

GLOBAL ANALYTICAL SOLUTIONS



**GERSTEL**

AppNote 11/2002

## Discrimination of Different Beer Sorts and Monitoring of the Effect of Aging by Determination of Flavor Constituents Using SPME and a Chemical Sensor

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### KEYWORDS

Chemical sensor, SPME, beer, flavor analysis, principal component analysis

### ABSTRACT

A new technique using a chemical sensor (Gerstel, SPME ChemSensor System) consisting of a GC-MS (Agilent 6890-5973N) system with a Solid Phase MicroExtraction (SPME) autosampler (Gerstel, MPS 2) was applied to the analysis of flavor compounds in different beer sorts.

The flavor compounds were extracted by SPME and analyzed with the GC-MS. The obtained GC-MS data were used to identify the particular compounds and characterize the chemical composition of the beer flavor. A chemometrics-pattern recognition software (Infometrix, Pirouette) was used for multivariate data analysis.

Freshly opened bottles from different beer sorts were used to build a model and represent the fresh grades. The variations in the fingerprint mass spectra of the different samples were analyzed using principal component analysis (PCA).

The composition spectrum of each sample becomes a dot on a 3-dimensional PCA plot. The dots from similar samp-

les cluster together on the plot. Samples that differ in their flavor components due to different composition (different beer sorts or aging processes) group in different clusters.

If samples were classified to be different, the chemical sensor provided hints which ions were responsible. Extracted ion chromatograms were used to locate and identify the compounds that caused the sample to be different respectively aged.

## INTRODUCTION

*Aim of the study.* The aim of utilizing SPME and chemical sensor technology for the analysis of flavors in beer was to illustrate the potential of the technique for classifications and monitoring of the effect of aging in food and beverage analysis.

*Why using SPME?* Flavors in food and beverages having different chemical natures (acids, alcohols, aldehydes, esters) in aqueous media are very difficult to analyze, because of the different polarities of the compounds and their interactions with the matrix. SPME has been proven to be a very sensitive method for the analysis of flavor and fragrance compounds [1]. The use of a fiber for extraction enhances the selectivity and sensitivity of the analysis compared to the generally used techniques of static or dynamic headspace sampling.

*Why using a chemical sensor?* The main applications for chemical sensors are in the field of quality control. Due to the relatively high number of sample lots that need to be analyzed daily, it is not possible to qualify all samples by classical investigations, e.g. GC-MS analysis.

The Gerstel ChemSensor (Figure 1) consists of a headspace and/or SPME autosampler (Gerstel, MPS 2) directly coupled to a mass spectrometer (Agilent 5973N). This system in combination with chemometrics data analysis provides fast pass/fail answers in quality control analysis.

The Gerstel ChemSensor System (Figure 2), like the one used in this study, consists of a headspace and/or SPME autosampler (Gerstel, MPS 2) and a GC-MS system (Agilent 6890-5973N). This system is highly flexible and can be used for both GC-MS and ChemSensor applications. The GC is normally kept isotherm and there is virtually no separation of the sample constituents. The mass spec gives rise to a single broad peak composed of all the constituents

from the sample. Mass spectra of all the compounds are overlaid. Multivariate analysis and pattern recognition software is used for data analysis (Infometrix, Pirouette). When “out of spec” samples are encountered, the combination of the sensor with a GC allows fast trouble shooting, since the system can be easily switched between ChemSensor and GC-MS mode. In this case, the GC is temperature programmed to separate the analytes to locate and identify the compounds that caused the particular sample to be different.



**Figure 1.** Gerstel ChemSensor.



**Figure 2.** Gerstel ChemSensor System.

In this work Principal Component Analysis (PCA) is used as a chemometric tool to detect the variations in the fingerprint mass spectra. The variables are the sums

of abundances of each m/z fragment over the run time of the analysis.

## THEORY

*Solid Phase MicroExtraction (SPME)*. SPME was introduced as a solvent-free sample preparation technique [1]. The principle approach of this technique is to use a small amount of an extracting material (usually less than 1  $\mu\text{L}$ ) to extract traces of organic compounds from aqueous samples. The extracting phase is either a high molecular weight polymeric liquid (e.g. PDMS) or it is a solid sorbent of high porosity to increase the surface area available for adsorption.

The SPME device utilizes a small fused silica fiber, usually coated with polymeric phase. The fiber is mounted in a syringe like device for protection. The analytes are absorbed or adsorbed by the fiber phase, depending on the nature of the coating until an equilibrium is reached in the system. The amount of an analyte extracted by the coating at equilibrium is determined by the magnitude of the partition coefficient of the analyte between the sample matrix and the coating material.

