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Discrimination of Beer Flavours by Analysis of Volatiles Using the Mass Spectrometer as an Electronic Nose

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ABSTRACT

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Entire mass spectra of beer headspace components were used as fingerprints for beer brand classification and differentiation of beer samples stored under various conditions. Chemometric analysis of the mass spectra allowed for the discrimination of beer brands and for the detection of beer aging and photodegradation. The numeric methods used include unsupervised PCA modelling and discrimination using *k*NN, LDA, and D-PLS methods.

INTRODUCTION

One of the key factors in the quality assessment of a beer is its flavour, determined to a great extent by a complex mixture of volatile constituents varying in chemical structures and concentration levels^{3,15}. Common methods to study beer volatiles are chromatographic separation techniques including GC and, particularly, sensory analyses (panel tests) in the brewing practice^{1,3,4,12,16}. Gas chromatography with mass spectrometry detection (GC-MS) offers a powerful tool to identify and quantify volatile beer compounds. Pattern recognition methods are often applied to the GC-MS results in order to elucidate the flavour characteristics. However, separation-based techniques are usually expensive and time-consuming; furthermore, trained personnel are required to adequately execute the measurements. An alternative approach is to analyze the overall composition of volatiles thereby omitting the separation steps⁹. Thus, “electronic noses” have

been developed as instruments that allow rapid and objective differentiation of samples. These can be categorised into either instruments equipped with solid-state gas sensors or a new generation of mass spectrometers applied as detectors. Both types of instruments have been used for the analyses of alcoholic beverages such as wines, whiskeys, cognacs, liquors, and beers^{8,10,11}. It has been shown that MS-based instruments have many advantages over sensor-based instruments, such as stability, sensitivity and versatility, as information is collected on the chemical composition of the analyzed mixtures. Moreover, the problem of interferences of ethanol with solid-state gas sensors is effortlessly eliminated in the MS analysis of alcoholic beverages.

We report on the application of a system consisting of a headspace sampler and a mass-spectral detector to the analysis of beer flavours. Our objective included fingerprinting of the volatile compounds in beers in order to discriminate between different beer samples, both in function of brand and of the storage conditions.

EXPERIMENTAL

Beers

Eight different beer brands (one ale, labelled number 1, and seven lagers, labelled numbers 2 to 8) from different breweries were bought in a local supermarket. These beers were chosen in order to investigate the capabilities of the techniques as regards brand discrimination and beer aging detection, being representative of those available to Polish consumers. Freshly opened samples were degassed using an ultrasonic bath prior to analysis. The characteristics of the beers are given Table I.

Beer aging

Beers 1, 2, 3 and 4 were chosen to be used in aging tests, in order to investigate the performance of the method in detecting aged beers. This subset included the only ale of the larger set and three lagers. The samples for aging tests were divided into three groups. After decapping the commercial bottles, beers (15 mL aliquots) were transferred into clear glass vials (20 mL), which were tightly closed. One group was stored in darkness at 4°C; the other samples were kept at 22°C, with one group stored in darkness, and another under diffuse lighting. The

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aging time for all samples was 21 days. The aging conditions reflect conditions that a beer bottle may undergo on a shelf in a shop (room temperature in light) or in storage (room temperature or cold, in darkness).

Methods

The analyses of volatile components were performed using an “electronic nose”, composed of a TurboMatrix HS-40 Headspace Sampler and a TurboMass Mass Spectrometer (Perkin Elmer, Norwalk, UK) and controlled by TMSOFT NT chemometric software (HKR Sensorsysteme, Munich, Germany).

The technique of headspace sampling provides solvent-free extraction of volatiles from a liquid mixture, while eliminating time-consuming and error-producing steps required in GC-sample preparation techniques such as purge-and-trap or solvent extraction.

Twenty replicates of each beer sample were analyzed using the “electronic nose” equipped to sample 2 mL aliquots sealed in headspace vials. Sampling parameters were as follows: sample temperature: 50°C, needle temperature: 100°C; time intervals: 30 min thermostating, 1 min pressurization, 20 min purge. The headspace of each sample was introduced into the mass spectrometer and the mass spectra of the constituents of each sample were acquired in the run: 30 s – zero level, 90 s – building-up and 30 s – signal level. Helium was used as a carrier gas. Full mass spectra of the beer headspace constituents were subjected to statistical analysis.

