

EVALUATION OF BEER AGING USING AN ELECTRONIC NOSE

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The studies of the applicability of headspace sampling coupled to mass spectrometry in discrimination of differently aged beers are reported. The entire mass spectra of headspace components provided "fingerprints" of the beer samples and were used for classification purposes. PCA analysis of the mass spectra revealed clustering of samples according to the aging procedure, allowing automated sample classification by appropriate chemometric methods.

INTRODUCTION

Food aromas are determined by complex mixtures of hundreds of volatile compounds with different chemical and sensory properties. The basic techniques available to assess the aroma of food include sensory analysis based on expert panel tests, and instrumental analytical techniques such as gas chromatography [Cortacero-Ramirez *et al.*, 2003; Daems & Delvaux, 1996; Meilgaard, 1982; Vanderhaegen *et al.*, 2003]. However, separation-based instrumental techniques are usually expensive and time-consuming, requiring trained personnel to execute the measurements. An alternative approach is to analyze an overall composition of volatiles thereby omitting the separation steps [Marsili, 2001]. Thus, "electronic noses" have been developed as instruments that allow rapid and objective analysis of the flavour of food products. The two types of electronic noses use either solid-state gas sensors or mass spectrometers as detectors. Both types had been used for beer flavour analysis, although mass-spectrometer-based instruments have several important advantages [Kojima *et al.*, 2005; Marti *et al.*, 2004, 2005; Sikorska *et al.*, 2007]. They provide signals containing useful information that can be used as a fingerprint of any given food based on its aroma. Some information on the chemical composition of the analysed mixtures can be derived as ions of certain m/z values, can be associated with the presence of particular compounds. Moreover, they are characterised by higher stability, sensitivity and versatility as compared to sensor-based instruments. The problem of interferences of ethanol with the solid-state gas sensors is naturally eliminated in the MS analysis of alcoholic beverages.

This article explores the possibility of application of an electronic nose in the analysis of beers. The objective of the

present study was to investigate volatile beer constituents in order to discriminate between differently aged beers. The analytical results were interpreted by using unsupervised principal components analysis (PCA).

MATERIALS AND METHODS

Beers. We analysed samples of a lager beer of a single brand, including those freshly manufactured and subjected to different aging treatments routinely used in brewery. All samples were supplied by a local brewery. The samples and the respective aging procedures are listed in Table 1.

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-generating steps required in GC-sample preparation techniques.

TABLE 1. Samples and the respective aging procedures.

Sample	Aging procedure
A1, A2	fresh beer from two different production batches
B1	forced aging, 7 cycles, according to PN-A-79093-9
B2	forced aging, 8 cycles, according to PN-A-79093-9
C1	3 months, 20°C, darkness
C2	7 months, 20°C, darkness

Nine replicates of each beer sample were analyzed using the “electronic nose” equipped to sample 2 mL aliquots. Samples were 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, through a transfer line heated to 120°C, and an open split interface. The total mass spectra of all the volatile constituents of each sample were recorded in the m/z range of 50–150 amu. The spectra were measured in the run: the building-up time, which includes the zero-level time (30 s) and the time required for signal build-up (90 s), after the ion intensities reached a stable plateau, the signal was acquired for 30 s (signal-level time). Helium was used as a carrier gas.

Full mass-spectra of the beer headspace constituents were subjected to a statistical analysis.

Data analysis. ‘Principal Component Analysis’ (PCA), a multivariate technique acting in unsupervised manner, was used to analyse the inherent structure of the data sets [Smilde *et al.*, 2004; Wold *et al.*, 1987]. PCA reduces the dimensionality of a data set by finding an alternative set of coordinates, the 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 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 in centring the spectra to eliminate any common spectral information; in this procedure the average of each variable is subtracted from a variable, for each data point. Full cross-validation was applied to the models.

Data analyses were performed using Unscrambler ver. 9.0 (Camo AS, Oslo, Norway).

RESULTS AND DISCUSSION

Presently, the volatiles of the beers were transferred directly from the sample vial, using the headspace sampler, into the mass-spectrometer detector, excluding any chromatographic separation. The recorded mass spectra contained numerous characteristic features that provided “fingerprints” of the beer samples.