After extraction, the SPME device is transferred to the analytical instrument. In the case of GC analysis, the fiber is inserted into a hot inlet, where thermal desorption of the trapped analytes takes place [2]. Several kinds of coatings with different film thickness for different applications have become commercially available from Supelco. In this study we used a Carboxene/PDMS (75  $\mu\text{m}$ ) coating which has a strong affinity to volatile compounds of different polarity.

SPME sampling can be performed in 3 basic modes: direct extraction, headspace extraction, and extraction with membrane protection. In this study, SPME extraction is performed in headspace mode. The Carboxene/PDMS fiber can provide a more efficient headspace SPME method sensitivity for more volatile analytes [1, 2].

*Principal Component Analysis (PCA)*. PCA is a mathematical tool to represent the variation present in the data in many variables using a small number of "factors". The variations in the samples are plotted by redefining the axes using factors rather than the original measurement variables.

These new axes, referred to as factors or principal components (PCs), allow to probe matrices with many variables and view the true multivariate nature of the data in a relatively small number of dimensions [3].

All principal components have the following properties [3]:

1. All PCs explain the maximum amount of variation possible in the data set in one direction. It is the direction that describes the maximum spread of data points. The first PCs are calculated such that describe the largest variation in the data set.
2. Each sample has coordinates defined by the original measurement variables. It also has coordinates with respect to the new PC axes. The coordinates of the samples relative to the PC axes are typically termed "scores".
3. Each PC is constructed from combinations of the original measurement variables. The extent to which a measurement variable contributes to a PC depends on the relative orientation in space of the PC and variable axes. The variable axes, which are potentially the best at discriminating between samples are those which contribute most significantly to the individual principal components.
4. Excluding non-significant PCs can be used to filter noise from a data set. The first PC describes the largest variation in the data set. Each PC contains also noise, but the noise is spread out amongst all of the PCs. The ratio of signal-to-noise is highest in the first PC and decreases as subsequent PCs are calculated. The result is that non-significant PCs typically describe more noise than signal and, therefore, their exclusion filters noise from the data set.
5. The maximum number of PCs that can be calculated is smaller than the number of samples or variables.
6. PCs are orthogonal to each other and therefore not correlated.

## MATERIALS & METHODS

*Materials*. 5 different German pilsener beer sorts in glass bottles were purchased at a local store: Krombacher, Warsteiner, Veltins, Koenig Pilsener, and Brinkshoffs No. 1. Furthermore, Koenig Pilsener purchased in an aluminum can was also analyzed to prove that a discrimination of the same beer sort from the same vendor filled in glass bottle and aluminum can is possible.

**Instrumentation.** The SPME ChemSensor System consisting of a SPME autosampler (Gerstel, MPS 2) and a GC-MS (Agilent 6890-5973N) was used for conventional GC-MS and ChemSensor analysis. The GC-MS measurements were performed using a DB-Wax column (30 m x 0.25 mm x 0.25  $\mu$ m). The GC oven had an initial temperature of 40°C (1 min) and was ramped with 5°C/min to a final temperature of 300°C (8 min).

The PTV inlet (Gerstel, CIS 4) was set to the hot split mode (220°C, split 5:1) and the EPC pneumatics to the constant flow mode (1 mL/min).

The MS was used in the scan mode (45-300 amu, 5.46 scans/sec).

The chemometrics software Pirouette from Infometrix was used for Principal Component Analysis. Gerstel ChemSensor software was used to format the GC-MS data in order to be compatible with Pirouette.