Data analysis

‘Principal Component Analysis’ (PCA), a multivariate technique acting in unsupervised manner, was used to analyze the inherent structure of the MS data sets^{14,17}. PCA reduces the dimensionality of a data set by finding an alternative set of coordinates, so-called ‘Principal Components’ (PCs). The PCs are linear combinations of the original variables, orthogonal to each other and designed in such a way that each variable successively accounts for the maximum variability in the data set. The principal component scores, when plotted, reveal relationship between samples such as natural sample clustering present in the data or outlier samples. The technique provides insights into how effective pattern recognition algorithms are in classifying the data. Data pre-treatment consisted of average-centring the spectra to eliminate any common spectral information.

Three methods of discriminant analysis were subsequently used for the purpose of group classification: ‘*k*-

Nearest Neighbours method (*k*NN)^{14,18}, ‘Linear Discriminant Analysis’ (LDA)^{7,13,14} and ‘Discriminant Partial-Least Squares’ regression (D-PLS)¹⁴.

The *k*-Nearest Neighbours method is a well-known non-parametric classification method. The test object is assigned to the cluster, which is the most represented in the set of *k* nearest training objects. The closest neighbouring data points are identified for each of the data points, called “*k*-nearest neighbours”, and then the decision is made according to the values of these neighbours. *k*NN is one of the simplest learning techniques. The *k* values were chosen in the range of *k* = 1,...,10 due to the size of the sample set, which was too small for higher values of *k*. This non-parametrical method was used because common parametrical methods like ‘Linear Discriminant Analysis’ and ‘Quadratic Discriminant Analysis’ are often unsuitable for datasets with a number of variables higher than the number of objects, due to matrix singularity or non-normality of the data set. The *k*-nearest neighbours method allows one to analyse full spectra without any previous reduction of the datasets.

Another method that enables analysis of full spectra is ‘Discriminant Partial Least-Squares Regression’ (D-PLS). In D-PLS, the spectral data are projected onto orthogonal factors called D-PLS components that describe the maximum covariance between the spectral information and the reference values. D-PLS is a method for relating variations in one or several response variables (Y-variables) to the variations of several predictors (X-variables), with explanatory or predictive purposes². D-PLS models both the X- and Y-matrices simultaneously to find the latent variables in X that will best predict the latent variables in Y. These D-PLS-components are similar to principal components. Interpretation of the relationship between X-data and Y-data is simplified, as this relationship concentrates on the smallest possible number of components. By plotting the first D-PLS components, one can view the main associations between the X-variables and the Y-variables, and also inter-relationships within the X- and Y-data sets.

Full cross-validation was applied for all of the regression models. Cross-validation is a strategy to validate calibration models based on systematically removing groups of samples in the modelling and testing the left-out samples in a model based on the remaining samples. The regression model was evaluated using the correlation coefficient (*r*) and the validation parameter, ‘Root Mean Square Error of Cross-Validation’ (RMSECV) as a term to indicate the error of the model. The RMSECV is defined as follows:

$$RMSECV = \sqrt{\frac{\sum_{i=1}^N (y_i^{pred} - y_i^{ref})^2}{N}}$$

where y_i^{pred} is the predicted concentration value for the sample in the cross-validation procedure, y_i^{ref} – the reference value, *N* – the number of samples.

The bootstrap method was used to estimate the classification error in LDA and *k*NN analyses^{5,6}. The dataset was randomly split into two independent sets: a training set and a test set. The training set was used to construct a rule, and the test set – to test it. This procedure was repeated many times. The version 0.632+ of the method was

Table I. Beer characteristics.

Beer number	Beer type	Alcohol content* [% v]	Extract content* [% w]	Bottle colour
1	Ale	7.5	17.48	Amber
2	Lager	5.0	11.0	Amber
3	Lager	5.0	11.9	Green
4	Lager	4.5	14.5	Amber
5	Lager	4.4	—	Green
6	Lager	5.8	—	Can
7	Lager	—	—	Clear
8	Lager	4.6	11.2	Clear

* Information taken from the beer labels.

