall in the 3.7−4.4 range.

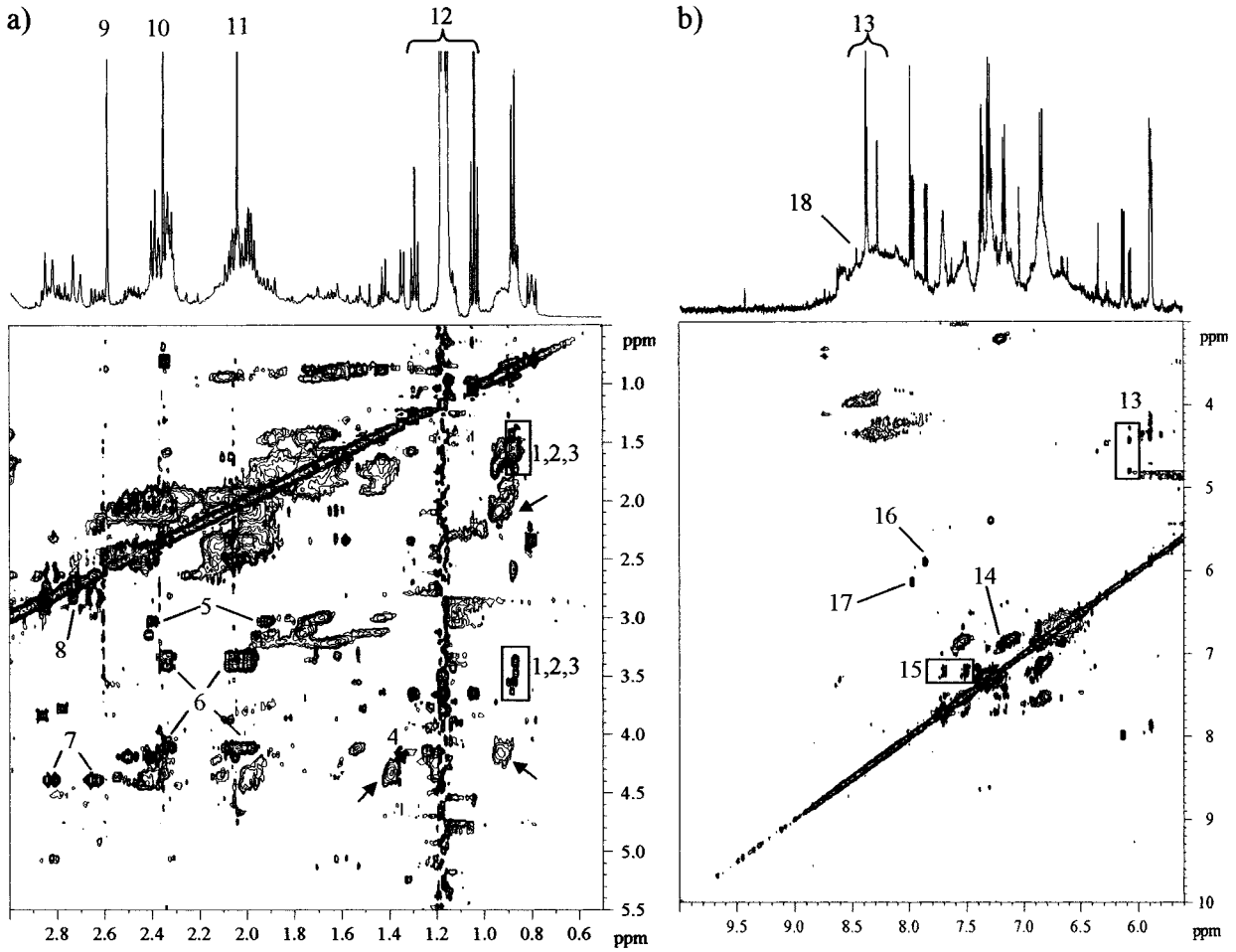

Figure 2. TOCSY spectrum of the same beer sample as in **Figure 1**, recorded at 500 MHz: (a) aliphatic region; (b) aromatic region. Some assignments are indicated: 1, propanol; 2, isobutanol; 3, isopentanol; 4, lactic acid; 5, GABA; 6, proline; 7, malic acid; 8, citric acid; 9, succinic acid; 10, pyruvic acid and/or diacetyl; 11, acetic acid and acetates; 12, ethanol and ^{13}C satellites; 13, adenosine and/or inosine; 14, tyrosine; 15, tryptophan; 16, uridine; 17, cytosine; 18, formic acid.