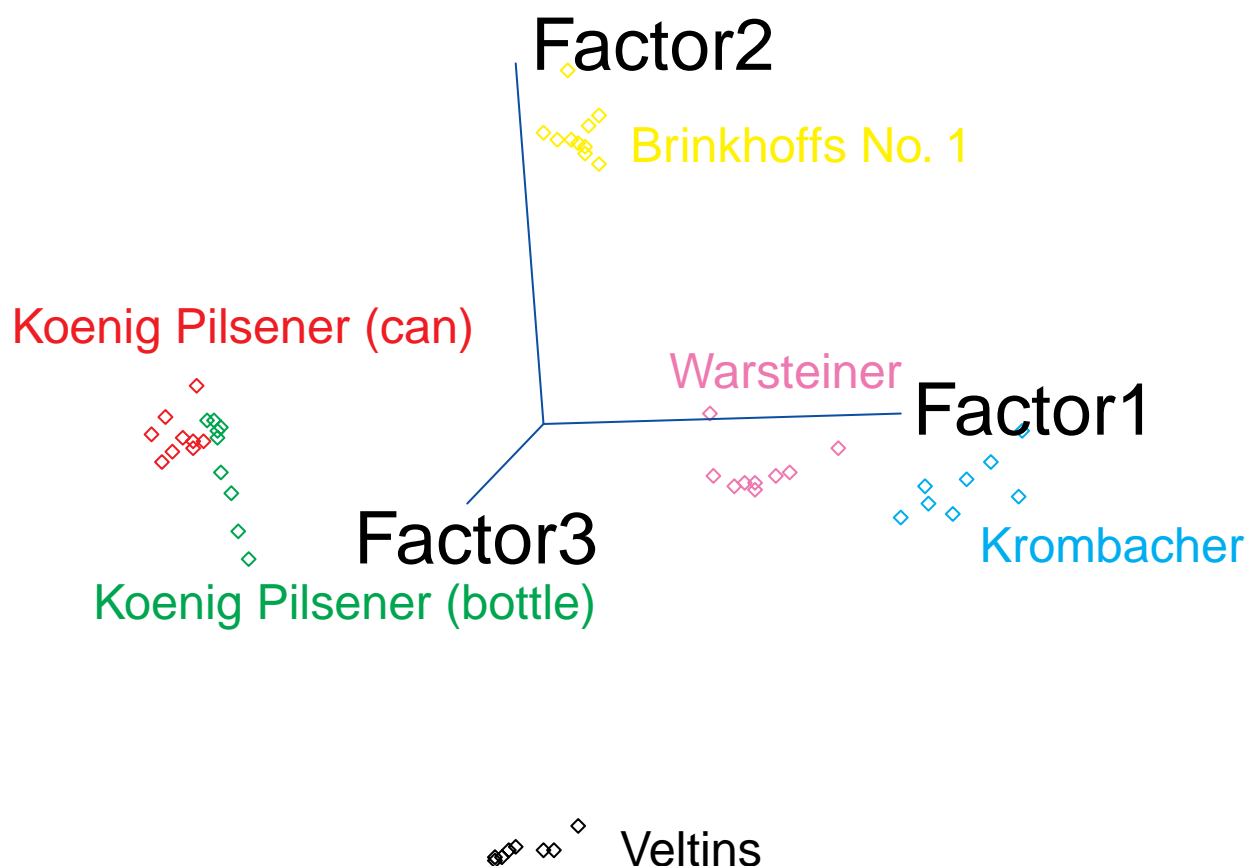
**Sampling.** Ten replicates of each sample were analyzed using 5 mL aliquots in 10 mL vials, which were crimped and equilibrated 2 min at 45°C before SPME

sampling. SPME headspace extractions were performed using a 75  $\mu$ m Carboxene/PDMS fiber within 15 min at 45°C and sample introduction was achieved in the GC inlet at 220°C within 3 min.

For the aging experiment, the 6 different beer sorts were filled in open transparent glass bottles and were stored in the lab in front of the window. After 3 and 6 days all the beer sorts were sampled to be analyzed by the ChemSensor System to prove if it is possible to discriminate between the freshly opened beer and those which have been opened and lighted up for few days.