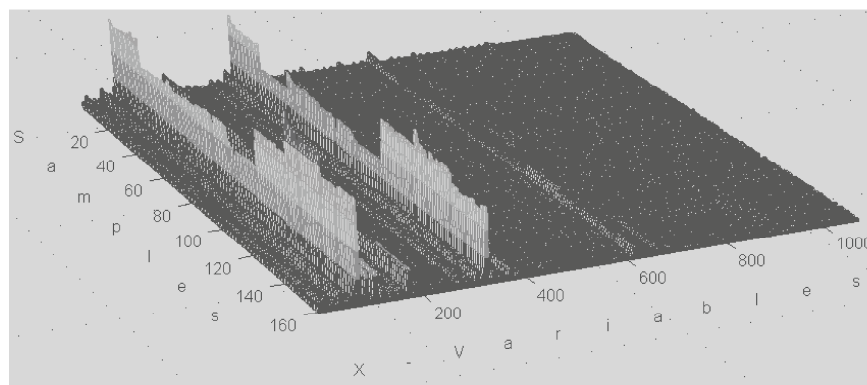


Fig. 1. Data matrix of mass spectra of 160 analyzed beer samples.

applied, which has a small bias and a small variance, and 50 bootstrap replications were found to be sufficient. Larger numbers of bootstrap replications did not improve the classification error estimates.

Data analyses were performed using The Unscrambler version 9.0 (Camo AS, Oslo, Norway) and Matlab 6.5.

RESULTS AND DISCUSSION

Fresh beers

Fig. 1 shows the data matrix consisting of mass spectra of 160 analyzed beer samples collected using the “electronic nose” and used for analyses of fresh beer samples. Similar ions were found in the mass spectra of all the beers studied, however, the spectra differed in the sample-dependent ion abundances. The most intense peaks correspond to the ions m/z 55 and 70. These peaks were reported to be part of higher alcohols and esters that are important for the aroma of beers⁸.

The mass spectra of the headspace volatiles of the beers were analyzed by various chemometric approaches. The unsupervised ‘Principal Component Analysis’ (PCA) was first applied to evaluate the data structure. The results are shown in Figs. 2 and 3, with Fig. 3 showing results for beer 2 as an illustrative example.

About 96% of the data variability is described by five principal components. Projection of the beer samples onto the plane defined by the first two PCs, which explain 90% of the variance, did not reveal any grouping of samples according to the beer brand. Therefore, other PCs were analyzed in order to discriminate the beers. Figure 2B shows the projection of the beer samples onto a plane defined by the PC1 and PC3 components, which, among the two-dimensional projections, provides the best separation of the samples studied. Marked sample clustering according to the beer brand is evident, although some groups are located close to each other or even partly overlap. The best results were achieved for beers 6, 7, and 8, which are well separated from the others. Clusters corresponding to beers 2 and 4, and 3 and 5 overlapped partially. Interestingly, beer 1, the only ale beer included in the data set, had PC1, PC2, and PC3 values very close to zero, as opposed to the lager beers. This signifies that other principal components should be used to characterize and differentiate the ale beers, which have an essentially different set of major volatiles, when compared to lager beers.

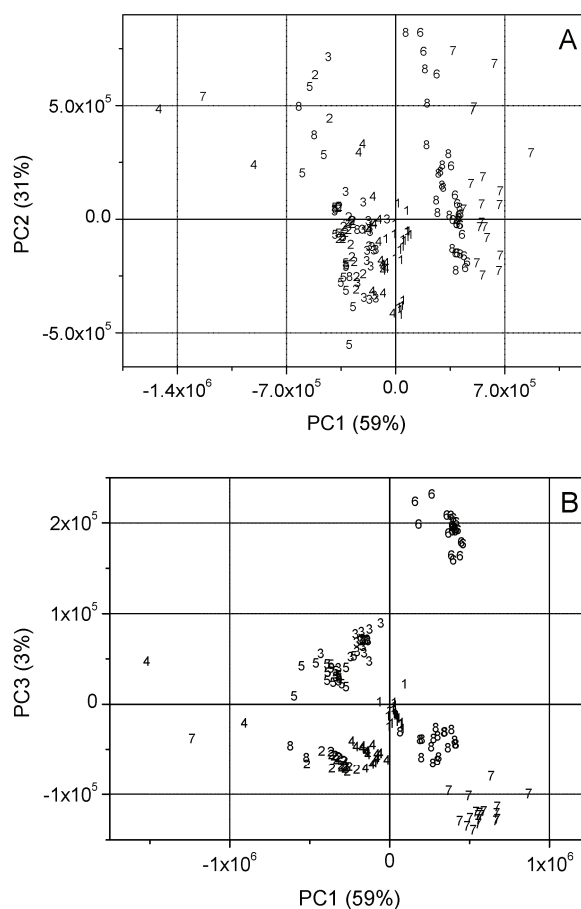


Fig. 2. Score plots of ‘Principal Component Analysis’ (PCA) of mass spectra of different fresh beers. Top: PC2 vs PC1. Bottom: PC3 vs PC1.