Similar ion sets were found in the mass spectra of all the beers studied, however, the spectra differed in sample-dependent ion abundances. The most intense peaks corresponded to the m/z 70 and 55 ions. The same peaks have been reported recently in the mass-spectra of various beers [Kojima *et al.*, 2005; Sikorska *et al.*, 2007]. These peaks have contributions from several alcohols, including propyl alcohol, isobutyl alcohol, and isoamyl alcohol, and esters, including ethyl acetate and isoamyl acetate, all of which are important for the aroma of beers [Kojima *et al.*, 2005].

The mass-spectra of the headspace volatiles of the beer samples were analysed by multivariate methods. The unsu-

pervised ‘Principal Component Analysis’ (PCA) was applied to evaluate the data structure. An entire set of spectra including those of fresh and aged beer samples was analysed. However, the analysis of these raw spectra revealed only minor grouping of samples. In contrast, the analysis of spectra after normalization and outlier removal revealed definite trends in the data set, corresponding to the beer freshness and the aging procedure used. The results of the PCA are shown in Figures 1 and 2.

About 98% of the data variability is described by the first five principal components. Projection of the beer samples onto the plane defined by the first two PCs, which already explain 95% of the total variance, revealed grouping of samples according to the production batch and aging procedure.

Certain distinct patterns are evident in the sample location on the scores plot. The fresh samples, A1 and A2, are characterised by the higher values of PC1 and lower values of PC2 components. Interestingly, fresh samples from the two different production batches can be clearly differentiated. The samples subjected to forced aging process, B1 and B2, differ from fresh samples mainly by the higher values of the PC2 component. In contrast, the beers stored in darkness at 20°C,

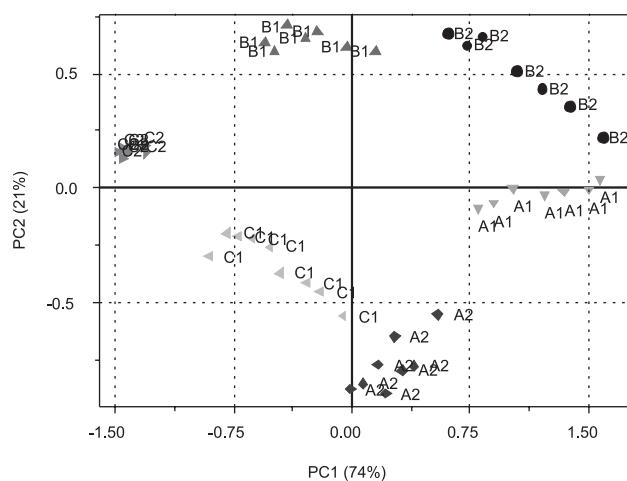


FIGURE 1. PCA score plots of the mass-spectra of the beers studied.

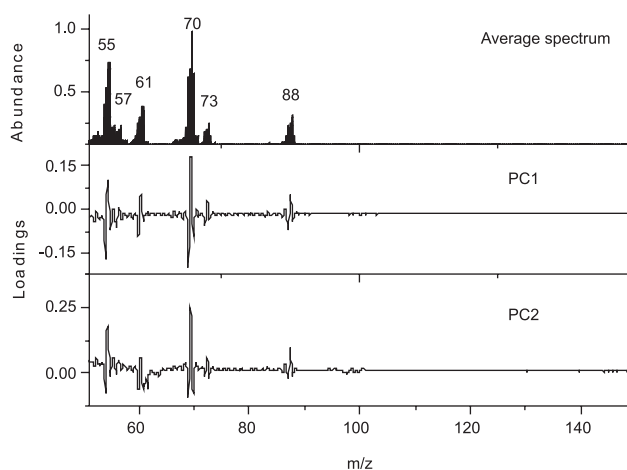


FIGURE 2. Average mass-spectrum of the beers studied and the loading plots for the two first PCs of the PCA.

