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Headspace analysis with large sample volumes

Influence of sampling device volume, analyte concentration and sample matrix

Frank Bohnenstengel, Nasrollah Soltani and Werner Baltes*

Institut für Lebensmittelchemie, Technische Universität Berlin, Gustav-Meyer-Allee 25, 13555 Berlin (Germany)

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ABSTRACT

In three series of experiments the factors influencing headspace gas chromatography (HSGC) with large sample volumes were investigated. The main factors studied were sampling device volume and interactions between the substances and between the volatiles and the sample matrix. It could be shown that variation in any of these factors causes a dramatic change in the resulting headspace composition. The results of these experiments are compared, and the reasons for the different behaviour of the substances are discussed. It is demonstrated that the mechanisms of interaction are complicated and difficult to estimate, especially in complex samples such as food flavours.

INTRODUCTION

Headspace gas chromatography (HSGC) has been shown to be a mostly objective analytical method for investigating food flavours. It has therefore become a widely used and important tool for aroma analysts. Originally developed by Machata [1] for the measurement of blood alcohol concentration, this method has undergone many modifications and improvements for the analysis of volatile food components, including flavour compounds, in the last 20 years.

A dramatic increase in sensitivity after injection of large headspace volumes can be obtained when the volatiles are simultaneously concentrated by cryofocusing directly on the gas chromatographic column or on a deactivated precolumn [2]. The applicability of this method for the analysis of complex food flavours has been demonstrated by several examples [3–9]. When evaluating the results of HSGC, influencing factors such as interaction of the volatile compounds with the sample matrix and the vapour pressure of the substances at a given temperature must be considered. These compound-specific properties can be expressed by the activity coefficient, γ, which depends on the partial pressure of the substance according to Henry's law [10]:

$$p_i' = x_i \gamma p_i^0$$

where p_i' is the partial pressure of compound i, x_i is the mole fraction of i, p_i^0 is the saturated vapour pressure of compound i at a given temperature.

The aim of this work was to determine to what extent the final headspace composition of a model solution is influenced by interaction of compounds with the sample matrix and with each other. The test compounds were chosen for

* Corresponding author.

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TABLE I

COMPOSITION OF THE INVESTIGATED TEST SOLUTION

No.	Substance	Boiling point (°C) [11,12]	Amount (mg/l)
1	2-Methylpropanal	64.2–64.6	199
2	3-Methylbutanal	92.5	102
3	2-Methylbutanal	92–93	137
4	Butanol	117.2	177
5	2,5-Dimethylfuran	93–94	193
6	1-Methylpyrrole	114–115	138
7	Dimethyldisulphide	109.7	147
8	3-Methylthiophene	115.4	201
9	Butylbutyrate	166.6	119
10	3-Methylpyridine	144.1	283
11	2,4-Dimethylthiazole	144–145	105
12	2,6-Dimethylpyrazine	155.6	129
13	2-Acetylpyrazine		114
14	Phenylacetaldehyde	193–194	188
15	Limonene	177–178	126
16	β-Caryophyllene	258–259	124

their differences in chemical structure (different substance classes), volatility and polarity, representing the complexity of food flavours.

MATERIALS AND METHODS

Sample preparation

Large headspace volumes were collected using a gas-tight syringe with a deactivated fused-silica needle according to the sampling procedure described by Wittkowski *et al.* [4]. The headspace sampling device (a 14-ml vial or a 250-ml or 1000-ml Erlenmeyer flask) was filled with 1 ml of the test solution in diethyl ether (for details of the chosen compounds and their concentrations, see Table I). This system was spiked with one of the substances in a great excess or with one of the test matrices listed in Table II. Afterwards the headspace sampling unit was connected to the flask and the sample was stirred for 20 min and finally maintained at exactly 20°C for 1 h.

Headspace injection

A 1-ml aliquot of the vapour phase was withdrawn from the thermostated headspace apparatus using a gas-tight syringe with a fused-silica needle and injected "on-column" with an injection speed of 0.5 ml/min. During the injection, the first loop of the deactivated precolumn was cooled with liquid nitrogen.