## RESULTS & DISCUSSION

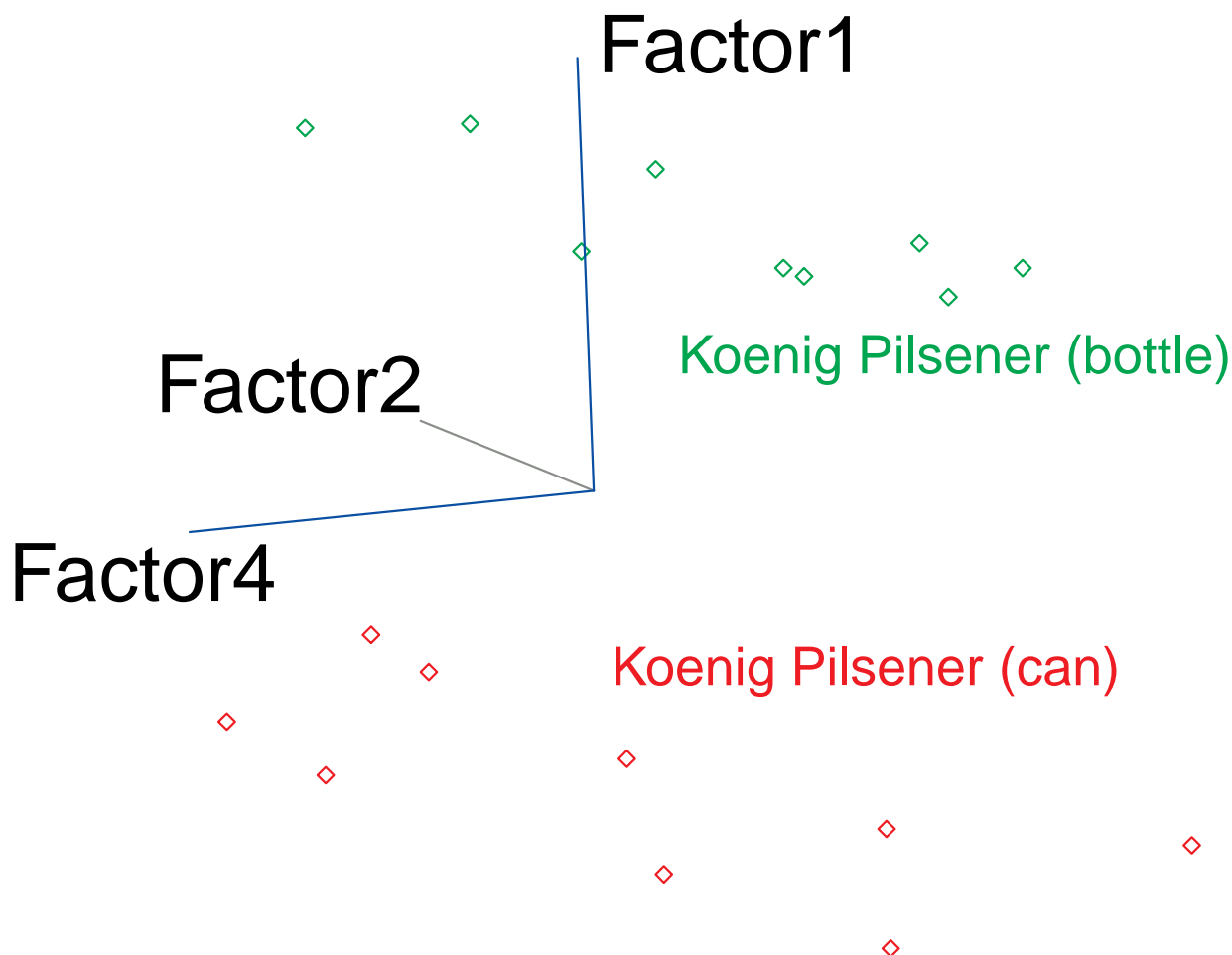
The projection of the 6 different samples into the 3-dimensional space of a PCA scores plot (4 factors) is shown in figure 3. 98-99% of the data variability is described by the first 3 factors (PCs), whereas the first PC always describes over 80% of the data variability. As shown all 5 beer sorts can be discriminated by the model. The group of the 2 sample types of Koenig Pilsener beer (bottle and can) cluster together.



**Figure 3.** PCA scores plot (4 factors) of the 6 beers fingerprint mass spectra.

Figure 4 shows the projection of the 2 sample types Koenig Pilsener bottle and Koenig Pilsener can into the 3-dimensional space of the first 3 of 5 factors. It is

