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Determination of aging-relevant substances in beer by solid phase microextraction/capillar gaschromatography (SPME/GC)

A microanalytical method is described that enables the quantitative determination of a whole spectrum of aging-relevant carbonyl components in beer. The carbonyls are separated from the beer matrix using the headspace-SPME-technique and are determined by gas chromatography after derivatization on the SPME-fiber.

Descriptors: Beer, carbonyl components, headspace-SPME-technique, gas chromatography.

1 Introduction

During the aging of beer, alterations in flavour occur which influence considerably the beer quality. Besides other trace compounds, several carbonyls are known to be responsible for this impairment of beer flavour. With respect to this, especially the very low threshold levels of alkanals of mid-chainlength are of critical interest. Flavour stability is therefore an important criterium of beer quality. To assure this the breweries routinely control analytically their beers.

Most commonly the following analytical procedure is applied in brewers laboratories (1):

Steam distillation of the interesting carbonyl compounds from a beer sample of about 200 ml by water, extraction of the distillate with dichloromethane, concentrating of the extract and successive separation and detection by gas chromatography. This procedure is time-consuming and expensive at the same time. A reduction of operating expense can be achieved by application of the solid phase extraction method (SPE), where a separation of the carbonyls occurs on a solid phase (SPE-column). After detachment and concentration gas chromatography is again used for determination.

But the investments in time and money are still considerable.

Recently, a new procedure has been developed which enables the adsorption of the analytes of interest directly from the sample on an especially coated silica fiber, followed by their estimation in the gas chromatograph after thermal desorption. This procedure called "Solid-Phase Microextraction (SPME)" was introduced for the first time by Belardi, and Pawliszyn (2) and Pawliszyn (3) in 1989. SPME is a simple and time-saving enrichment procedure which works without solvents, which is highly automatizable and which is suitable for a great number of more or less volatile organic compounds (4,5).

First of all, like in the SPE an adsorption of analytes takes place using an adsorbent of suitable polarity. But in difference to the SPE. the analytes are not completely extracted from the sample. It works in such a way that a balance of distribution is established between the analyte molecules on the stationary polymer phase and in the sample direct or in the gas area ("headspace") above the sample.

For an analysis a coated SPME fiber is exposed either directly to the sample ("direct immersion") or to the gas area over the sample. Proportional to the concentration of the substances in the sample their loading on the fiber takes place, which is again temperature and time-dependent. Thus the SPME combines extraction and enrichment in one elegant step The sample should be heavily agitated, because otherwise only an insufficient quantity of the analytes is adsorbed on the fiber due to a lack of balancing (6).

After completion of the extraction procedure the SPME fiber is heated in the injector of the gas chromatograph and the adsorbed substances are thermally desorbed. Subsequently, their chromatographic separation and detection takes place.

The solid phase microextraction exhibits the following advantages in relation to other extraction techniques:

- ☐ less work procedures;
- ☐ work without organic solvents;
- ☐ small sample volumes;
- ☐ low matrix influences;
- ☐ automizable procedure (an appropriate autosampler is available);
- ☐ reusable fibers.

Subsequently the application of the SPME technique in connection with gas chromatography to determine the aging-relevant carbonyls in the beer is described.

To increase the selectivity and detector response the carbonyls are transferred by means of pentafluorobenzylhydroxylamine into

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their appropriate oximes. For the detection of the derivatives in GC the electron capture detector (ECD) can be used (7).

2 Material und Methods

2.1 SPME equipment

SPME Sampling Stand with magnetic stir plate, SPME Fiber Holder for use with manual sampling and SPME Inlet Guide and the red SPME fiber (100 mm Polydimethylsiloxan (PDMS)), purchased from Supelco.

All standards and also the derivatization agent were purchased from Aldrich.