Gas chromatography

The gas chromatographic conditions were as follows: gas chromatograph, Carlo Erba HRGC 5160 Mega Series with an MFC 500 programming unit and an EL 480 electrometer; integrator, Shimadzu CR3-A; column, J&W DB-1, 60 m × 0.323 mm × 1 μm, connected to a 2-m deactivated precolumn; carrier gas, helium, average linear velocity 30 cm/s; temperature pro-

TABLE II

LIST OF INVESTIGATED MODEL MATRICES

Sampling device volume (ml)	Investigated model matrices
250	3 ml of glycerine
250	3 ml of paraffin
250	3 ml of water
250	1.4 g of gelatine + 10 ml of water
1000	3 ml of glycerine
1000	3 ml of paraffin
1000	3 ml of water
1000	1.6 g of gelatine + 10 ml of water

gramme, 30°C for 5 min, 30–40°C at 1°C/min, 40°C for 1 min, 40–260°C at 3°C/min, 260°C for 60 min; injector, on-column, room temperature; detector; flame ionization detector at a temperature of 280°C.

RESULTS AND DISCUSSION

To examine the interactions between the substances in headspace analysis we chose a model solution of test compounds dissolved in diethyl ether. It was necessary always to add the same amount of each component to the headspace vessel. Otherwise, without adding solvent, a similar dosing of the substances was not practicable. In the first series of experiments the volume of the headspace sampling device was varied. As depicted in Fig. 1 there were great differences in the resulting headspace chromatograms. Some of the higher-boiling substances could only be detected in larger amounts when sampling was carried out in the 1000-ml Erlenmeyer flask. For instance, phenylacetaldehyde and β-caryophyllene were detectable in the 14-ml vial only as trace components.

The peak areas shown graphically in Fig. 2 confirm these qualitative results. As shown, the peak areas of the higher-boiling substances were greatly increased when the 1000-ml Erlenmeyer flask was used, whereas the decrease in the low-boiling compounds was only small. This fact indicates the usefulness of increasing the sampling device volume for the investigation of substances with low volatility or decreasing the sampling device volume for the separation of high-boiling substances for the fast routine analysis of highly volatile compounds. Of course, diethyl ether as a low-boiling solvent would have some effect on the composition of the headspace compared with experiments without solvent. However, because it is present in large excess over the test solutes this effect will be the same in every experiment and can therefore be neglected.

On the basis of these results the 1000-ml configuration was preferred as the sampling device for further studies of headspace composition. In a second series of experiments the strength of interactions between the tested components was examined. For this the test solution

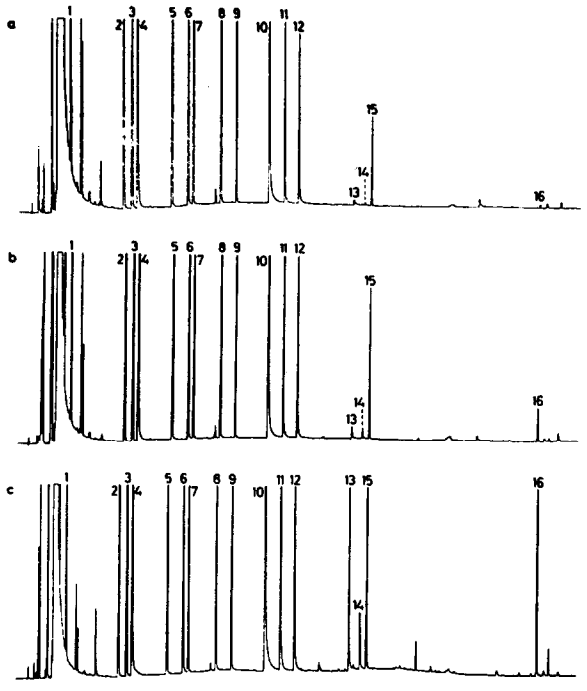

Fig. 1. Headspace gas chromatograms of a test solution after sampling from different sampling devices: (a) 14-ml vial; (b) 250-ml Erlenmeyer flask; (c) 1000-ml Erlenmeyer flask. (The peak numbers refer to compounds listed in Table I.)

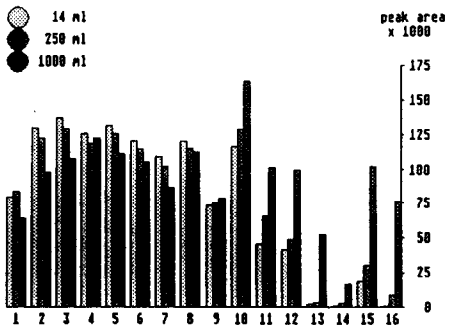

Fig. 2. Comparison of peak areas of reference compounds after HSGC with different sampling device volumes. For compound numbers, see Table I.

was spiked with an excess of one of the model compounds and the effect on the peak areas of the other substances was observed. The HSGC procedure was the same as described above.