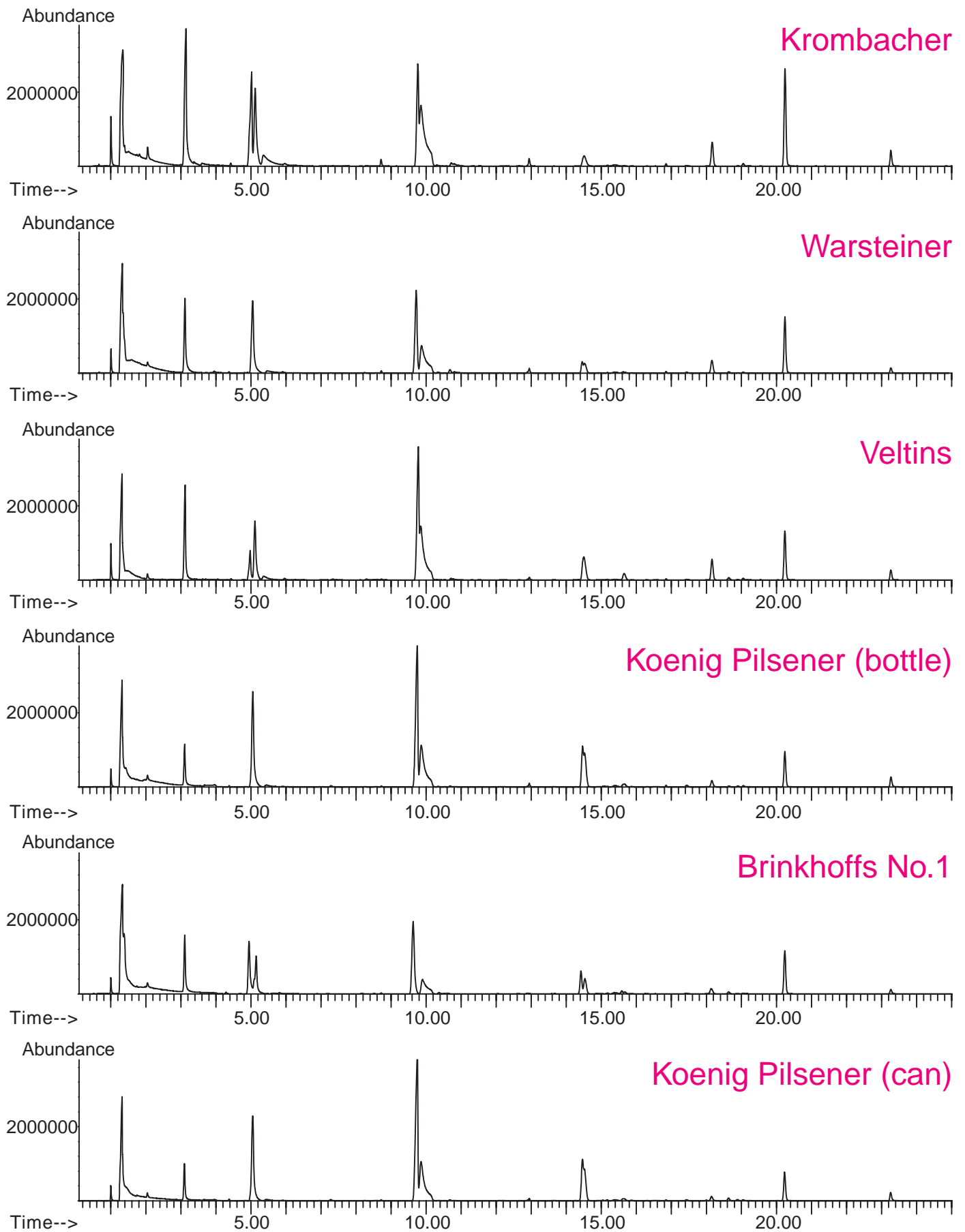
even possible to discriminate between the same beer sort stored in bottle and can.



**Figure 4.** PCA scores plot (5 factors) of Koenig Pilsener in bottle and can.

The GC-MS total ion chromatograms of the 6 samples are very similar (figure 5). It is obvious how difficult it is to discriminate the samples only by GC-MS analysis, apart from the time consumption needed for the chromatographic separation. The chromatographic separation took 41 min in our study. Using the System in the ChemSensor mode (GC column is kept isotherm) the sample throughput is determined by the SPME sampling time, which was 17 min (2 min

