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Flavor Profiling of Beverages by Stir Bar Sorptive Extraction (SBSE) and Thermal Desorption GC/MS/PFPD

Andreas Hoffmann, Arnd Heiden
*Gerstel GmbH & Co. KG, Eberhard-Gerstel-Platz 1,
D-45473 Mülheim an der Ruhr, Germany*

Edward Pfannkoch
*Gerstel, Inc., 701 Digital Drive, Suite J,
Linthicum, MD 21090, USA*

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ABSTRACT

The analysis of flavor compounds in beverages such as coffee, tea, soft drinks and alcoholic beverages usually requires cumbersome sample preparation steps such as liquid/liquid extraction, solid phase extraction or distillation techniques, often with the drawback of organic solvent use. Headspace and purge & trap methods do not use organic solvents, but their analyte range is restricted to volatile compounds and therefore characterize compounds that contribute to the aroma/smell of a sample, not flavor/taste. The sensitivity of solid phase microextraction (SPME) is limited by the small amount of sorptive material that can be coated on the fibers.

A new extraction technique, Stir Bar Sorptive Extraction (SBSE), recently described by Pat Sandra et.al., that overcomes the major problems with classical extraction techniques is applied in this paper. With this technique, a small stir bar (10-20mm length, 1.3mm OD) is coated with polydimethylsiloxane (1mm d.f.), placed directly in the sample, and stirred for about 1 hour. During this time, analytes are extracted into the PDMS phase, which acts as an immobilized liquid phase. The stir bar is removed, rinsed with distilled water, and placed into a thermal desorption unit. Due to the hydrophobic character of PDMS, a drying step is not necessary. Heating the stir bar releases the extracted compounds into a GC-MS system for subsequent analysis with very low detection limits (parts per trillion).

INTRODUCTION

Beverages contain a complex mix of compounds that contribute to the aroma and flavor profile characteristic for each beverage. Dozens, or even hundreds of compounds contribute to the aromas and flavors perceived by the consumer.

Aroma is perceived when volatile compounds interact with receptors in the nasal passages. This mechanism generally limits the compounds contributing to aroma to volatile molecules with detectable levels in the headspace above the liquid. In addition, the structures of the compounds play a major role in receptor binding and the intensity of the perceived odor. Odor thresholds can differ by 6 orders of magnitude or more, therefore it is possible for trace components to contribute significantly to the aroma profile.

Flavor, on the other hand, is perceived as a combination of aroma and taste. The four basic taste receptors (sweet, sour, bitter, salty) are found on the tongue, which requires the liquid beverage to be sampled in the mouth. Many of the compounds that stimulate these receptors are either non-volatile or semivolatile, therefore they may not be represented in the headspace of the beverage. Furthermore, when the beverage enters the mouth it is warmed to body temperature, which can volatilize additional compounds and contribute to the aroma component of flavor.

In addition to the compounds comprising the desirable aroma and flavor profiles in a beverage, trace components can contribute off-flavors and odors. These compounds can be generated a variety of ways. They can enter as contaminants in raw materials used in

the beverage, for example, in the water or sugar. They can migrate into the beverage from process equipment or packaging materials. Finally, they can be generated by degradation of naturally occurring flavor compounds due to oxidation, or exposure to light or heat. Even changes in the relative concentrations of flavor components may result in an undesirable change in the flavor of the beverage.

It is therefore desirable to be able to accurately profile the compounds contributing to flavor and aroma, which can span a wide range of volatility. Most beverages consist of a water matrix, which can have additional compounds present at relatively high levels (e.g. alcohol, sugar, and plant pulp) in addition to the trace flavor and aroma components. To facilitate analysis of the volatile fraction in these matrices, Static Headspace, SPME and Purge & Trap GC are often used. These techniques rely on the volatiles partitioning into the gas phase to eliminate matrix interference, and therefore are biased toward profiling the more highly volatile compounds. To try to profile a broader range of flavor compounds, sometimes liquid/liquid extraction with water immiscible liquids like ethyl acetate or pentane can be used. Beverage components like alcohol and plant pulp can significantly interfere with this approach, however.

In this paper, we describe the use of a new extraction technique, Stir Bar Sorptive Extraction (SBSE) to extract the flavor and aroma components from a variety of beverages. Compounds are recovered by thermal desorption and are analyzed by GC/MS. This technique is highly reproducible and sensitive, and requires no solvents.



Figure 1. Gerstel Twister.

EXPERIMENTAL

Instrumentation. All analyses were performed on a GC (6890, Agilent Technologies) with mass selective detection (5973, Agilent Technologies). The GC was equipped with a Thermal Desorption unit with autosampling capacity (TDS 2 & TDS A, Gerstel), a PTV (CIS 4, Gerstel) and a PFPD (O.I. Analytical).

Operation. Samples were transferred to 10 ml-headspace vials leaving minimal headspace. One Gerstel Twister stir bar was added to the vial before capping with PTFE faced silicone crimp caps. Samples were stirred for either 0.75 to 2 hrs or overnight (16 hrs).

Stir bars were removed with forceps, rinsed briefly in distilled water, blotted dry and placed into clean glass thermal desorption tubes. Analytes were desorbed at 200°C for 5 minutes with a 50 ml/min gas flow and cold trapped in the CIS 4 inlet packed with a glass wool liner at -150°C.

Samples were transferred to the column splitless or in the split mode (see chromatogram) and analyzed by GC-MSD on a 30m x 0.25mm x 0.25um HP-5 column (Agilent) except where noted in the figures.

RESULTS AND DISCUSSION

Effect of extraction time on flavor component profiles. One tea bag was added to 250 ml boiling water and covered with a watch glass to brew for 6 minutes. The tea bag was removed, and the tea was allowed to cool, covered, for 30 minutes. Ten aliquots were transferred to vials and one stir bar added to each of them.

Volatile flavor component profiles were found to be remarkably similar when 1.5 hr and 16 hr extractions were compared (Figure 2). The largest differences were seen in the peak areas for late eluting components, which increased in the longer extraction. It is not yet known whether this represents a slower partitioning into the PDMS phase, or an actual increase in these components over time, perhaps due to oxidation.