Generally, more than 2 PCs may be required to properly discriminate a given set of samples. In our case three PCs seemed to be sufficient, as seen later in the results section, where 3 latent components (similar to PCs in their nature) were required for proper D-PLS classification of the fresh beers. However, a visual representation of the samples classified by 3 or more PCs is quite intractable; therefore, we used two-dimensional plots to illustrate the results. Note that this method was only employed for general evaluation of the data structure and qualitative

(visual) discrimination, whereas quantitative discrimination was subsequently performed using other numeric techniques.

Analysis of the loadings plots allows choosing the variables that are most useful for beer discrimination. Thus, most important for the model studied are the variables associated with PC1 that primarily correspond to the m/z 55 and 70 peaks from the mass spectra. Other important variables, associated with PC3, correspond to the peaks with m/z 61, 73, and 88, with contributions from esters, and 70, with contributions from esters and alcohols⁸. Moreover, PCA analysis of the raw data enabled identification of some outlying samples, which were excluded from further analysis.

With respect to quality monitoring, it is desirable to separate products into classes. In order to test the feasibility

of such classification based on mass spectra, three statistical methods were employed: LDA, k NN, and D-PLS.

'Linear Discriminant Analysis' (LDA) was performed on simplified data sets. The m/z values for this purpose were selected based on the PCA analyses. The LDA method provided good results, while being very straightforward in calculation and interpretation. In the LDA method, only selected variables, corresponding to the ions with m/z 55 and 70, were extracted from the spectra, and used in the analyses. Table II shows the results of LDA for individual beers.

The discrimination was performed with 9.75% error for the entire sample set. The discrimination with an effectiveness exceeding 95% was achieved for beers 6, 8, and 1 (ale beer). The highest error was found for the classification of beer 4, which was most frequently wrongly classified as beer 2. These results show that even a relatively simple LDA method, based on only two of the most intense peaks, enables confident discrimination of beer brands.

The k NN method – in contrast to LDA – uses collective mass spectra for the analysis. The results of the k NN analyses are shown in Table III.

In the k NN methods, the classification error depends on the number of the nearest neighbours chosen for the analysis (k), showing a tendency to increase with increased k values. The classification error for fresh beers changed from 8.99% for $k = 1$ to 18.98% for $k = 10$. The overall classification error obtained at $k = 3$ for fresh beers was 10.87% with a standard deviation of 4.8%. The best performance was achieved for beer 1, which was discriminated with 100% efficiency. This result may be due to the fact that beer 1 was the only ale beer among the samples studied, and as seen earlier, quite distinct from the others. Beers 4, 6, and 7 were discriminated with an efficiency exceeding 90%. The lowest percentage of correct classification was achieved for beer 5, which was most often wrongly classified as beer 2 or beer 3.

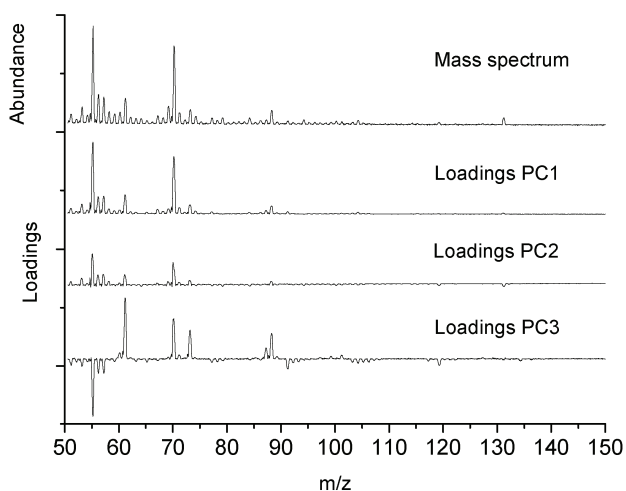


Fig. 3. Mass spectrum of a beer 2 and loading plots for the first three PCs of the 'Principal Component Analysis' (PCA) derived from the mass spectra of different fresh beers.