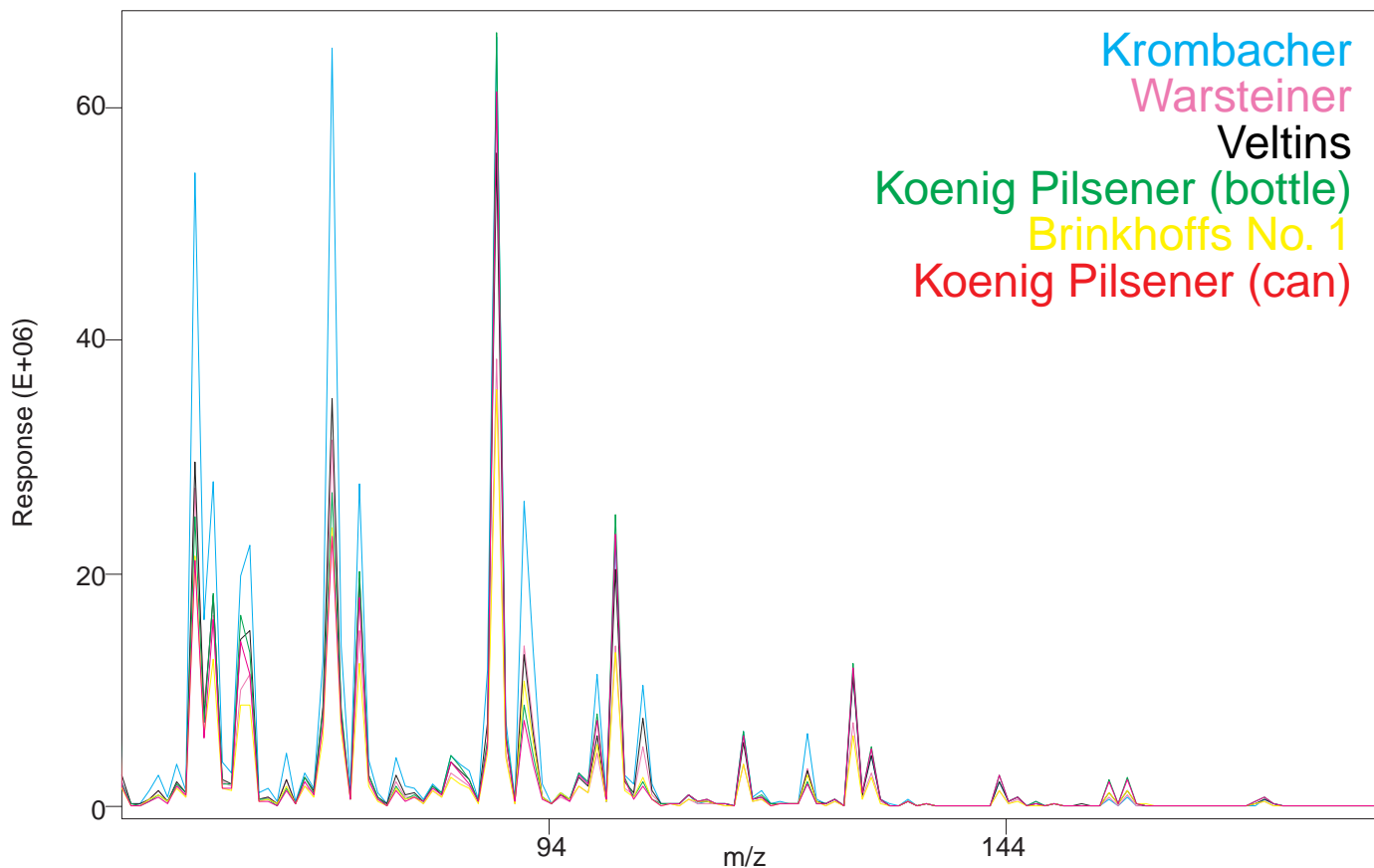
equilibration time and 15 min extraction time), and the MS analysis (around 4 min). The most advantageous mode of the ChemSensor System in combination with SPME would be to perform a fast separation within the sampling time, e.g. using “Fast-GC”. Since sample equilibration can be performed overlaid using the MPS 2, sample throughput is determined only by SPME extraction time.



**Figure 5.** GC-MS total ion chromatograms of the different beer sorts.

The mass spectra of the samples however, can be used as their fingerprints. As shown in figure 6, the mass spectra of the different beer sorts indicate differences in the abundances of some ions, mainly  $m/z$ : 55, 61, 70, 88, 91, and 104. The extracted chromatograms of these ions give hints to the compounds responsible for the differences in the different samples. These com-