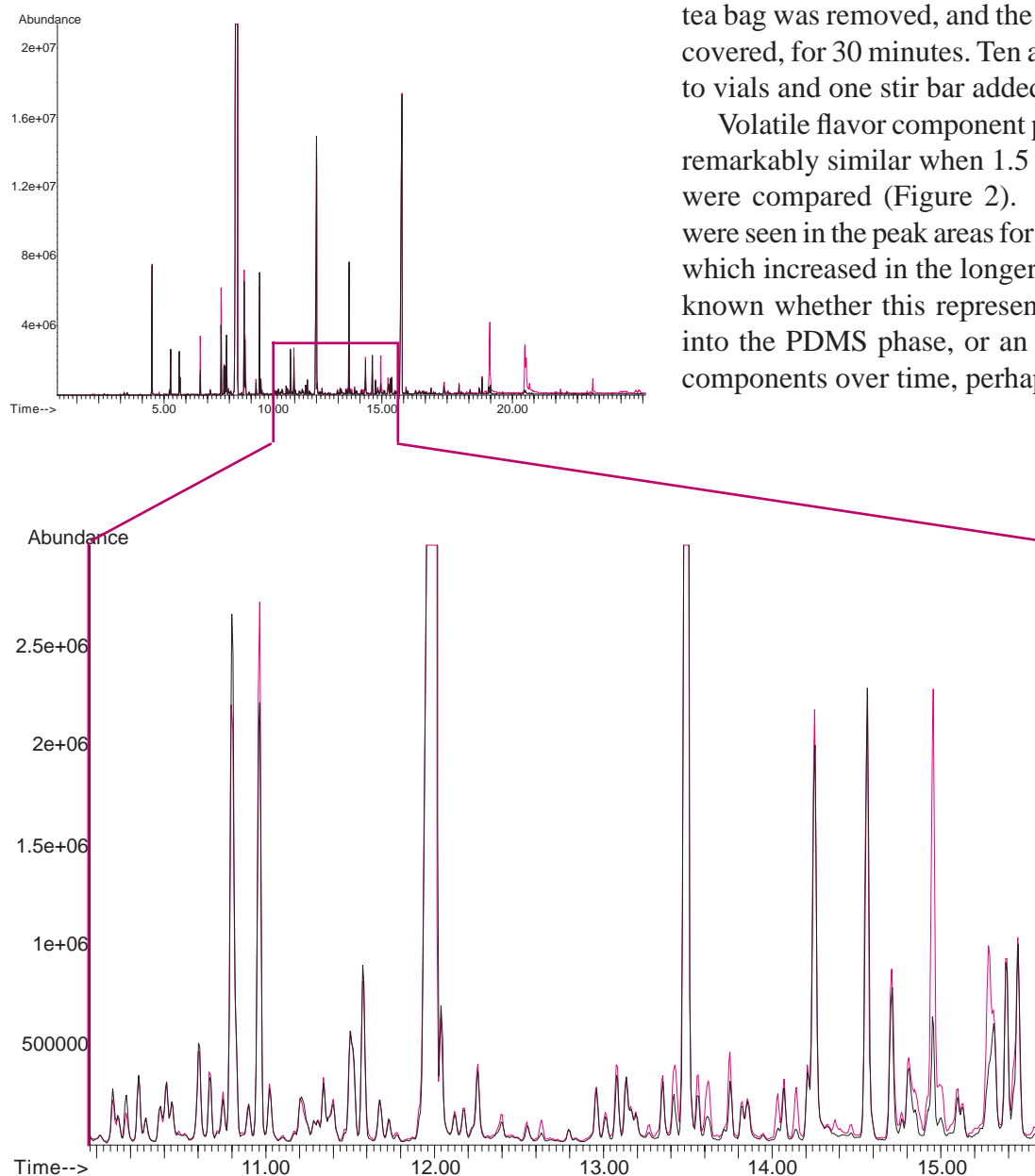


Figure 2. Flavor components in brewed herbal tea, split 1:20, effect of different extraction times.

Comparison of peak area reproducibility for herbal tea extracts (1.5hr extraction). Figure 3 shows 5 representative chromatograms overlaid to illustrate the reproducibility of the flavor profile obtained using the Gerstel Twister technique.

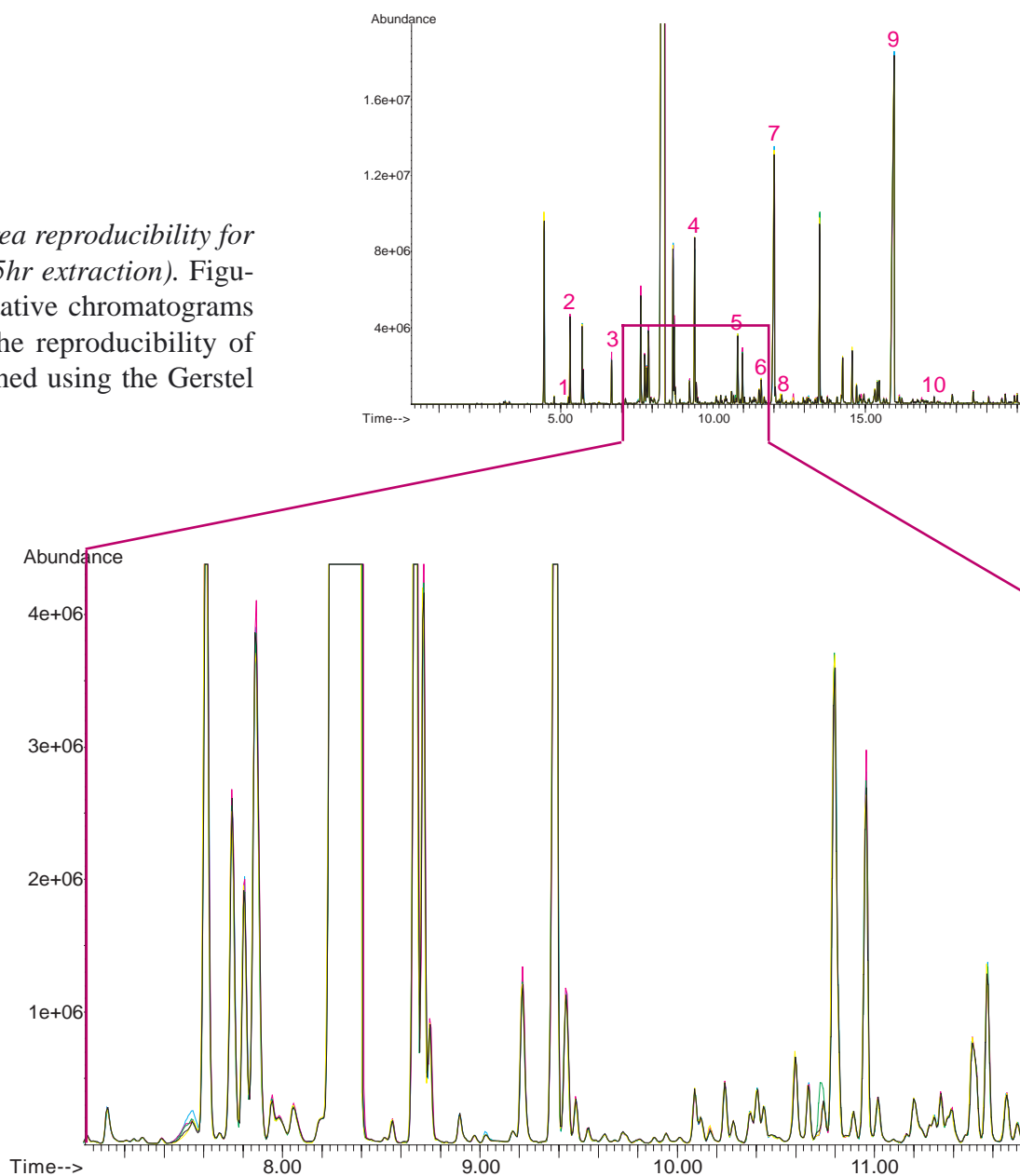


Figure 3. Flavor components in brewed herbal tea, split 1:20, reproducibility test.

Table I. Peak area precision of representative flavor components in herbal tea (1.5 h extraction).

No.	Compound	% RSD
1	Ethyl 2-Methylbutanoate	2.55
2	Ethyl 3-Methylbutanoate	2.08
3	α -Pinene	6.92
4	Pentyl 3-Methylbutanoate	3.57
5	α -Terpineole	2.23
6	Geraniol	2.05
7	Cinnamyl Aldehyde	3.93
8	α -Fenchyl Acetate	3.60
9	5-Heptyldihydro2(3H)-Furanone	1.50
10	β -Sinensal	3.16
	Average	3.16

Orange Juice. Terpenes like α -pinene and myrcene are of great importance in citrus fruits with limonene as major component of citrus oils. Other odorants contributing to the flavor are several aldehydes and esters, whereas furaneol and α -terpineol (from limonene) are more regarded as aroma defects.