NMR Measurements. The assignment work was carried out on a selected beer sample, whereas the whole group of samples was used for the chemometrics study.

One-dimensional (1D) and two-dimensional (2D) NMR spectra of the sample selected for assignment were recorded on a Bruker Avance DRX-500 spectrometer, operating at 500.13 MHz for proton and at 125.77 MHz for carbon. The ^{1}H 1D spectra were acquired using a pulse sequence based on the 2D nuclear Overhauser effect (NOE) experiment (*33*), with a 90° pulse of 8.5 μs. Water (4.77 ppm) and ethanol signals (1.17 and 3.64 ppm) were suppressed by applying a shaped pulse, with triple offset and amplitude scaling, during relaxation delay and mixing time (100 ms). One hundred and twenty-eight transients were collected into 16K data points with a spectral width of 5482 Hz.

The TOCSY spectra were acquired in the phase sensitive mode using time proportional phase incrementation (TPPI), the MLEV17 pulse sequence, and a shaped pulse for presaturation of water and ethanol resonances (*34*, *35*). Sixteen scans were collected for each of the 512 increments, using a spectral width of 5482 Hz in both dimensions, 2K data points, a mixing time of 100 ms, and a relaxation delay of 1.5 s. ^{1}H−^{13}C phase sensitive (echo/antiecho-TPPI) heteronuclear multiple quantum correlation (HMQC) spectra were recorded with inverse detection and ^{13}C decoupling during acquisition (*36*, *37*). Two thousand and forty-eight data points with 64 scans per increment and 300 increments were acquired with spectral widths of 5482 and 25157 Hz in the proton and carbon dimensions, respectively. *J*-resolved spectra were measured with a shaped pulse suppression of H_2O/ethanol and a spectral width of 8012 Hz in the proton dimension and 32 Hz in the *J* dimension. Eight thousand data points were acquired with 8 scans for each of 128 increments.

The ^{1}H 1D spectra of the 17 beers studied by PCA were recorded on a Bruker Avance DRX-600 spectrometer, operating at 599.87 MHz for proton. The 1D NOESY pulse sequence was used, with suppression of water and ethanol signals by a shaped pulse during the NOESY mixing time (100 ms). One hundred and twenty-eight transients were collected into 64K data points with a spectral width of 10000 Hz.

Principal Component Analysis (PCA). PCA is essentially a descriptive method. This method is, normally, the first step in data exploration, which allows the main variability aspects of a data set to be visualized, without the constraint of an initial hypothesis concerning the relationship within samples and between samples and responses (variables). The main goals of this procedure are to find relationships between the different parameters (objects and variables) and to detect possible clusters within objects or/and variables.

To find the main sources of data variability and the relationship between or within objects and variables, the initial matrix [defined as $\mathbf{X}(n, m)$] is converted into an object space matrix (samples), a variable space matrix (chemical shifts, in this case), and an error matrix (representing the variation not accounted for by the extracted principal components). The decomposition is formalized by

$$\mathbf{X}_{(n,m)} = \mathbf{T}_{(n,k)}\mathbf{P}_{(k,m)}{}^{\mathbf{T}} + \mathbf{E}_{(n,m)}$$

where **T** is the scores matrix, **P** is the loadings matrix, **E** is the error matrix, n is the number of objects (samples), m is the number of variables (in this study, chemical shifts), and k is the number of principal components used (*38*).

In this work, the FIDs were Fourier transformed (with 0.3 Hz line broadening) and the spectra phased, baseline corrected, and calibrated

by the TSP signal at 0.0 ppm. The resulting spectra were converted to JCAMP files and PCA analyzed on a PC workstation.

RESULTS AND DISCUSSION

Figure 1 shows the 1D ^{1}H spectrum of an ale, recorded at 500 MHz. Because the two major components in beer are water and ethanol, triple suppression was employed by irradiation at 1.17, 3.64, and 4.77 ppm. The three strongest peaks in the spectrum reflect these two components, and the sugar region (3−5 ppm) shows a large number of overlapped and relatively broad peaks with medium intensity. The aliphatic region (0−3 ppm) shows weaker signals, again with large overlap in certain regions. The aromatic region (6−9 ppm), enlarged in the figure insert, shows a considerable number of peaks arising from many different types of aromatic compounds. The occurrence of some underlying broader signals should be noted as probably arising from higher molecular weight aromatic compounds. The high spectral complexity clearly shows the potential of the technique to enable identification of many different compounds, present in different concentrations.