2.2 Gaschromatography

Instrument: Varian GP 3800 with ECD;

Fused silica capillary column: Optima 5 (length 50 m, 0,25 mm i. d., film thickness 0,25 mm) from Macherey-Nagel

Condition for GC operation: carrier gas: hydrogen 5.0 (60 cm/s), detector: ECD (300 °C), split/splitless- injector (260 °C), split ratio 10:1 (opened after 6 min);

GC oven temperature program: 80 °C hold 1 min, 5 °C/min to 100 °C, 3 °C/min to 150 °C, 5 °C/min to 200 °C, 20 °C/min to 280 °C hold 10 min.

3 Elaboration of the analysis method

At first 20 of the important aging-relevant carbonyl compounds in beer were selected (Table 1).

Table 1 Selected aging-relevant carbonyl compounds in beer

1	3-methylbutan-2-one	11	heptanal
2	propanal	12	5-methylfurfural
3	2-methylpropanal	13	octanal
4	2-acetylfuran	14	benzaldehyde
5	butanal	15	2,4-trans, trans-heptadienal
6	2-methylbutanal	16	phenylacetaldehyde
7	3-methylbutanal	17	nonanal
8	pentanal	18	trans-2-nonenal
9	hexanal	19	decanal
10	2-furfural	20	trans, trans-2,4-nonadienal

The derivatisation took place first with standard solutions of the carbonyls in the presence of o-(2,3,4,5,6 pentafluorobenzyl) hydoxylamine (PFBHA).

PFBHA is particularly suited due to its selective reaction with carbonyl groups. As reaction products the appropriate cis and trans PFB carbonyl oximes are formed (Fig. 1). They can than be separated by gas chromatography and detected by means of different detectors, like FID, MS or ECD.

In the present study the ECD was preferred, because the ECD detects the halogenated derivatives selectively and in addition, its response is higher than that of the other detectors (8).

The formed cis /trans derivatives of the different carbonyl compounds are generally well separated on a suitable capillary column. For evaluation both signals are added and the sum of the peak areas is computed.

With regard to the derivatization technique several variations are possible:

1. Derivatization in the sample matrix followed by adsorption of the derivatives on the SPME fiber (direct immersion or headspace technique).
2. Loading of the SPME fiber with carbonyls in the headspace of the sample vessel followed by derivatization on the fiber in the headspace of a solution of the derivatization agent.
3. Initial loading of the fiber with the derivatization agent (in liquid or vapor phase) followed by reaction of the carbonyls directly on the fiber in the headspace of the sample ("on-fiber-derivatisation").

Extensive tests showed that the latter variant has to be preferred (7). In addition, different fiber types were tested. As particularly suitably proved a coating with 100mm Polydimethylsiloxan (PDMS) (7).

In the next step several ancillary conditions were tested:

- ☐ Optimal loading time of the fiber with the derivatization agent;
- ☐ time dependence of the reaction of carbonyls with derivatization agent on the fiber;
- ☐ desorption time of the PFB derivatives from the SPME fiber in the gaschromatograph.

The reaction conditions were optimized in each case with standard concentrations of the 20 carbonyls in water after addition of 5% ethanol (simulation of conditions in beer).

After numerous test series (7) the following optimal conditions were found:

- ☐ Loading time of PFBHA: 6 min headspace at ambient temperature;
- ☐ reaction time: 40 min headspace at 40°C;
- ☐ desorption of the PFB derivatives during GC: 6 min at 260°C.

Under these conditions calibration data of the carbonyls were obtained: Therefore standard solutions of the 20 selected carbonyls were set in concentrations from 0,5 to 50mg/l and their PFB-oximes were determined by SPME/GC as described above. Each standard was measured five times.