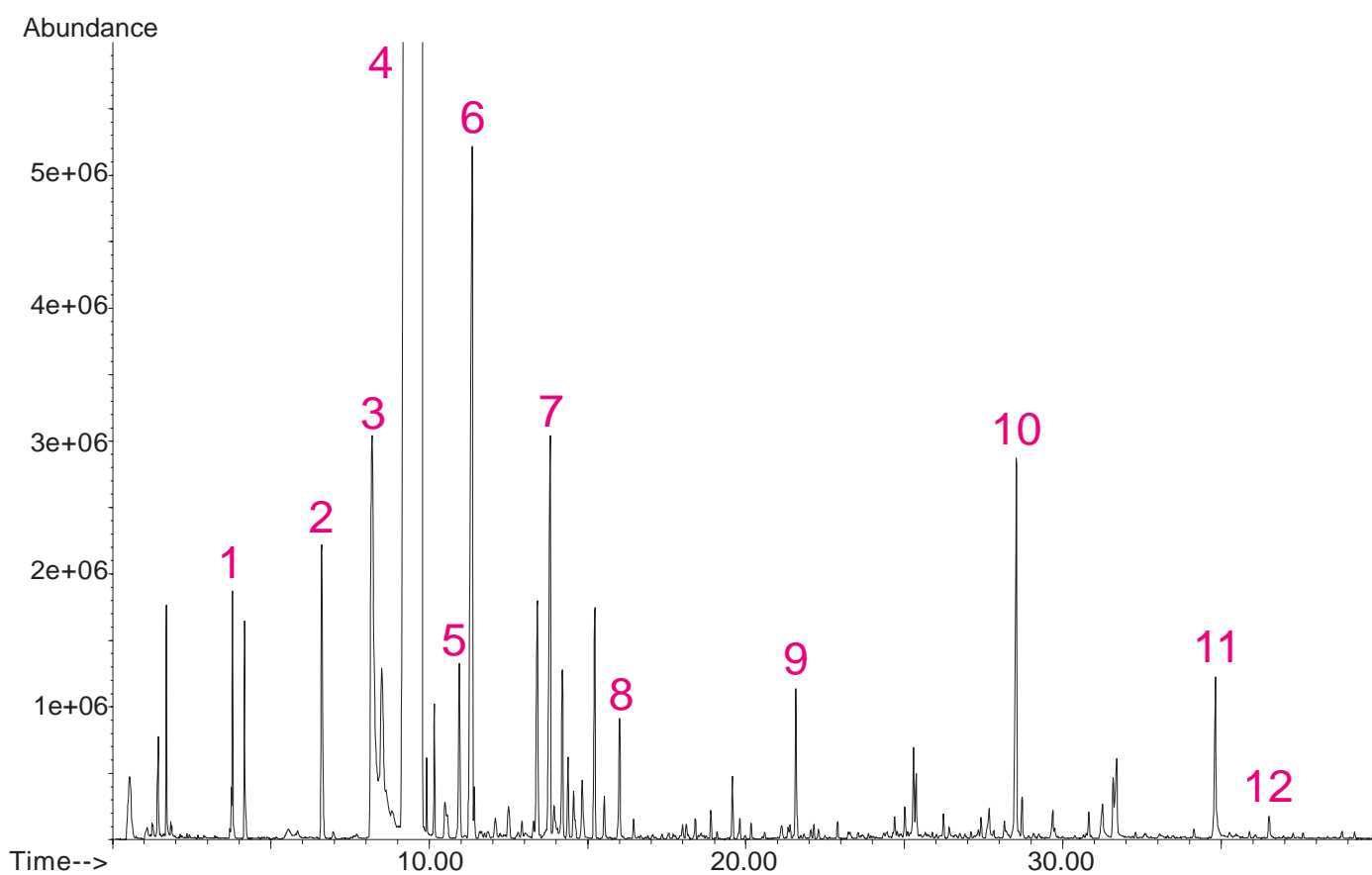


Figure 4. Orange juice, split 1:20.

Table II. Orange juice, list of compounds.

No.	Compound	No.	Compound
1	Ethyl Butanoate	7	α -Terpineol
2	α -Pinene	8	Perilla Aldehyde
3	Myrcene	9	Valencene
4	Limonene	10	Nootkatone
5	Terpinolene	11	7-Methoxy-8-isopentyl Coumarin
6	Linalool	12	7-Methoxy-8-(2-oxo-3-methylbutyl) Coumarin

Apple Juice. The aroma of apples is determined by esters, aldehydes and alcohols, not so much by terpenes as in citrus fruits.

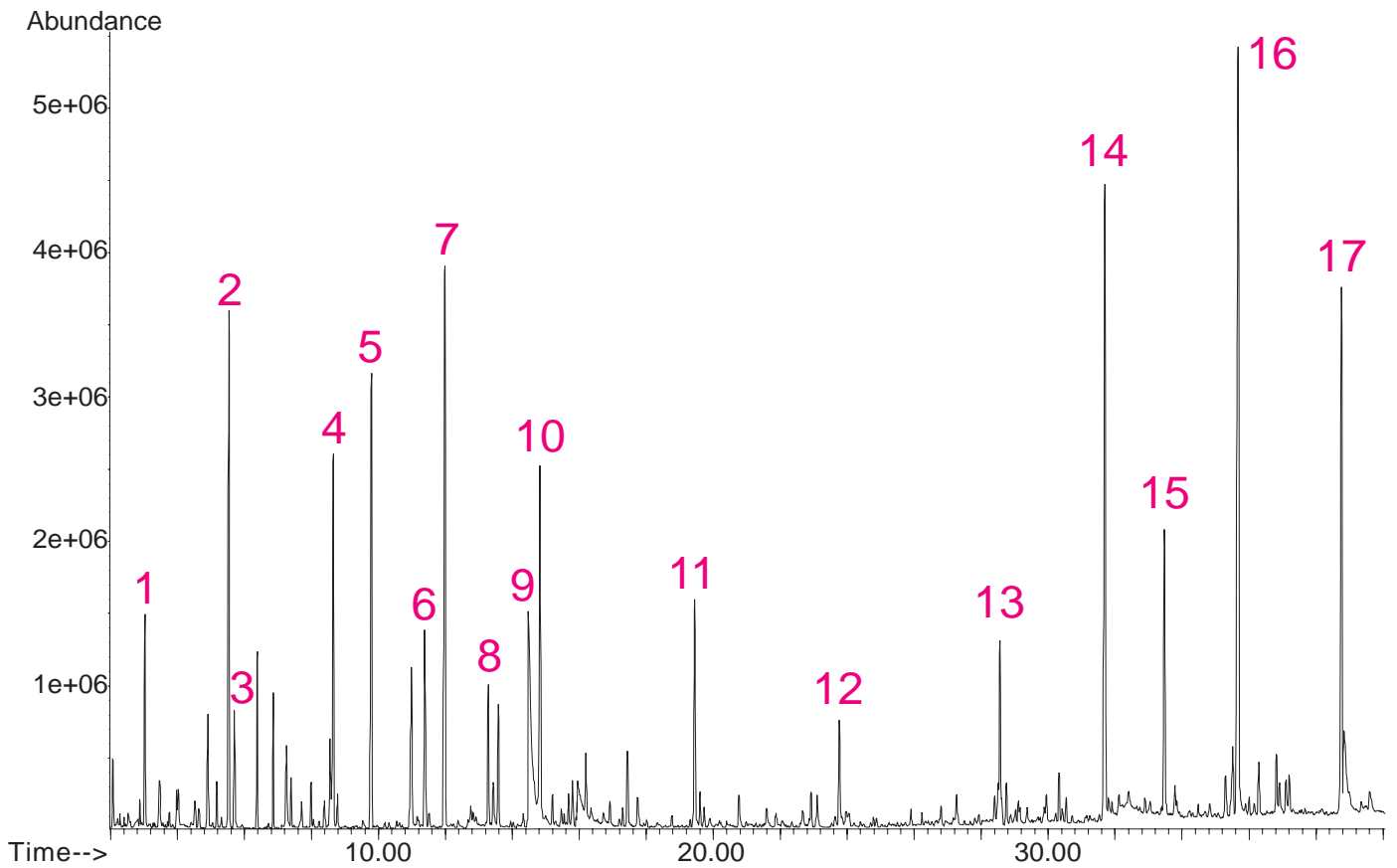


Figure 5. Apple juice, split 1:20.

Table III. Apple juice, list of compounds.

No.	Compound	No.	Compound
1	Ethyl Acetate	10	Furfural
2	Butyl Acetate	11	Furfuryl Alcohol
3	Hexanal	12	Furaneol
4	Trans-2-Hexenal	13	2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one
5	Hexyl Acetate	14	2,3-Dihydro-3,5-Dihydroxy-6-methyl-4H-pyran-4-one
6	2-Hexenyl Acetate	15	Diethylphthalate
7	Hexanol	16	5-Hydroxymethyl Furfural
8	2-Hexene-1-ol	17	Dibutylphthalate
9	Acetic Acid		

Cola. Cola drinks contain extracts from the cola-nut or aromatic extracts from ginger, orange blossoms, carob and tonka-beans or lime-peels. The sugar content averages 10-11%.