There are two possible approaches to interpretation of such complex spectra. One approach is to pursue extensive assignment work so as to eventually achieve individual assignment of most peaks observed. Naturally, this calls for extensive use of 2D NMR experiments, but it will be shown that, in many cases, many difficulties still arise due to the large spectral complexity and overlap, even in the 2D displays. A second possible approach involves the correlation of the overall spectral profile, without requiring individual and complete assignment, with a known nature or characteristic of the beer sample. This approach calls for chemometric methods such as PCA, and its potential to characterize and distinguish beers will be described in a later part of this paper.

Assignment of High-Resolution ^{1}H NMR Spectrum of Beer. To assign most of the peaks observed in the spectrum shown in **Figure 1**, the results obtained from the TOCSY, ^{1}H−^{13}C correlation, and *J*-resolved experiments were combined. Parts a and b of **Figure 2** show the aliphatic and aromatic sections of the TOCSY spectrum, respectively, where relatively less intense signals are observed. The overlap in the sugar region is clear in all spectra. The figures show some of the assignments, but a more complete list is indicated in **Table 1**.

In the aliphatic region, peaks from alcohols (propanol, isobutanol, and isopentanol), aliphatic organic acids (citric, malic, lactic, pyruvic, acetic, and succinic), and amino acids (alanine, γ-aminobutyric acid, and proline) are observable. In other beer samples, valine, leucine, and isoleucine were also detected. Furthermore, a few spin systems are identified involving very broad resonances below 1 ppm and at ~1.4 ppm (indicated with arrows in **Figure 2a**). These resonances should arise from motionally restricted molecules, possibly high molecular weight compounds such as lipids or other polymeric compounds. In addition, two more aliphatic spin systems have been identified (bottom of **Table 1**) as probably arising from lipids. As these peaks show enhanced resolution, compared to the broad features mentioned above, it is possible that they arise from smaller molecules, such as medium-chain fatty acids that are known to occur in beer. These compounds contribute importantly to the characteristic flavor of beer, being also responsible for off-flavors if present in excessive concentrations (*16*). None of these spin systems show correlations with unsaturated proton or carbon resonances, which suggests that the lipids detected are mostly of the saturated type.

The 3−6 ppm region of the spectrum shows the strong contribution of the beer carbohydrates. Because most carbohydrates (fermentable sugars and dextrins) are mainly constituted of glucose monomers, spectral distinction is extremely difficult due to peak overlap, even in the 2D spectra. This is illustrated by the set of 1D ^{1}H NMR spectra of standard solutions of maltose, maltotriose, maltoheptaose, and potato dextrin (**Figure 3**). As expected, spectral resolution is strongly dependent on molecular size, reflecting the shortening of transverse relaxation times for larger molecules such as maltoheptaose and potato dextrins (**Figure 3c**,**d**). Furthermore, the intensity ratio of the 5.4 ppm (glycosidic linkage H1) and 5.2 ppm (α anomer H1) peaks varies according to molecular size, giving an indication of the average degree of polymerization (DP). For instance, values of 2.5, 4.6, and 11.2 have been measured for maltose, maltotriose, and maltoheptaose, respectively, in broad agreement with the expected values of 2, 4, and 12. Comparison of the anomeric profile and spectral resolution of the beer spectrum (**Figure 3e**) and those of the standard solutions (**Figure 3a**−**d**) suggests that carbohydrates of DP > 3 predominate in the beer sample studied, along with a strong, yet unassigned, contribution of a spin system observed at 3.59, 3.86, 4.01, and 4.96 ppm. It should be noted, however, that different beers may show very different sugar profiles, a subject that will be discussed later. The characterization of beer carbohydrates is clearly a problematic issue for high-resolution NMR, thus calling for improved analytical methods.