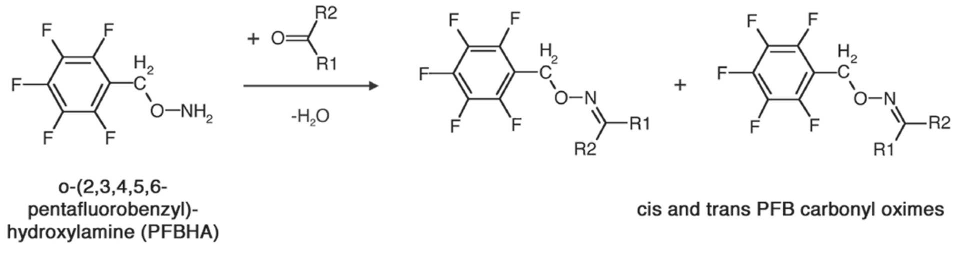

Fig. 1 Schematic diagram of the derivatization of carbonyl compounds with o-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA)

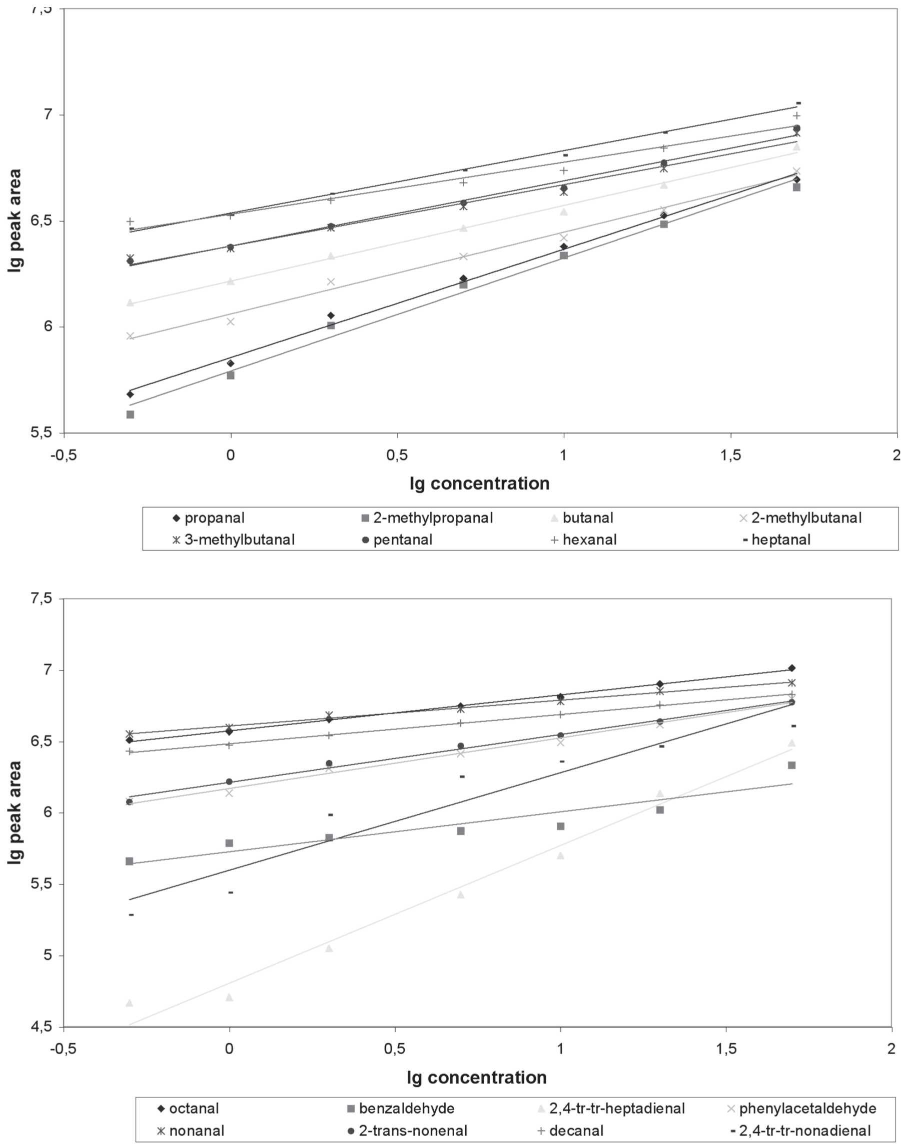

Fig. 2 Double logarithmic plot of the peak areas of the carbonyl derivatives against their concentrations after linear regression