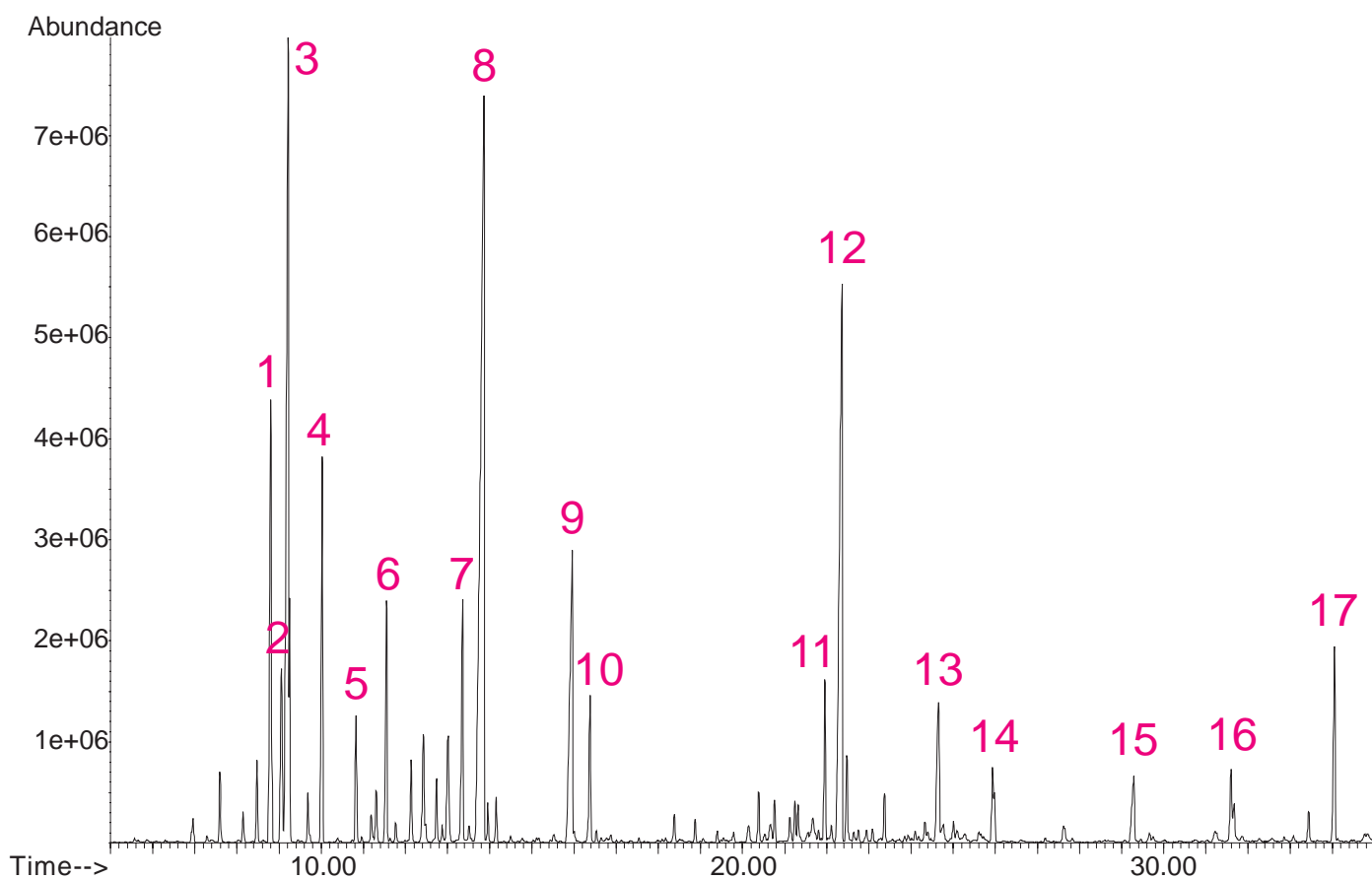


Figure 6. Cola, split 1:20.

Table IV. Cola, list of compounds.

No.	Compound	No.	Compound
1	Isocineole	10	Safrole
2	p-Cymene	11	β -Bisabolene
3	Limonene	12	Myristicin
4	γ -Terpinene	13	γ -Gurjunene (?)
5	Terpinolene	14	α -Bisabolol
6	Fenchol	15	Caffeine
7	Terpinen-4-ol	16	Dibutylphthalate
8	α -Terpineole	17	Terpene mw 268
9	Cinnamic Aldehyde		

Multi-fruit beverage. These drinks are prepared from fruit juices or their mixtures, from fruit juice concentrates, natural and artificial fruit essences, and are diluted with water or soda or mineral water.

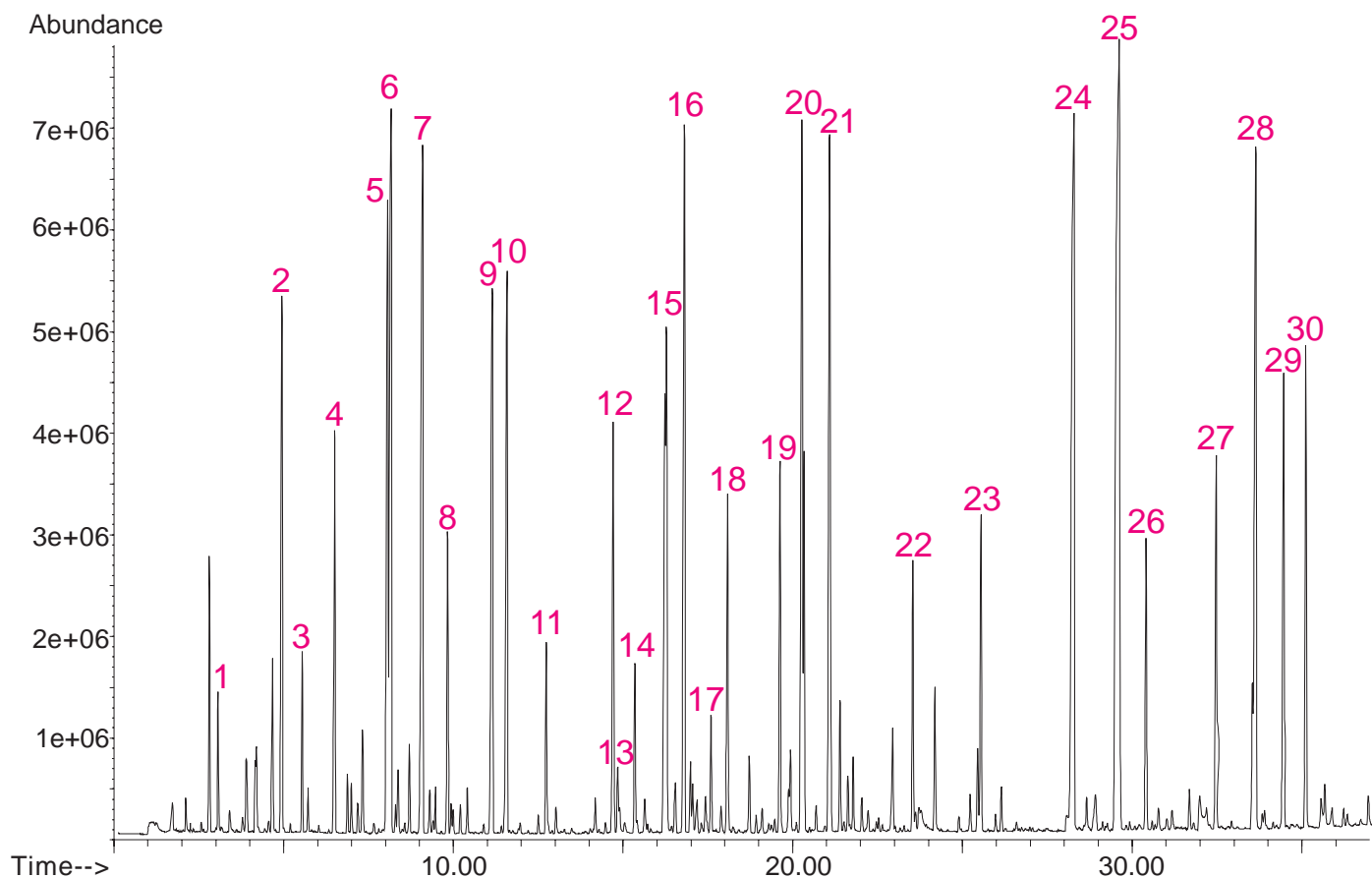


Figure 7. Multi-fruit beverage, split 1:20.

Table V. Multi-fruit beverage, list of compounds.