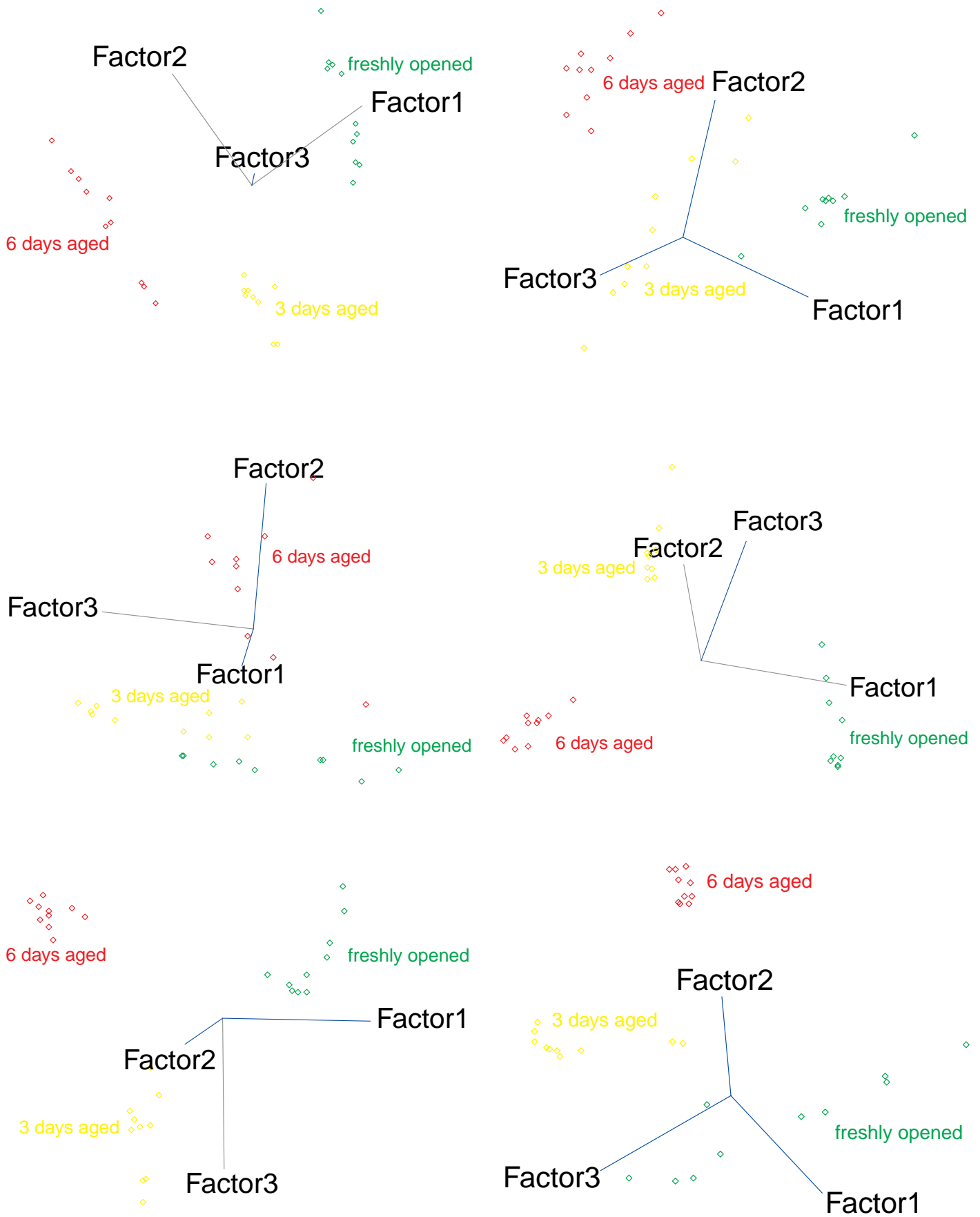
pounds are ethyl acetate ( $m/z$ : 61), 1-butanol 3-methyl acetate ( $m/z$ : 70), ethyl caproate ( $m/z$ : 55), octanoic acid ethyl ester ( $m/z$ : 88), decanoic acid ethyl ester ( $m/z$ : 88), acetic acid 2-phenylethyl ester ( $m/z$ : 104), and phenylethyl alcohol ( $m/z$ : 91). As an example for compound identification, a Warsteiner beer chromatogram can be seen in figure 8.