Table 2 Equations of the regression curves and their coeffizients of correlation for the quantitative determination of carbonyls (concentration range 0,5 – 50 mg/l) by SPME GC/ECD after derivatization with PFBHA.

substance	Equation of regression curves	Coefficient of correlation R
propanal	y = 0,5111 x + 5,8548	0,9973
2-methylpropanal	y = 0,5335 x + 5,7901	0,9954
butanal	y = 0,3570 x + 6,2155	0,9975
2-methylbutanal	y = 0,3862 x + 6,0590	0,9958
3-methylbutanal	y = 0,2901 x + 6,3795	0,9916
pentanal	y = 0,3077 x + 6,3810	0,9947
hexanal	y = 0,2454 x + 6,5305	0,9881
heptanal	y = 0,2943 x + 6,5360	0,9980
octanal	y = 0,2522 x + 6,5743	0,9987
benzaldehyde	y = 0,2782 x + 5,7282	0,9274
trans, trans-2,4-heptadienal	y = 0,9661 x + 4,8052	0,9915
phenylacetaldehyde	y = 0,3548 x + 6,1691	0,9941
nonanal	y = 0,1811 x + 6,6079	0,9958
trans-2-nonenal	y = 0,3365 x + 6,2123	0,9958
decanal	y = 0,2055 x + 6,4831	0,9990
trans, trans-2,4-nonadienal	y = 0,6848 x + 5,5966	0,9582

During the experiments it was shown that from the 20 selected carbonyls only 16 could be reliably determined. The derivatization of 3-metylbutan-2-one, 2-acetylfuran, 2-furfural and 5-methylfurfural was not reproducible and as a consequence, the determination of these compounds was therefore no further considered.

The evaluation took place for each substance by means of the addition of the two cis-trans PFB carbonyl oximes and averaging five measurements. In the following the determined peak areas are plotted against the concentrations of the substances in the sample.

Alltogether the received calibration curves show particularly in the lower concentration range curved characteristics, which can be easily linearised using a double logarithmic plot (Fig. 2, Double logarithmic plot of the peak areas of the carbonyl derivatives against their concentrations after linear regression).

The equations of the different regression curves and their coefficients of correlation are shown in Table 2.

4 Application to beer

The calibration experiments have shown that the elaborated method supplies good results with respect to the determination of

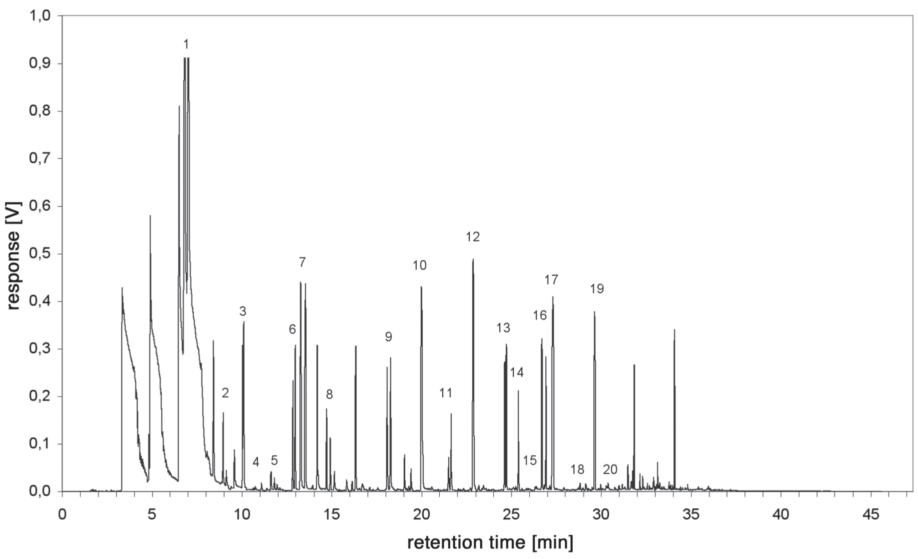

Fig. 3 GC-ECD chromatogram obtained after PFBHA derivatization of the carbonyls from fresh Pilsener beer

the volatile carbonyl compounds by SPME/GC in alcoholic solution. In the next step this method was applied to the matrix beer.