No.	Compound	No.	Compound
1	Ethyl Acetate	16	Linalool
2	Ethyl Butyrate	17	Diethyl Malonate
3	Butyl Acetate	18	Terpineole-4
4	Isoamyl Acetate	19	Ethyl Benzoate
5	Isobutyl Isovalerate	20	α -Terpineole
6	Limonene	21	Benzyl Acetate
7	Ethyl Caproate	22	Geraniol
8	Amyl Butyrate	23	cis-Jasmone
9	cis-3-Hexenyl Acetate & Isoamyl Butyrate	24	Triacetin
10	trans-3-Hexenyl Acetate	25	γ -Decalactone
11	cis-3-Hexenol	26	δ -Decalactone
12	cis-3-Hexenyl Isobutyrate	27	δ -Undecalactone
13	Furfural	28	γ -Dodecalactone
14	2-Ethyl Hexanol	29	δ -Dodecalactone
15	Benzaldehyde & Unknown	30	Triethyl Citrate

Coffee. The volatile fraction of roasted coffee has a very complex composition. More than 650 different compounds have been identified in coffee so far. In order to obtain an aromatic brewed coffee with a high content of flavoring and stimulant constituents the quality of the ground coffee and the way of brewing are of importance. Since the aroma of coffee is not stable analysis of a fresh brew is very difficult. SBSE offers here a possibility to extract aroma compounds directly from the hot brew, without the necessity of time consuming extraction steps.

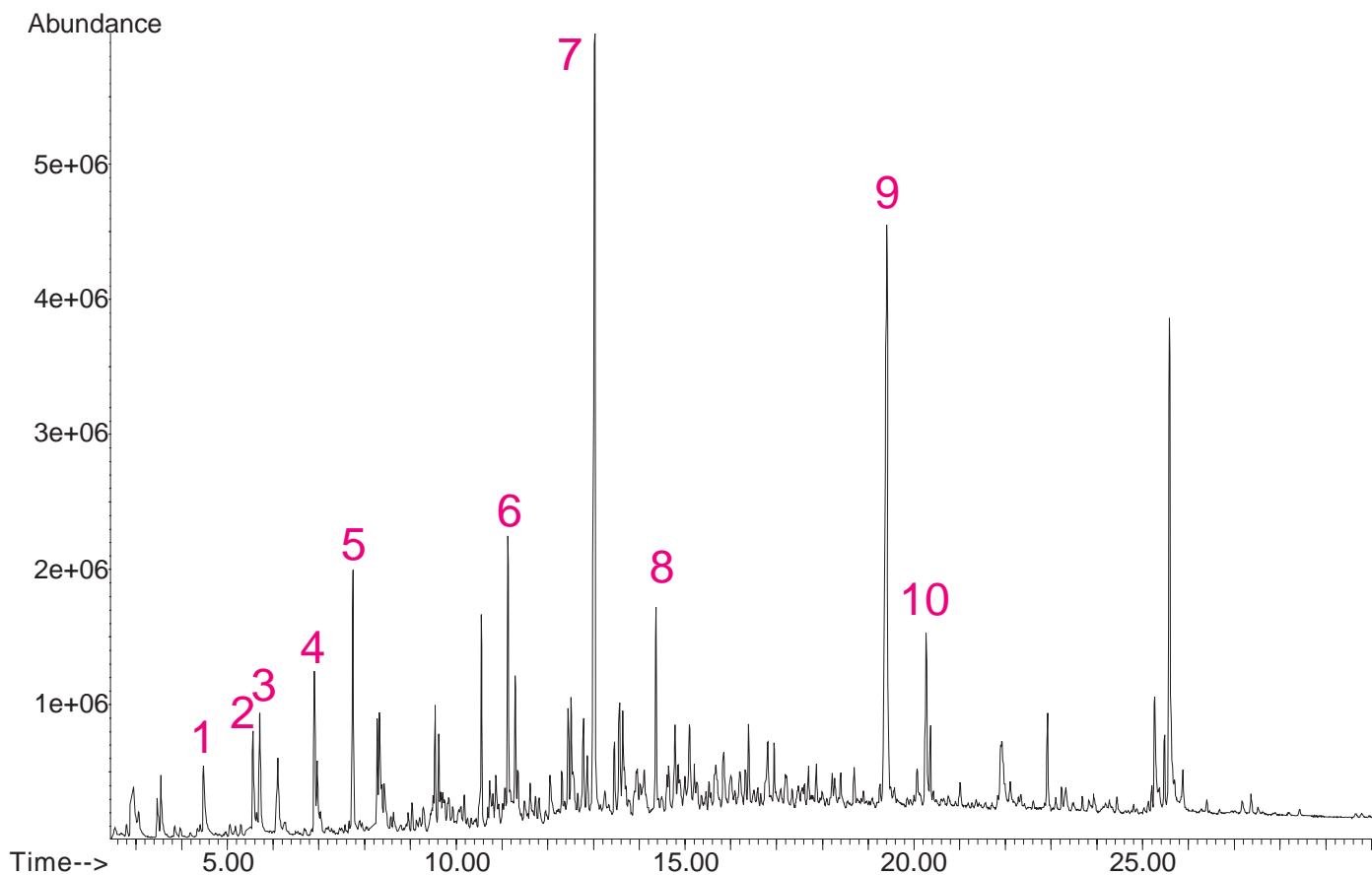


Figure 8. Brewed coffee, split 1:20.

Table VI. Brewed coffee, list of compounds.

No.	Compound	No.	Compound
1	Pyridine	6	N-Furfuryl Pyrrole
2	2-Methyl Pyrazine	7	4-Vinyl Guaiacol
3	Furfural	8	1-Furfuryl-2-formyl Pyrrole
4	2,6-Dimethyl Pyrazine	9	Caffeine
5	5-Methyl Furfural	10	Palmitic Acid