Table II. Classification of beers using 'Linear Discriminant Analysis' (LDA).

Beer brand	Predicted beer brand							
	1	2	3	4	5	6	7	8
1	96.23	0.00	3.50	0.27	0.00	0.00	0.00	0.00
2	0.00	90.41	0.00	9.59	0.00	0.00	0.00	0.00
3	0.00	0.00	91.83	0.82	7.08	0.27	0.00	0.00
4	0.27	22.31	0.00	71.51	5.91	0.00	0.00	0.00
5	0.00	0.80	3.45	5.04	90.72	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
7	0.00	0.93	0.00	2.47	1.85	0.00	84.57	10.19
8	0.00	0.85	1.13	0.56	0.28	0.00	0.56	96.62

Table III. Classification of fresh beers using collective mass spectra in the k -nearest neighbours method, $k = 3$.

Beer brand	Predicted beer brand, $k = 3$							
	1	2	3	4	5	6	7	8
1	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	86.73	0.89	1.47	9.44	0.00	0.00	1.47
3	0.00	0.91	85.98	2.44	10.67	0.00	0.00	0.00
4	0.00	4.19	2.40	92.22	1.20	0.00	0.00	0.00
5r	0.00	12.46	8.70	0.00	76.81	0.00	0.00	2.03
6	0.00	0.00	0.00	0.00	0.00	94.51	0.30	5.18
7	0.00	0.00	0.00	0.00	0.00	0.00	93.42	6.58
8	0.00	3.13	0.85	0.85	3.70	5.13	3.42	82.91

The discriminant PLS models were developed using non-metric dummy variables (set to 1 for the discriminated samples and to -1 for the others). Separate models were analyzed for discrimination of a particular beer brand from other brands, as well as for discrimination of beers stored in selected conditions from other beers. The results of the D-PLS analysis are shown in Table IV.

For five beers, namely, 4, 7, 8, 1, and 6, the D-PLS models gave correlation coefficients between predicted and actual beer brands exceeding 0.95 and relatively low RMSEP values. The poorest statistical parameters were obtained for models that discriminated beers 5 and 3 from all other beers.

Aged beers

Our objective here was to test whether mass spectra of volatiles allow discriminating between differently stored beer samples. PCA methods used for exploratory spectral analysis revealed clustering of the samples into 4 strongly overlapping classes corresponding to the three different aging conditions and fresh beers, respectively. With respect to quality monitoring, it is desirable to separate products into classes, for example, fresh and aged samples. In order to test the feasibility of such classification

Table IV. Results of 'Discriminant Partial Least-Squares Regression' (D-PLS) analysis used for discrimination between beer brands.

PLS model	Latent variables	<i>r</i>	RMSECV	Explained Y variance (%)
Beer 1	8	0.9748	0.153	95.07
Beer 2	8	0.9291	0.255	85
Beer 3	9	0.8014	0.406	63
Beer 4	6	0.9915	0.088	98
Beer 5	9	0.8738	0.325	75.5
Beer 6	8	0.9622	0.178	92.7
Beer 7	7	0.9865	0.107	97.3
Beer 8	8	0.9795	0.143	95.5

Table V. Classification of beers 1 to 4 stored in different conditions using collective mass spectra in Linear Discriminant Analysis (LDA).

Aging conditions	Predicted aging conditions			
	Fresh	Light 22°C	Darkness 22°C	Darkness 4°C
Beer 1: 12% (7%)*				
Fresh	100.00	0.00	0.00	0.00
Light 22°C	1.67	84.44	12.22	1.67
Darkness 22°C	0.44	6.22	73.78	19.56
Darkness 4°C	0.00	0.00	15.38	84.62
Beer 2: 14% (7%)*				
Fresh	100.00	0.00	0.00	0.00
Light 22°C	0.00	97.51	2.49	0.00
Darkness 22°C	0.00	8.18	60.45	31.36
Darkness 4°C	0.00	0.41	19.83	79.75
Beer 3: 14% (7%)*				
Fresh	100.00	0.00	0.00	0.00
Light 22°C	0.00	90.71	8.57	0.71
Darkness 22°C	0.00	2.81	75.84	21.35
Darkness 4°C	0.00	11.39	24.26	64.36
Beer 4: 6% (5%)*				
Fresh	100.00	0.00	0.00	0.00
Light 22°C	0.00	85.56	14.44	0.00
Darkness 22°C	0.00	10.62	89.38	0.00
Darkness 4°C	0.00	0.39	4.33	95.28

* Classification error (standard deviation).

based on mass spectra, the same three statistical methods were employed: LDA, *k*NN and D-PLS methods.