Fresh Pilsener beer (within the expiration date) and aged Pilsener beer (2 years after the expiration date) were compared. The application of our SPME method to fresh beer shows clearly the GC-signals of the expected carbonyl derivatives (Fig. 3, GC-ECD chromatogram obtained after PFBHA derivatization of the carbonyls from fresh Pilsener beer). Peak matching was achieved by heightening experiments (7).

A similar test with aged beer (Fig. 4, GC-ECD chromatogram obtained after PFBHA derivatization of the carbonyls of aged pilsener beer) shows a similar spectrum of the carbonyl derivatives, but with partially substantially deviating intensities, most probably due to the expected changes during the aging process.

The comparison of the two experiments shows that the concentrations of the 16 carbonyl compounds evaluated here are generally higher in the aged beer. This is especially the case with the homologous n-alkanals. Altogether it is shown that the new method of the determination of carbonyl compounds by SPME/GC can be successfully applied to the matrix beer. This opens new possibilities for the breweries to replace their time consuming and complex carbonyl analysis by a much simpler procedure.

5 Summary

An analytical method is presented, which allows to determine simultaneously a whole spectrum of aging-relevant carbonyl compounds in beer quantitatively down to the ppb-range at relatively small expenses.

The method is based on the modern solid phase microextraction (SPME) technique and is used in headspace application. After on-fiber derivatization of the carbonyl compounds with o-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine using headspace extraction above the beer sample, the carbonyl derivatives are separated and determined in the gas chromatograph by means of an electron capture detector.

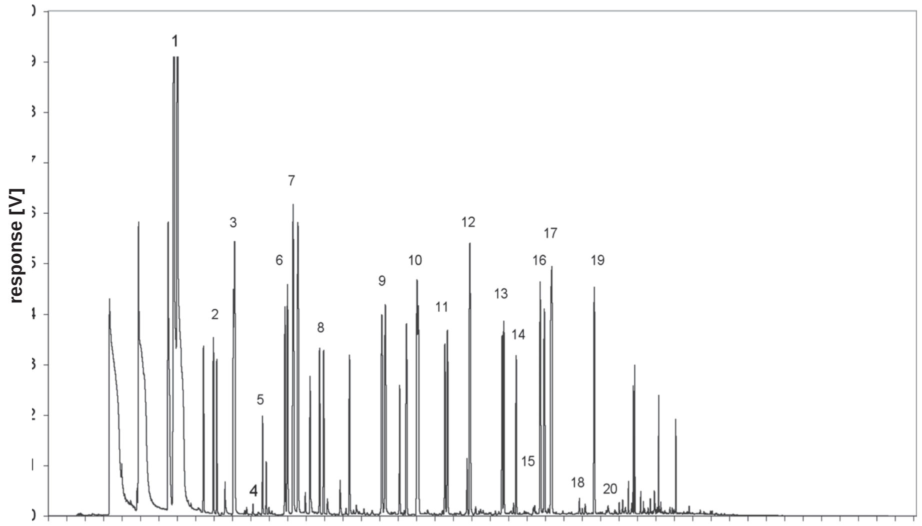

Fig. 4 GC-ECD chromatogram obtained after PFBHA derivatization of the carbonyls of aged pilsener beer

The method is first successfully established in 5% ethanol/water and then applied to beer samples of different age.

Acknowledgements

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