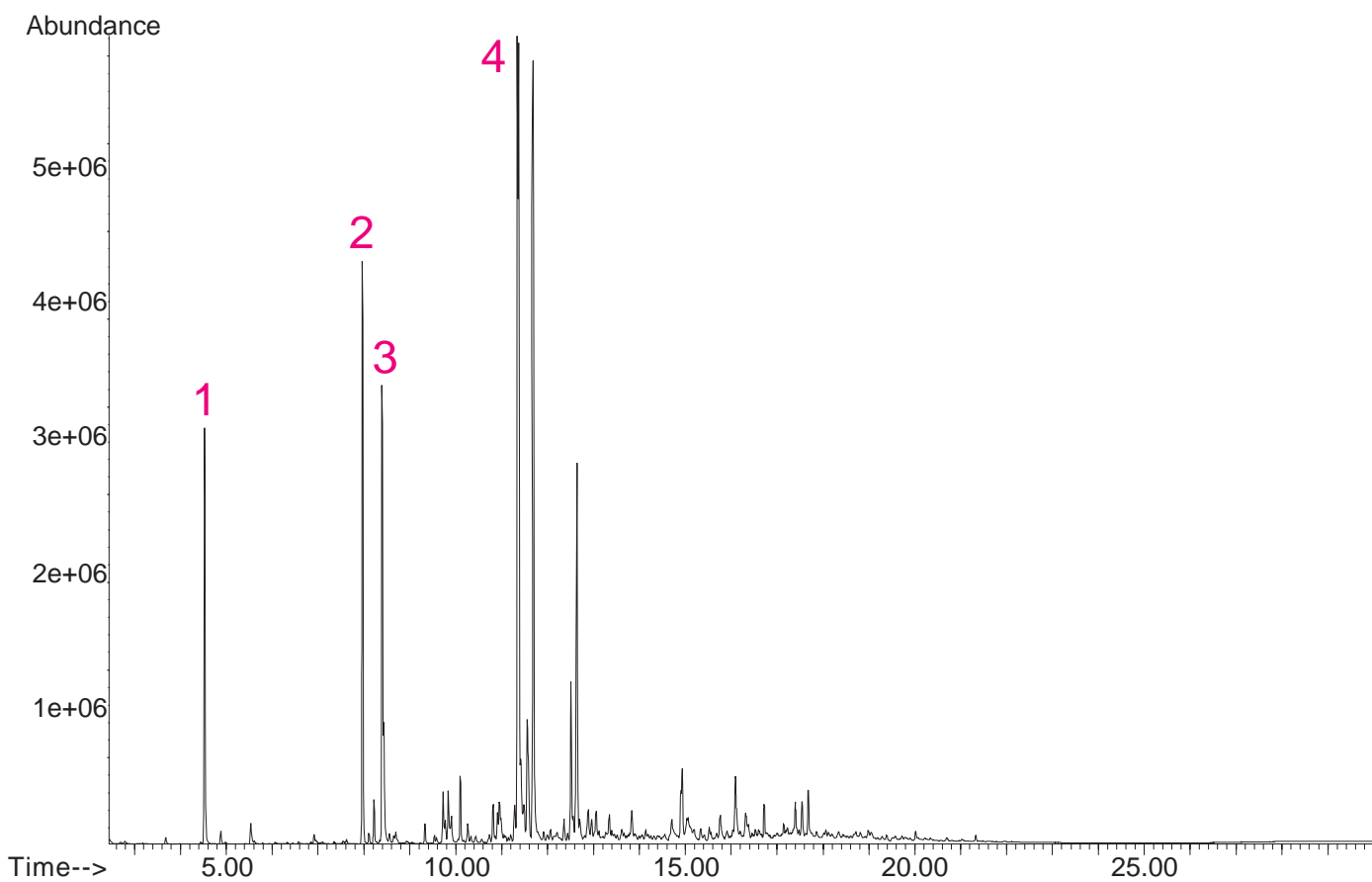


Figure 9. Brewed coffee, PFPD, sulfur-trace, split 1:20.

Table VII. Brewed coffee, list of sulfur compounds.

No.	Compound	No.	Compound
1	Dimethyl Disulfide	3	2-[(Methylthio)methyl]-Furan
2	Dimethyl Trisulfide	4	Kahweofuran

Beer: Beer brewing involves the use of germinated barley (malt), hops, yeast and water. Beer owes its aroma, flavor and bitter taste to hops (primarily due to compounds of the humulon fraction), kiln-dried products and numerous aroma constituents formed during fermentation.

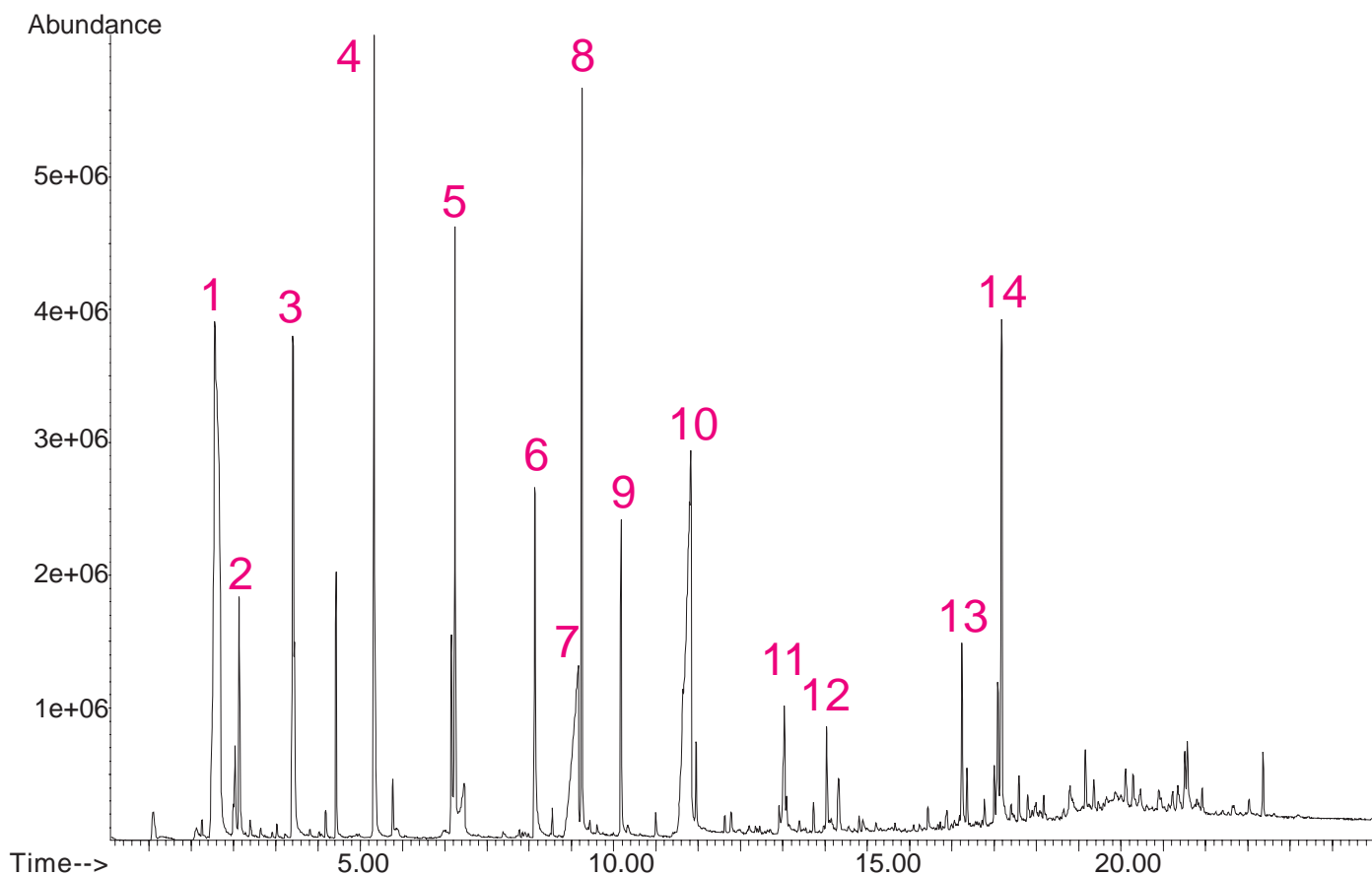


Figure 10. Pilsener beer, DB-Wax, split 1:20.

Table VIII. Pilsener beer, list of compounds.

No.	Compound	No.	Compound
1	Ethanol	8	Ethyl Caprylate
2	Ethyl Acetate	9	Phenylethyl Propionate
3	Isoamyl Alcohol	10	Capric Acid
4	Isoamyl Acetate	11	Lauric Acid
5	Ethyl Caproate	12	Phenylethyl Isovalerate
6	Phenylethyl Alcohol	13	Dehydro-Cohumulonic Acid
7	Caprylic Acid	14	Dehydro-Isohumulonic Acid

Beer is very sensitive to light and oxidation. The "light" taste is due to the formation of 3-methyl-2-buten-1-thiol from hop-constituents.

Figure 11 shows a sulfur-trace of a freshly bottled pilsener-type beer, figure 12 shows the trace of the same beer, but after several hours of exposure to UV-light.