C1 and C2, are distinguished from the fresh samples by lower values of the PC1 component. Moreover, the sample C2, stored for a longer period of time (7 months), shows a shift along the PC2 axis in the direction of the samples B1 and B2, subjected to forced aging.

The analysis of the loadings plots shows that similar ions are associated with both the PC1 and PC2 components. The most important ions contributing to PC1 and PC2 are the m/z 70, 55, 61 and 88 peaks from the mass-spectra. The most significant difference in loadings between the first two PC components occurs for the peak corresponding to 61 m/z.

CONCLUSIONS

The present work demonstrated that mass-spectrometer based electronic nose is a valuable tool in the investigations of beer flavour changes during aging. Discrimination between differently aged beers can be achieved using mass-spectra of beer headspace volatiles in combination with Principal Component Analysis. The proposed approach may be routinely used for quality control and flavour analysis of beers.

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REFERENCES

1. Cortacero-Ramirez S., de Castro M.H.B., Segura-Carretero A., Cruces-Blanco C., Fernandez-Gutierrez A., Analysis of beer components by capillary electrophoretic methods. *Trends Anal. Chem.*, 2003, 22, 440-455.
2. Daems V., Delvaux F., Multivariate analysis of descriptive sensory data on 40 commercial beers. *Food Qual. Pref.*, 1996, 8, 373-380.
3. Kojima H., Araki S., Kaneda H., Takashio M., Application of a new electronic nose with fingerprint mass spectrometry to brewing. *J. Am. Soc. Brew. Chem.*, 2005, 63, 151-156.
4. Marsili R.T., SPME-MS-MVA as a rapid technique for assessing oxidation off-flavors in foods. *Headspace Analysis of Foods and Flavors*, 2001, 488, 89-100.
5. Marti M.P., Boque R., Busto O., Guasch J., Electronic noses in the quality control of alcoholic beverages. *Trac-Trends Anal. Chem.*, 2005, 24, 57-66.
6. Marti M.P., Busto O., Guasch J., Application of a headspace mass spectrometry system to the differentiation and classification of wines according to their origin, variety and ageing. *J. Chromatogr. A*, 2004, 1057, 211-217.
7. Meilgaard M.C., Prediction of flavor differences between beers from their chemical composition. *J. Agric. Food Chem.*, 1982, 30, 1009-1017.
8. Polish Standard PN-A-79093-9. Beer. Methods of analysis. Determination of bottles and cans filling, clarity assay and stability determination with the forcing method (in Polish).
9. Sikorska E., Chmielewski J., Gorecki T., Khmelinskii I.V., Sikorski M., De Keukeleire D., Discrimination of beer flavours by analysis of volatiles using the mass spectrometer as an electronic nose. *J. Inst. Brewing*, 2007, 113, 110-116.
10. Smilde A., Bro R., Geladi P., *Multi-Way Analysis with Applications in the Chemical Sciences*. 2004, Chichester, John Wiley.
11. Vanderhaegen B., Neven H., Coghe S., Verstrepen K.J., Verachtert H., Derdelinckx G., Evolution of chemical and sensory properties during aging of top-fermented beer. *J. Agric. Food Chem.*, 2003, 51, 6782-6790.
12. Wold S., Esbensen K., Geladi P., *Principal component analysis. Chemometrics & Intelligent Laboratory Systems*, 1987, 2, 37-52.

OCENA PROCESU STARZENIA PIWA Z ZASTOSOWANIEM NOSA ELEKTRONICZNEGO

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W pracy przedstawiono wyniki badań nad możliwością wykorzystania „nosa elektronicznego”, ze spektrometrem mas w roli detektora, do rozróżnienia piw poddanych procesom starzenia. Mieszanina związków lotnych uwalnianych przez badaną próbę analizowana była w spektrometrze mas z pominięciem etapu rozdziału na poszczególne składniki. Otrzymane widma mas stanowią swoisty chemiczny „odcisk palca” i mogą zostać wykorzystane w celu klasyfikacji prób. W celu interpretacji uzyskanych wyników zastosowano metodę analizy głównych składowych.