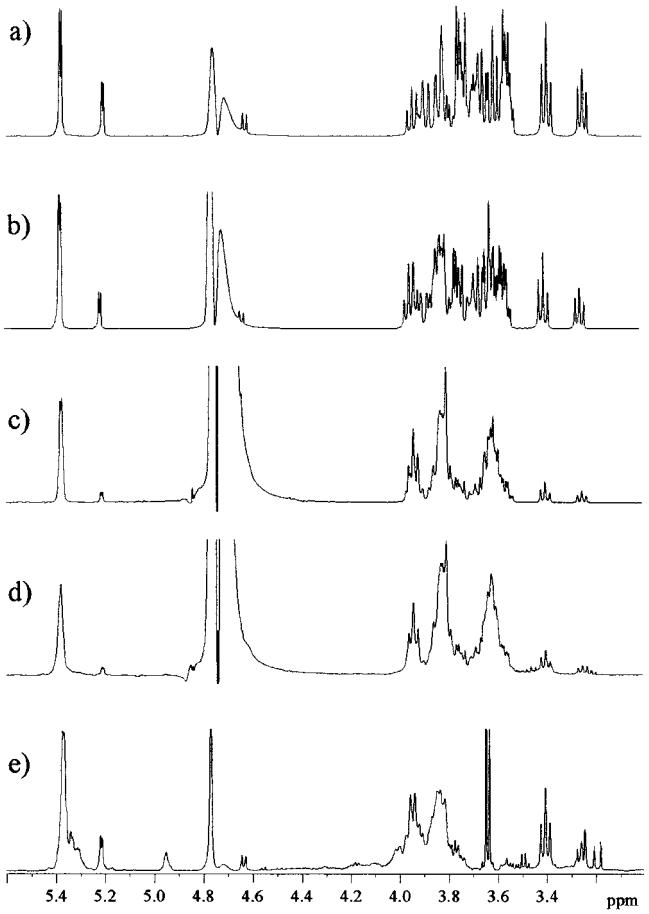

Figure 3. ^{1}H NMR spectra of standard solutions of (a) maltose, (b) maltotriose, (c) maltoheptaose, (d) potato dextrin, and (e) the ale sample selected for assignment. All spectra were recorded at 500 MHz.

The aromatic spectral region is one of the most interesting due to the very low relative abundance of the metabolites shown. As shown in the corresponding TOCSY section (**Figure 2b**), quite a few homonuclear correlations are detected, either within

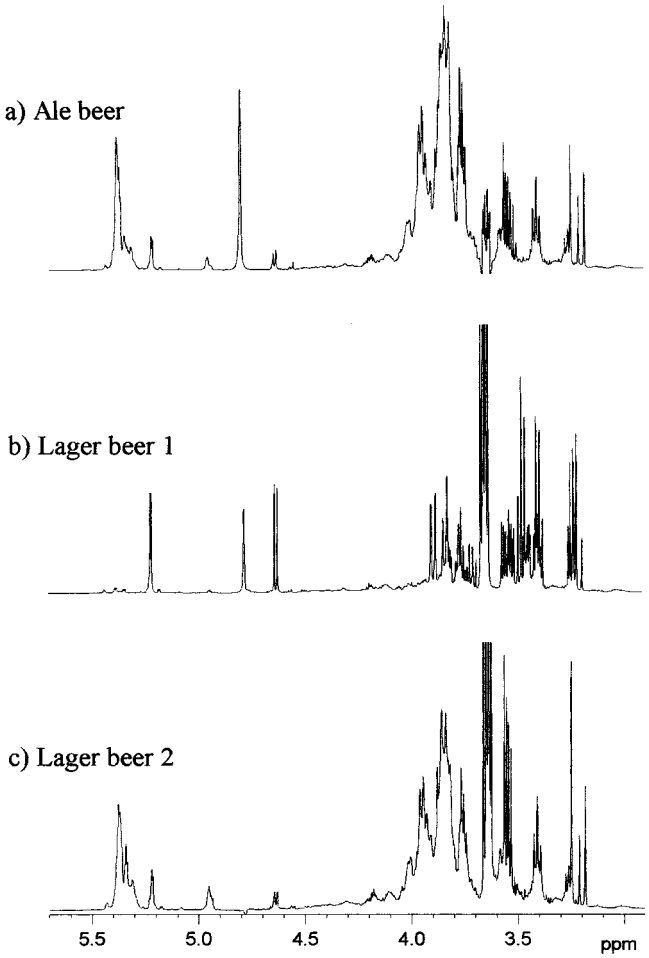

Figure 4. Sugar regions of the ^{1}H NMR spectra of different beer samples, recorded at 600 MHz.