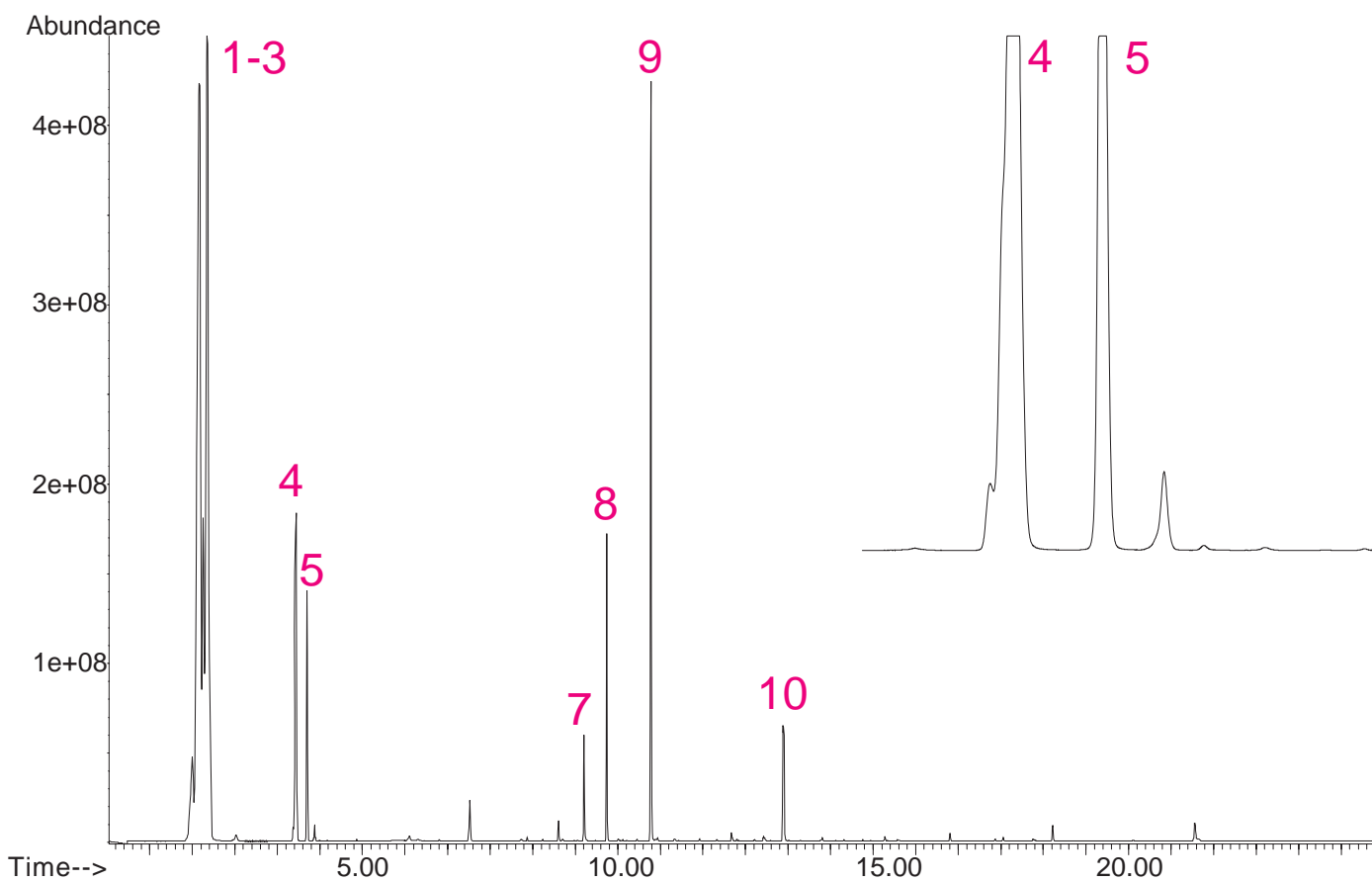


Figure 11. Fresh Pilsener beer, DB-Wax, splitless, PFPD, sulfur-trace.

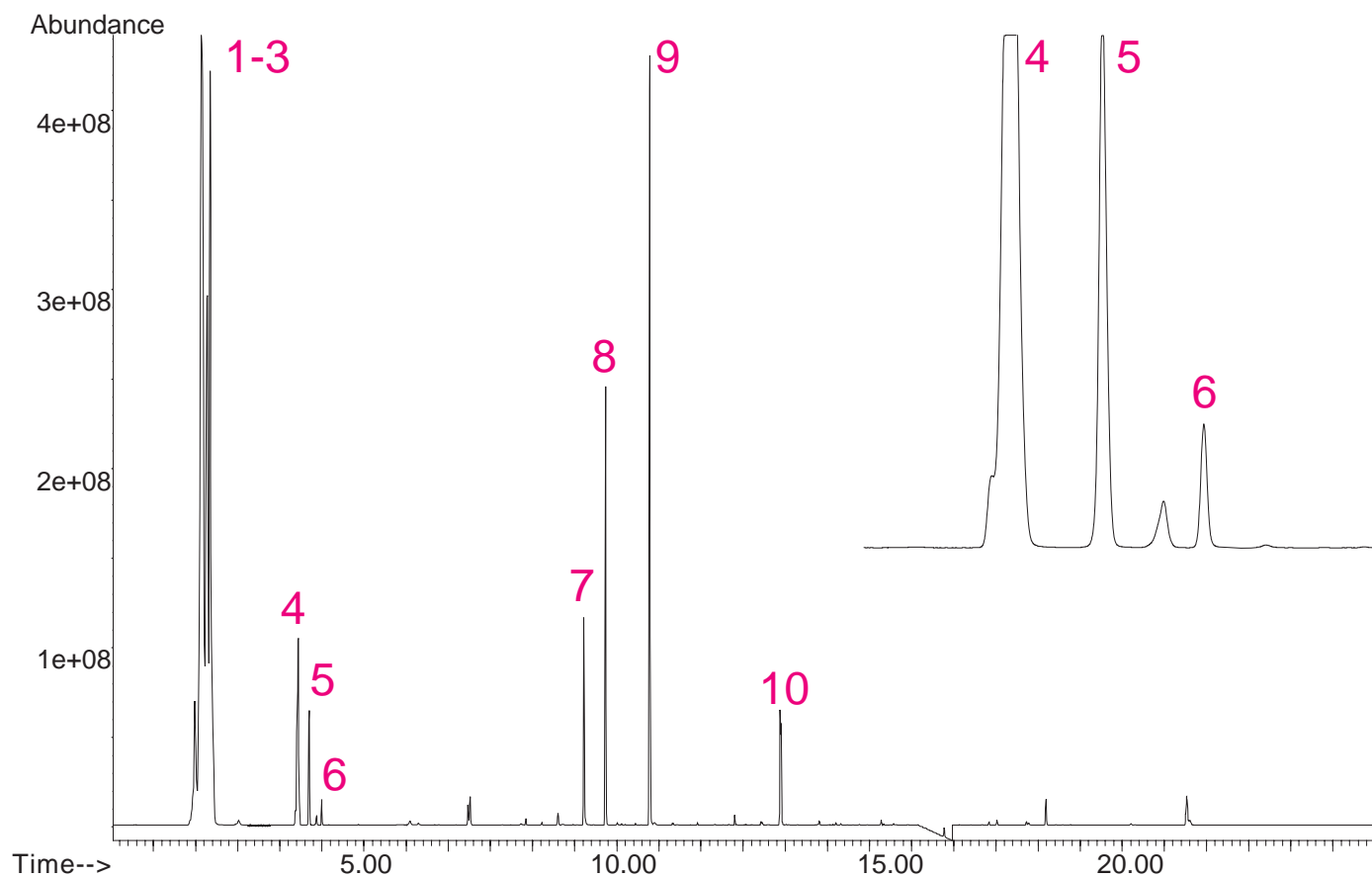


Figure 12. Fresh Pilsener beer exposed to UV-light, DB-Wax, splitless, PFPD, sulfur-trace.

Table IX. Pilsener Beer, list of sulfur compounds.

No.	Compound	No.	Compound
1	H ₂ S / SO ₂ / COS / Methyl Mercaptane	6	3-Methyl-2-butene-1-thiol
2	Ethyl Mercaptane	7	Dimethyl Sulfoxide
3	Dimethyl Sulfide	8	3-(Methylthio)-propyl Acetate
4	Methylthio Acetate	9	Methionol
5	Dimethyl Disulfide	10	2(2-Furanyl)thiazole

Spumante. Spumante is an italian sparkling wine, where young wines from suitable regions are used to provide the fresh and fruity bouquet desired for production. Blending of wines from different localities, often with older wines, is aimed at obtaining a uniform end-product to fulfill customers expectations of a specific brand. Controlling the uniformity of such a product therefore is mandatory for quality control.