Fig. 3 shows as an example, headspace gas chromatograms obtained after addition of 2-methylpropanal or 2,4-dimethylthiazole and, for comparison, the chromatogram of the original test solution headspace. These results are shown graphically in Fig. 4. In both cases all peak areas decreased drastically, but especially those of the higher-boiling substances. In the case of the addition of large amounts of 2-methylpropanal, some of the compounds (3-methylpyridine, 2-acetylpyrazine and β-caryophyllene) could no longer be detected.

The results of all substance addition experiments are summarized in Table III. As expected, there are some interactions between the compounds in the headspace above a sample. But it is surprising how strong these effects can be. On the one hand, a displacement of substances by others primarily takes place in the low-volatile fraction of the compounds investigated. These components are listed at the bottom of Table III. Several substances could only be detected as traces, or, if a greater excess of the additional compound (No. 1b, 2-methylpropanal; No. 3, 2-methylbutanal) was added, they disappeared. On the other hand, these interactions can also influence the headspace concentrations of higher volatile polar substances, such as butanol, which is displaced much more than other low-boiling compounds.

The addition of a low-volatile compound (*e.g.* 2-acetylpyrazine) influences the headspace composition only a little, as is shown in Table III, No. 6. The recoveries of all tested compounds were higher than 90%, except for butylbutyrate and phenylacetaldehyde. The recovery of the former was only a little less (79%), but the recovery of the latter was drastically reduced (6%).

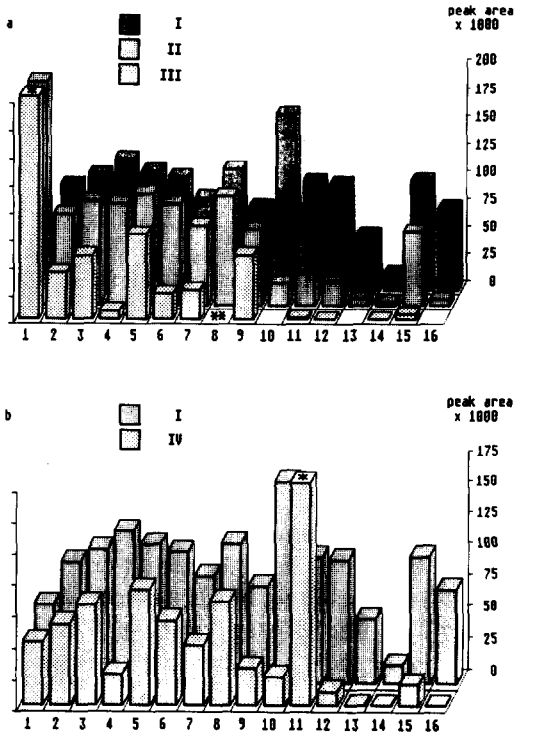

Fig. 4. Peak areas of test compounds. (a) After addition of 2-methylpropanal: I = original test solution; II = small excess; III = large excess. (b) After addition of 2,4-dimethylthiazole: I = original test solution; II = excess. * = Bars are not depicted at full height. ** = Peak overlapping with an impurity of 2-methylpropanal. (For compound numbers, see Table I.)

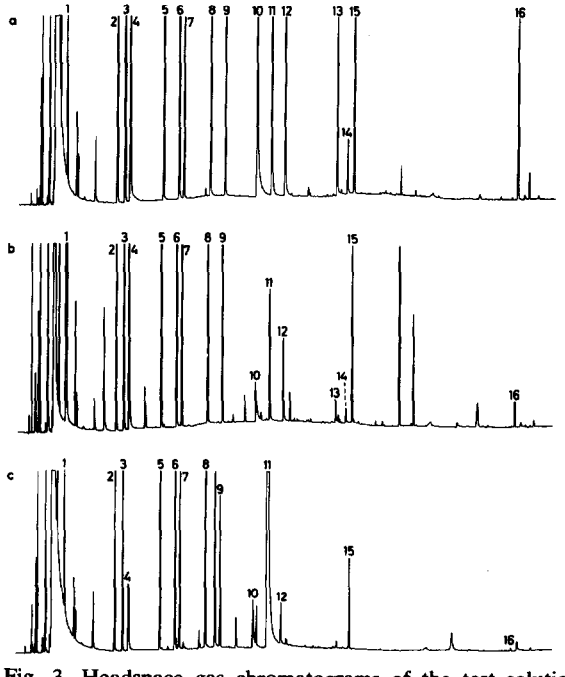

Fig. 3. Headspace gas chromatograms of the test solution before (a) and after addition of 2-methylpropanal (1) (b) or 2,4-dimethylthiazole [11] (c).