the aromatic region or with resonances appearing at higher field. With support from the ^{1}H−^{13}C and *J*-resolved spectra (not shown) the assignment of most of the stronger peaks has been carried out showing the presence of organic acids, nucleic acid bases, and amino acids (**Table 1**). The underlying broad humps between 6.7 and 8.7 ppm may result from large molecular weight aromatic compounds, possibly polyphenolics. Interestingly, the broad resonances at 7.9−8.7 ppm show weak correlations with broad resonances at 3.8−4.5 ppm. This suggests the presence of nonaromatic moieties in the polyphenolics fraction; however, alternative and/or complementary methods are required to characterize the nature of these moieties.

As shown in **Table 1**, ∼25 compounds have been assigned with certainty in the ale sample investigated, whereas ∼40 more spin systems have been identified, mostly situated in the aliphatic and sugar regions, but their assignment remains unknown mainly due to signal overlap even in the 2D spectra.

PCA of Spectral Profiles of Beers. The 1D ^{1}H NMR spectra of 17 beer samples (6 lagers and 11 ales) have been recorded, and the sugar regions of some of these are shown in **Figure 4**, indicating that spectral profiles can indeed vary significantly between different beer types and labels. A clear observation, for instance, is that one of the lager beers contains mostly glucose (**Figure 4b**), contrary to the two other beers shown, which clearly indicate the predominance of dextrins, as shown by the lower resolution and the anomeric peak profile. However, the question that arises at this stage is if the observed composition may be consistently correlated with beer origin. Beer origin is in this work merely indicated by beer type and

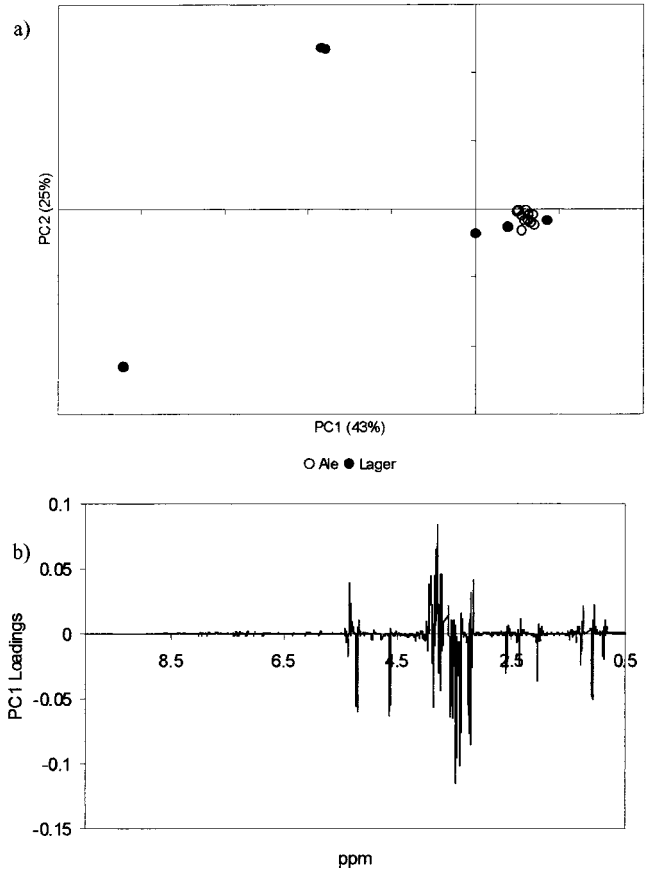

Figure 5. (a) PCA scores scatter plot and (b) loadings plot obtained when whole spectra are considered.

label but may be, in a widened study, extended to other important properties such as geographical origin and processing conditions.