The results of LDA are shown in Table V. Note that fresh beers could always be classified correctly, while the highest misclassification rate was obtained for samples aged in darkness, either at room temperature or in the cold, depending on the beer, with one class of the latter two frequently misclassified as the other. This indicates that the latter two classes are the most similar within those studied, with the temperature being a minor factor compared to aging time and illumination in the degradation of the beer aroma.

*k*NN-analysis was performed for each of the four beers studied, assuming the existence of four classes corresponding to the fresh samples and three different aging conditions. The overall classification error depended on the beer studied. The classification results for the *k*NN analysis at *k* = 3 are shown in Table VI. The overall classification errors and standard deviations are higher than those obtained in the LDA analysis, with consequently higher misclassification rates, even for the fresh samples.

For all of the beers studied, the highest proportion of correct classification was obtained for fresh samples. The beer samples exposed to light at 22°C were also classified quite well by LDA, with *k*NN producing slightly inferior results. These samples were most often misclassified as samples aged in darkness at 22°C, both by LDA and *k*NN. The samples with the highest misclassification rates are those aged in darkness, in both methods, with the most frequent classification error being the beer aged at 4°C in darkness classified as beer aged at 22°C in darkness, and vice versa. The classification errors for these analyses were generally higher than those obtained for fresh beers.

Fig. 4 shows score plots of PC1 vs PC2 from D-PLS analysis of the mass spectra of beers 1 (ale) and 2 (lager), including fresh and aged samples. The score plots for both beers reveal clustering of samples according to the aging

Table VI. Classification of beers 1 to 4 stored in different conditions using collective mass spectra in the *k*-nearest neighbours method, *k* = 3.

Aging conditions	Predicted aging conditions <i>k</i> = 3			
	Fresh	Light 22°C	Darkness 22°C	Darkness 4°C
Beer 1: 16% (9%)*				
Fresh	100.00	0.00	0.00	0.00
Light 22°C	0.00	76.47	22.94	0.59
Darkness 22°C	0.00	14.62	59.43	25.94
Darkness 4°C	0.98	0.00	16.59	82.44
Beer 2: 18% (9%)*				
Fresh	100.00	0.00	0.00	0.00
Light 22°C	0.00	97.38	2.62	0.00
Darkness 22°C	0.00	11.56	52.44	36.00
Darkness 4°C	0.43	0.00	26.07	73.50
Beer 3: 16% (7%)*				
Fresh	99.72	0.00	0.28	0.00
Light 22°C	0.00	73.38	21.58	5.04
Darkness 22°C	0.00	0.00	90.05	9.95
Darkness 4°C	0.00	3.08	37.95	58.97
Beer 4: 25% (9%)*				
Fresh	89.77	1.75	7.02	1.46
Light 22°C	1.00	74.13	14.43	10.45
Darkness 22°C	3.39	12.71	69.49	14.41
Darkness 4°C	1.31	20.52	17.90	60.26

* Classification error (standard deviation).