TABLE III

RELATIVE PEAK AREAS OF THE TEST COMPOUNDS IN DIFFERENT SUBSTANCE ADDITION EXPERIMENTS

Sampling device: 1000-ml Erlenmeyer flask. Std = Test solution; Ex = excess of the added compound; 1a/1b = small/great excess of 2-methylpropanal; 2 = excess of 3-methylbutanal; 3 = excess of 2-methylbutanal; 4 = excess of 3-methylpyridine; 5 = excess of 2,4-dimethylthiazole; 6 = excess of 2-acetylpyrazine.

Test compound	Std	1a	1b	2	3	4	5	6
2-Methylpropanal	100	Ex	Ex	68	46	85	81	90
3-Methylbutanal	100	87	46	Ex	29	61	67	96
2-Methylbutanal	100	90	55	67	Ex	72	76	95
Butanol	100	77	7	69	12	22	12	101
2,5-Dimethylfuran	100	93	71	78	42	82	84	93
1-Methylpyrrole	100	90	24	73	25	46	65	95
Dimethyldisulphide	100	86	33	60	18	47	57	100
3-Methylthiophene	100	91	[a]	79	29	68	76	96
Butylbutyrate	100	91	76	61	10	59	40	79
3-Methylpyridine	100	13	–	48	3	Ex	15	90
2,4-Dimethylthiazole	100	31	3	52	4	123	Ex	89
2,6-Dimethylpyrazine	100	22	1	47	4	13	12	102
2-Acetylpyrazine	100	14	–	24	–	2	2	Ex
Phenylacetaldehyde	100	22	7	11	–	6	6	6
Limonene	100	68	6	72	3	13	18	94
β-Caryophyllene	100	8	–	40	1	1	1	89

[a] Peak overlapping with an impurity of 2-methylpropanal.

These results indicate that there are strong interactions between the substances in the headspace over a complex sample. Even small changes in the sample composition can cause drastic changes in the resulting headspace composition, but the effects vary depending on the amounts and characters of the investigated substances. Therefore general rules cannot be given for the interactions of different compounds in the gas phase above complex samples because many different and unknown factors influence its composition.

We did not investigate the behaviour of individual test compounds. These data would only be of theoretical interest because they have no analytical relevance to the interpretation of the behaviour of complex mixtures. In such systems there are strong interactions between the compounds in the gas phase, as is shown in the substance addition experiments. The individual data are therefore not useful tools for interpreting the results.

Another important factor in HSGC is the sample matrix, which can also influence the headspace composition [13]. To measure the degree of these interactions, a third series of experiments was undertaken, in which the sample matrix was varied. This was done by adding one of the test matrices (water, glycerol, paraffin or gelatine). After equilibration the sample was treated as described above.

The relative peak areas of two subseries of experiments (250- or 1000-ml Erlenmeyer flask) are summarized in Table IV. Here the peak areas in the matrix investigations are related to those of the original standard solution. To aid interpretation, the results of the 1000-ml experiments are additionally depicted as a bar graph in Fig. 5. As shown, all test matrices greatly influence the headspace composition. This effect cannot be explained by an incomplete equilibration due to the short equilibration time of 1 h, since longer times (2 h) did not affect the results.

The results of the matrix variation experiments can be summarized as follows. Adding a model matrix to the test solution generally causes a

TABLE IV

RELATIVE PEAK AREAS OF THE TEST COMPOUNDS IN DIFFERENT MATRIX ADDITION EXPERIMENTS

Std = Test solution; Gly = glycerine; Par = paraffin; Wat = water; Ge = gelatine + 10 ml of water (1 = 1.4 g; 2 = 1.6 g).