PCA of the entire ^{1}H NMR spectra of the 17 samples was first attempted, giving the scores scatter plot shown in **Figure 5a**, where three lager beers are separated from the remaining samples. The corresponding loadings profile (**Figure 5b**) indicates that the signals responsible for this separation are the sugar resonances, rather than those of the less abundant compounds. This result may be easily interpreted by considering the differences in sugar profiles, as discussed before. Because, in this approach, the contribution of the minor components may be masked by that of the sugars, a more effective evaluation of the variability of minor metabolites may be carried out by considering particular spectral regions instead of the entire spectrum. Therefore, PCA was applied separately to the aliphatic and to the aromatic regions of the spectra. Interestingly, the same three lager samples differentiated by PCA from the entire spectra were also differentiated by considering the aliphatic region alone (results not shown); however, the dispersion was considerably larger than that in **Figure 5a**. On the other hand, satisfactory separation between ale and lager beers was achieved when only the aromatic region was considered (**Figure 6a**). However, in the present conditions no significant distinction between beer labels was noted. According to the loadings profile (not shown) and 1D spectra (**Figure 6b**), it is clear that some of the differences noted arise from shifts of some peaks due to different beer pH values, for example, peaks at ca. 7.2 and 7.8 ppm. However, and most importantly, the main peaks responsible for sample distinction in terms of type are two broad resonances at 6.8 and 7.5 ppm (indicated with arrows in the bottom spectrum of **Figure 6b**), probably arising from polyaro-

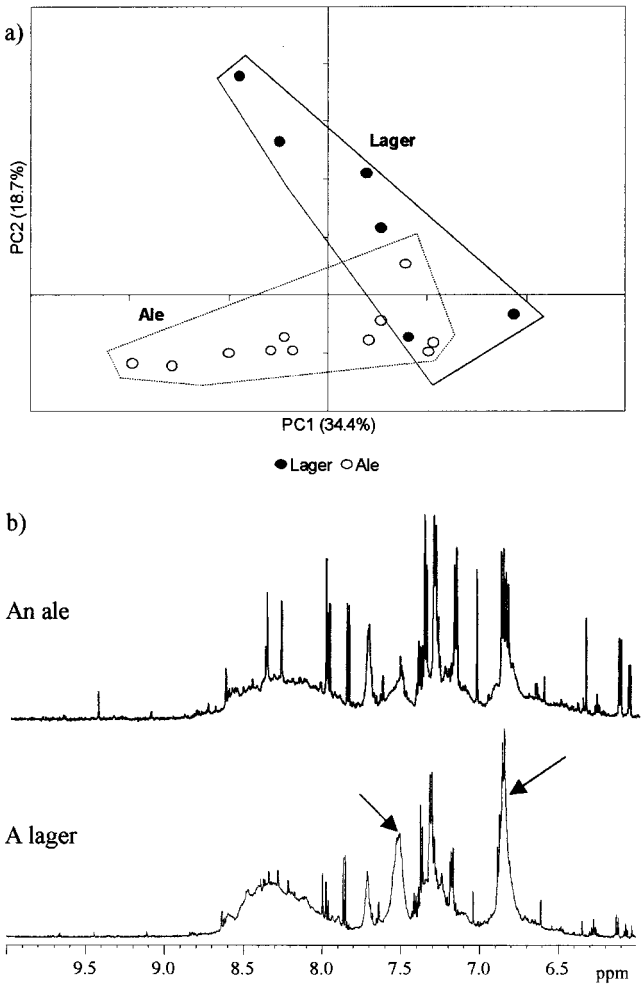

Figure 6. (a) PCA scores scatter plot obtained when only the aromatic regions are considered. (b) Aromatic regions of the ^{1}H NMR spectra of an ale and of a lager.

matic species such as polyphenols. These preliminary results suggest that beer distinction is possible at least in terms of beer types. The potential sensitivity of the method for label and other origin indicators will be investigated.

In conclusion, this work has shown that high-resolution NMR seems to be of great utility to characterize the complex chemical composition of beer, enabling the rapid identification of a large number of compounds, present in a range of concentrations. This is achieved in a noninvasive manner, requiring only the degassing of the beer sample. However, high-resolution NMR alone is insufficient to enable the full assignment of the beer spectra, mainly due to strong signal overlap, calling for alternative or complementary methods. The PCA results show that ales and lagers differ mainly in their aromatic compositions and can thus be distinguished on a PCA scores plot. These preliminary results open the possibility that the NMR/chemometrics method may be useful, when applied to a suitably enlarged group of samples, to provide rapid information about factors such as geographical origin, processing conditions, and reproducibility within different brewing sites.

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