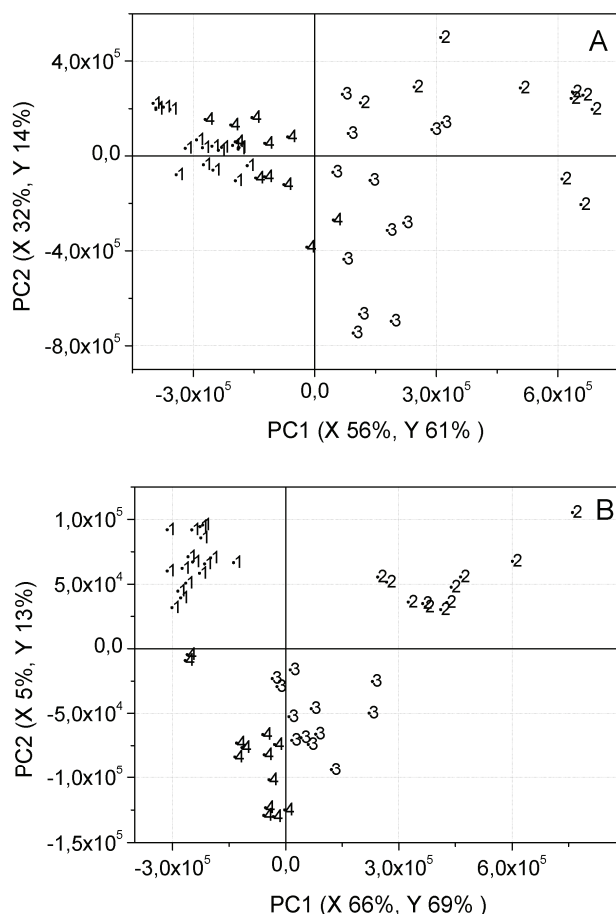


Fig. 4. Score plots of PC2 vs PC1 from 'Discriminant Partial Least-Squares Regression' (D-PLS) models for discriminating samples aged in light at 22°C from fresh beer and beer aged in other conditions. A. Beer 1 (ale). B. Beer 2 (lager). Samples: 1) fresh beer; 2) stored for 21 days in light at 22°C, 3) stored for 21 days in darkness at 22°C, 4) stored for 21 days in darkness at 4°C.

conditions. For beer 2 fresh samples are clearly separated from the others. The samples exposed to light also form a separate class. Samples stored in darkness at 22°C and 4°C are indeed quite close to each other, as we would expect from the classification results. Similar results were obtained for beer 1 and other beers, although the separation of groups corresponding to differently aged samples was less obvious in some cases.

Table VII shows the results of the D-PLS analyses for the four beers, used for discrimination of samples stored in various conditions. For all beers, the best models are those differentiating fresh beers from stored samples ($r > 0.98$). For beers 1, 2, and 3, relatively good models were also obtained for discrimination of beers exposed to light from all the other beer samples. Poorer models for these three beers referred to samples stored in darkness at 4°C and 22°C, the last case being the worst. Note that the number of latent variables required for aged beers is larger than that required for fresh beers; this very well reflects the additional difficulties encountered when classifying the same samples by other techniques and the generally higher classification errors for the aged beers.

Table VII. Results of 'Discriminant Partial Least-Squares Regression' (D-PLS) analyses used for discrimination of samples aged in various conditions.

PLS model	Latent variables	r	RMSECV	Explained Y variance (%)
Beer 1				
Fresh	3	0.9879	0.151	97.7
Light, 22°C	8	0.9106	0.326	80.9
Darkness, 22°C	8	0.7192	0.582	55.1
Darkness, 4°C	4	0.8249	0.459	67.3
Beer 2				
Fresh	4	0.9948	0.096	98.97
Light, 22°C	6	0.9236	0.311	85.7
Darkness, 22°C	6	0.3595	0.798	13
Darkness, 4°C	5	0.7269	0.596	53.8
Beer 3				
Fresh	3	0.9916	0.127	98.4
Light, 22°C	5	0.8225	0.423	68.8
Darkness, 22°C	7	0.4959	0.741	25.1
Darkness, 4°C	7	0.7328	0.568	56.4
Beer 4				
Fresh	3	0.9889	0.141	97.8
Light, 22°C	4	0.7922	0.493	63.9
Darkness, 22°C	6	0.8024	0.497	66.6
Darkness, 4°C	6	0.9335	0.311	87.2

CONCLUSIONS

Discrimination between different beer brands can be achieved using mass spectra of headspace beer volatiles in combination with chemometric analysis. Application of PCA methods for the analysis of collective mass spectra aids to select the key variables that are the most important for subsequent discrimination. LDA using the selected variables and D-PLS using full spectra enable accurate beer classification. The k NN method provided generally less robust results, although it should be more successful on less diverse sample sets.

The fresh beers were discriminated from each other at the highest confidence levels. Beers aged in different conditions were confidently discriminated from fresh samples. Aged samples were sometimes misclassified as samples aged in different conditions; the aging temperature variations (4°C vs 22°C) were found to cause smaller changes in the beer samples as opposed to the aging time (3 weeks) or the presence of light.

The proposed approach may be routinely used for screening, quality control, and flavour analysis of beers using non-assisted D-PLS chemometric analysis. It would be interesting to compare the results of panel tests to those presently obtained, which are potentially more objective and less prone to human error.

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