**Figure 6.** Line plot of the mass spectra of the different beer sorts.

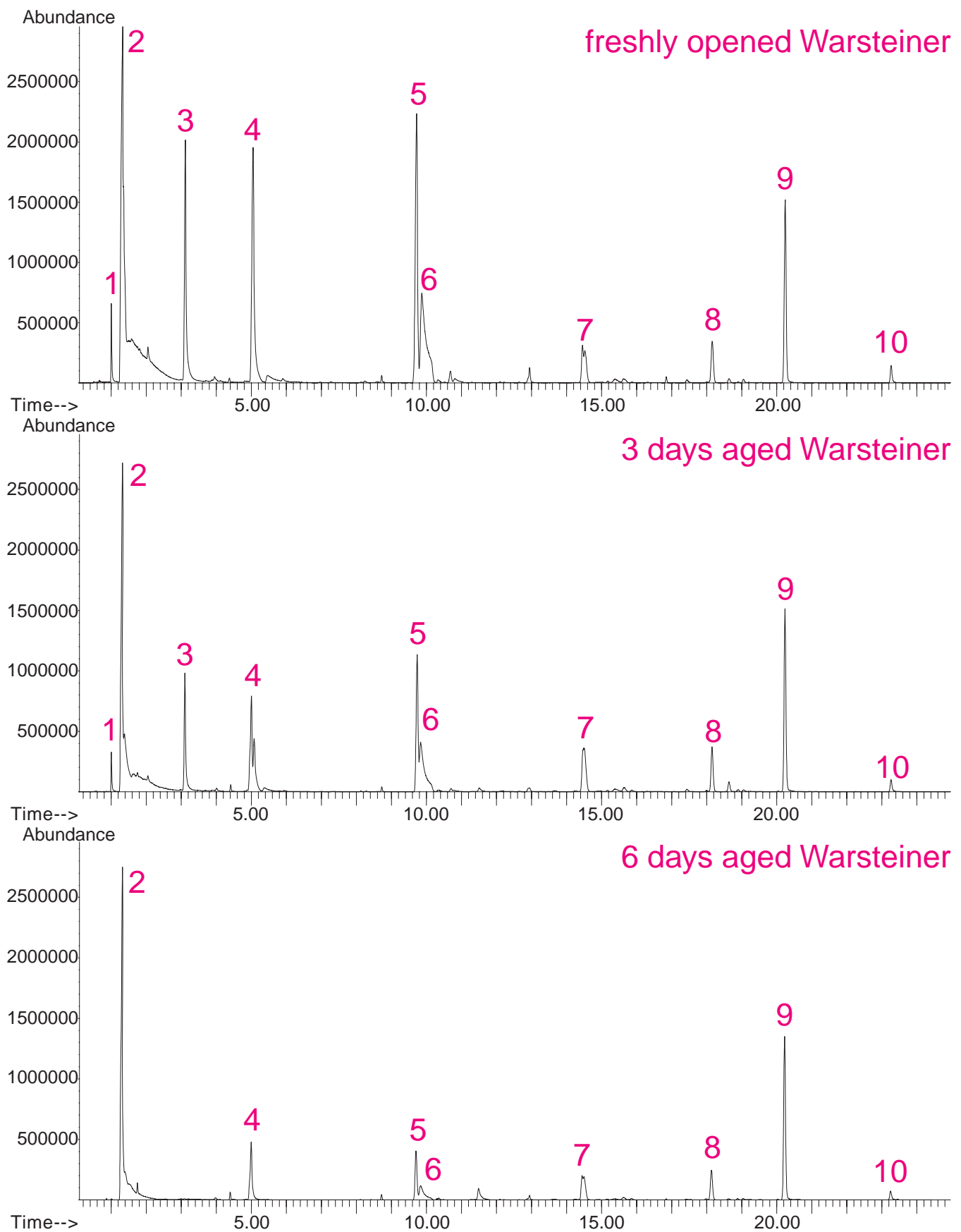
Figure 7 shows the projection of the mass spectra of the freshly opened and 3 and 6 days aged beers into the space of the first 3 factors (PCA scores plots). Since over 90% of the variability was captured within the first 3 PCs, it is confident that differences in the scores are differences in the flavor composition of the samples. Obviously, altering of the beer flavor during aging within 3 and 6 days can be observed by chemical

sensor analysis. As shown in figure 8, as example chromatograms of freshly opened and 3 and 6 days aged Warsteiner beer, the alteration of the flavor compounds is also evident from peak intensities of compounds like 1-butanol 3-methyl acetate, ethyl caproate, and the 2 isomers of octanoic acid ethyl ester (peaks 3, 4, 5, and 6).



**Figure 7.** PCA scores plots (3 factors) of the mass spectra of freshly opened and 3 and 6 days aged beers.





**Figure 8.** GC-MS total ion chromatograms of freshly opened, 3 and 6 days aged Warsteiner beer. Peak identification: 1: ethyl acetate, 2: ethanol, 3: 1-butanol 3-methyl acetate, 4: ethyl caproate, 5 and 6: 2 isomers of octanoic acid ethyl ester, 7: decanoic acid ethyl ester, 8: acetic acid, 2-phenylethyl ester, 9: phenylethyl alcohol, 10: octanoic acid.

## CONCLUSIONS

The classification of the different beer sorts and screening of aging effects was possible using chemometrics PCA models of fingerprint mass spectra.

The chromatographic separation of the samples enables identification of the compounds responsible for the differences in the samples.

The SPME ChemSensor System can be utilized for classification and quality control in food and flavor analysis. This system is very flexible and allows use of the instrumentation in conventional GC-MS and ChemSensor mode. The best advantage of using the system in the ChemSensor mode is saving the GC separation time in routine analysis. Separation of analytes can be performed by temperature programming of the GC column if “out of spec” samples are encountered.

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