Test compound	250-ml flask					1000-ml flask				
	Std	Par	Gly	Wat	Ge1	Std	Par	Gly	Wat	Ge2
2-Methylpropanal	100	56	66	60	35	100	80	71	81	43
3-Methylbutanal	100	29	69	69	28	100	46	84	77	73
2-Methylbutanal	100	33	76	74	44	100	50	87	80	78
Butanol	100	31	13	8	2	100	55	18	10	5
2,5-Dimethylfuran	100	28	83	86	80	100	42	89	86	94
1-Methylpyrrole	100	20	71	74	30	100	31	81	77	70
Dimethyldisulphide	100	12	76	87	72	100	19	87	91	94
3-Methylthiophene	100	14	86	96	89	100	22	89	88	97
Butylbutyrate	100	10	99	97	63	100	13	90	80	85
3-Methylpyridine	100	4	1	2	–	100	2	1	3	–
2,4-Dimethylthiazole	100	5	9	14	4	100	3	9	14	7
2,6-Dimethylpyrazine	100	5	3	2	–	100	2	2	1	–
2-Acetylpyrazine	100	–	–	3	–	100	–	–	2	–
Phenylacetaldehyde	100	–	–	–	–	100	–	–	6	–
Limonene	100	–	289	318	317	100	1	89	86	95
β-Caryophyllene	100	–	157	326	592	100	1	91	75	56

decrease in the peak areas of the reference compounds. Exceptions are limonene and β-caryophyllene, which were enriched after addition of water, glycerol or a gelatine solution in the 250-ml configuration. One reason for this behaviour could be that highly volatile substances, which would cause a displacement of these terpenes if no matrix were added, strongly interact with these test matrices. Therefore these substances no longer have any displacement power. This effect is additionally increased by only weak interactions of the non-polar terpenes with the more polar model matrices.

Looking at Table IV it is obvious that it can be divided into three parts. The substances at the top were only a little influenced by matrix addition, whereas the compounds in the middle show extreme interactions. These components were not detected or were detectable only in small concentrations in the test solution headspace. In contrast, the substances listed in the lower part of the table were only slightly influenced (or even enriched in the 250-ml configuration) in most matrix addition experiments.

A comparison between the tested matrices shows that the effect of displacement is stronger in the case of paraffin addition than in other cases. This effect is probably based on a better solubility of the test compounds in this matrix than in the others, as is shown by the extreme behaviour of the non-polar terpenes. These substances were hardly detectable in the paraffin addition experiments. On the contrary, butanol, a polar molecule, is less influenced by paraffin.

For the same reasons as in the substance addition experiments, no investigation was carried out in which the compounds were tested individually with one of the test matrices because these results cannot be extrapolated to the analysis of complex mixtures.

As in the case of substance addition, the matrix experiments show that it is not possible to predict the interactions between components in headspace analysis of a complex system.

CONCLUSIONS

The results of the experiments carried out show that there are strong interactions between substances in the headspace and between the

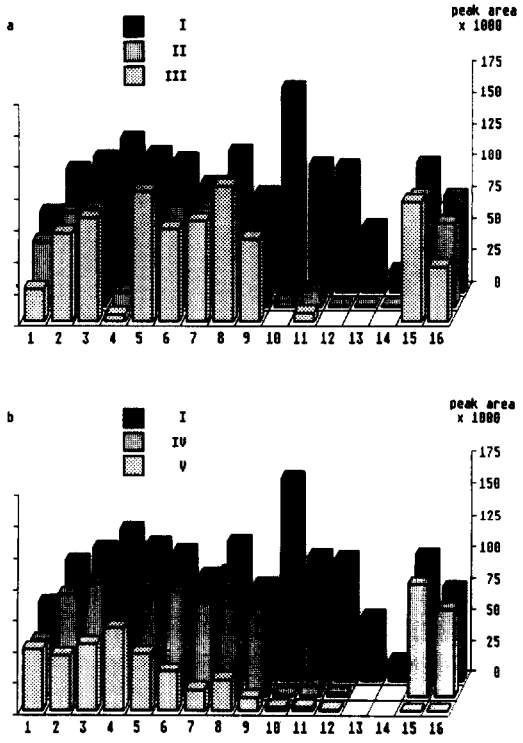

Fig. 5. Peak areas of test compounds before (I) and after addition of model matrices (sampling device: 1000-ml Erlenmeyer flask): (a) addition of water (II) or gelatine solution (III); (b) addition of glycerine (IV) or paraffin (V). (For compound numbers, see Table I.)

volatiles and the sample matrix. These factors and the sampling device volume influence the final headspace composition. Other influencing factors, such as the volatility and polarity of the analytes, their solubility in the sample matrix, etc., are also difficult to estimate, especially in HSGC with large sample volumes of complex samples. Nevertheless, HSGC is a suitable and easy method of investigating food flavours.

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