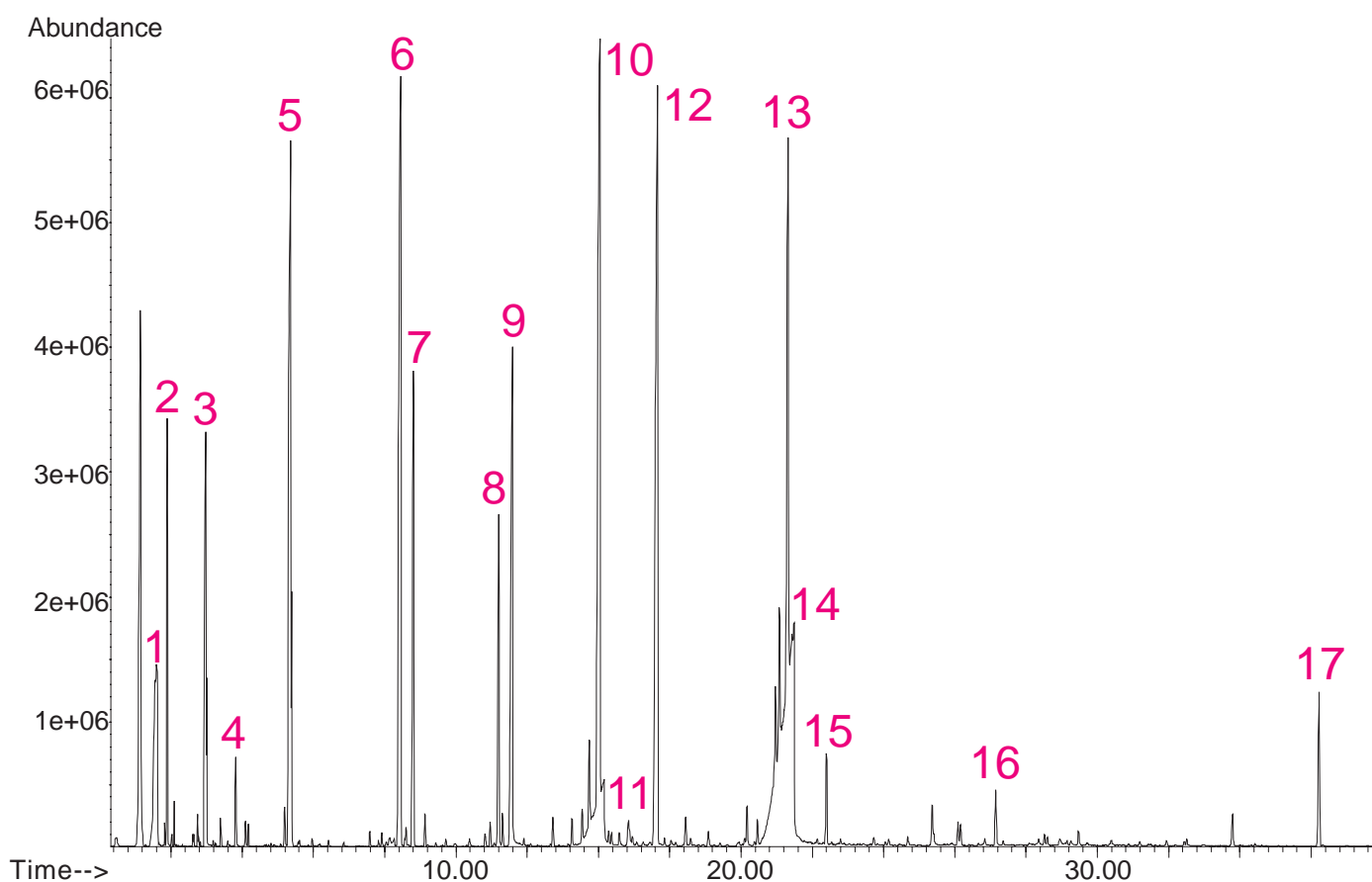


Figure 13. Spumante, split 1:20.

Table X. Spumante, list of compounds.

No.	Compound	No.	Compound
1	Ethanol	9	Phenylethyl Alcohol
2	Ethyl Acetate	10	Ethyl Caprylate
3	Isoamyl Alcohol	11	Citronellol
4	Ethyl Butyrate	12	Phenylethyl Acetate
5	Isoamyl Acetate	13	Ethyl Caprate
6	Ethyl Caproate	14	Capric Acid
7	Hexyl Acetate	15	Phenylethyl Butyrate
8	Linalool	16	Phenylethyl Isovalerate

Not only flavor compounds could be detected in this sample: Peak No. 17 could be identified as procymidone (figure 14), a fungicide commonly used in wineries to protect the grapes from botrytis cinerea. Repeating the analysis in splitless-mode, two additional fungicides could be detected (vinclozolin and iprodion).

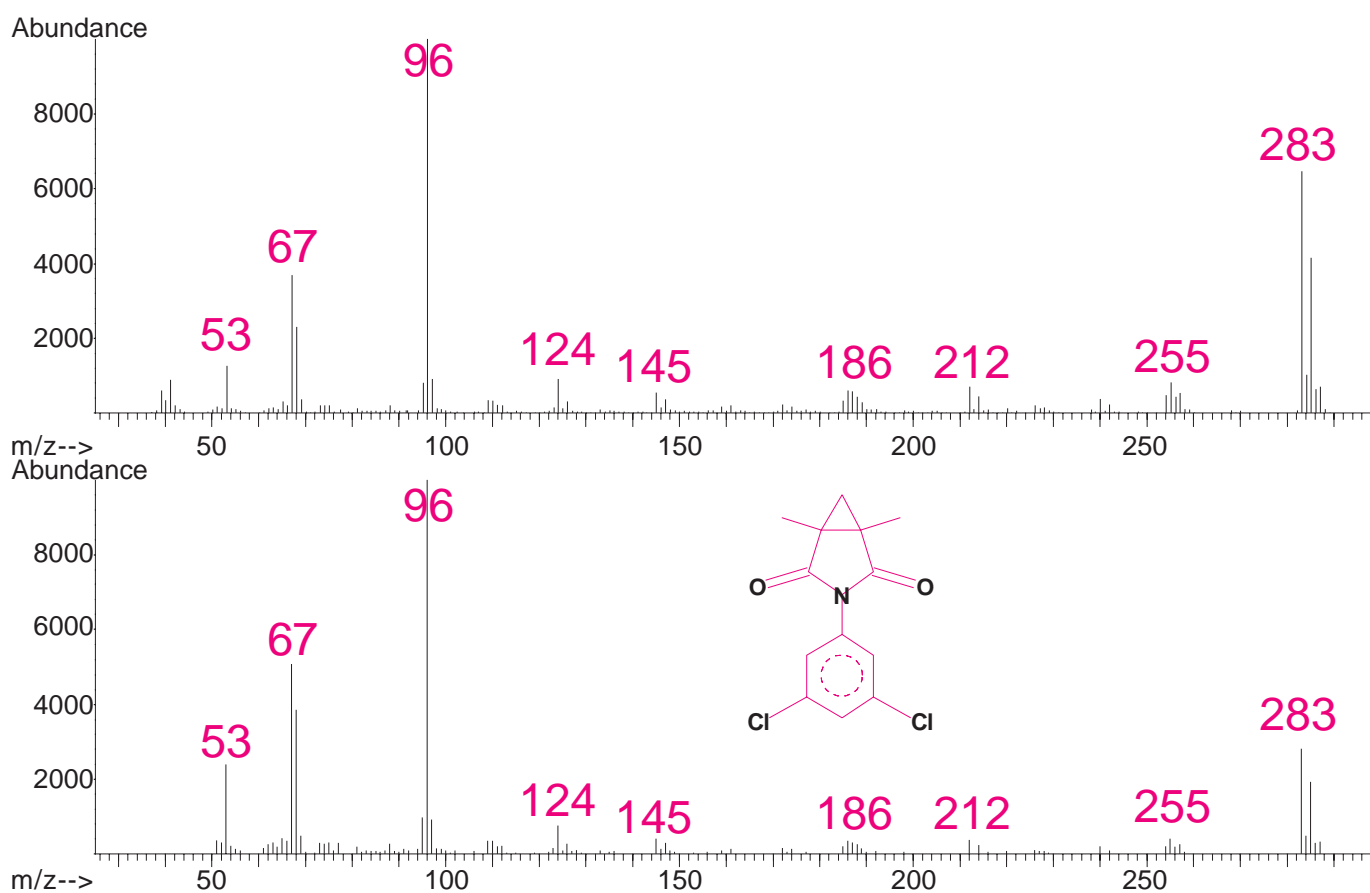


Figure 14. Spectrum of procymidone found in Spumante (top) compared to library spectrum.

Vermouth. For the production of vermouth, wormwood is extracted with the fermenting must or wine, or it is made from a concentrate of plant extracts added to wine. Other herbs or spices are additionally used, such as seeds, bark, leaves or roots like thyme or calamus.

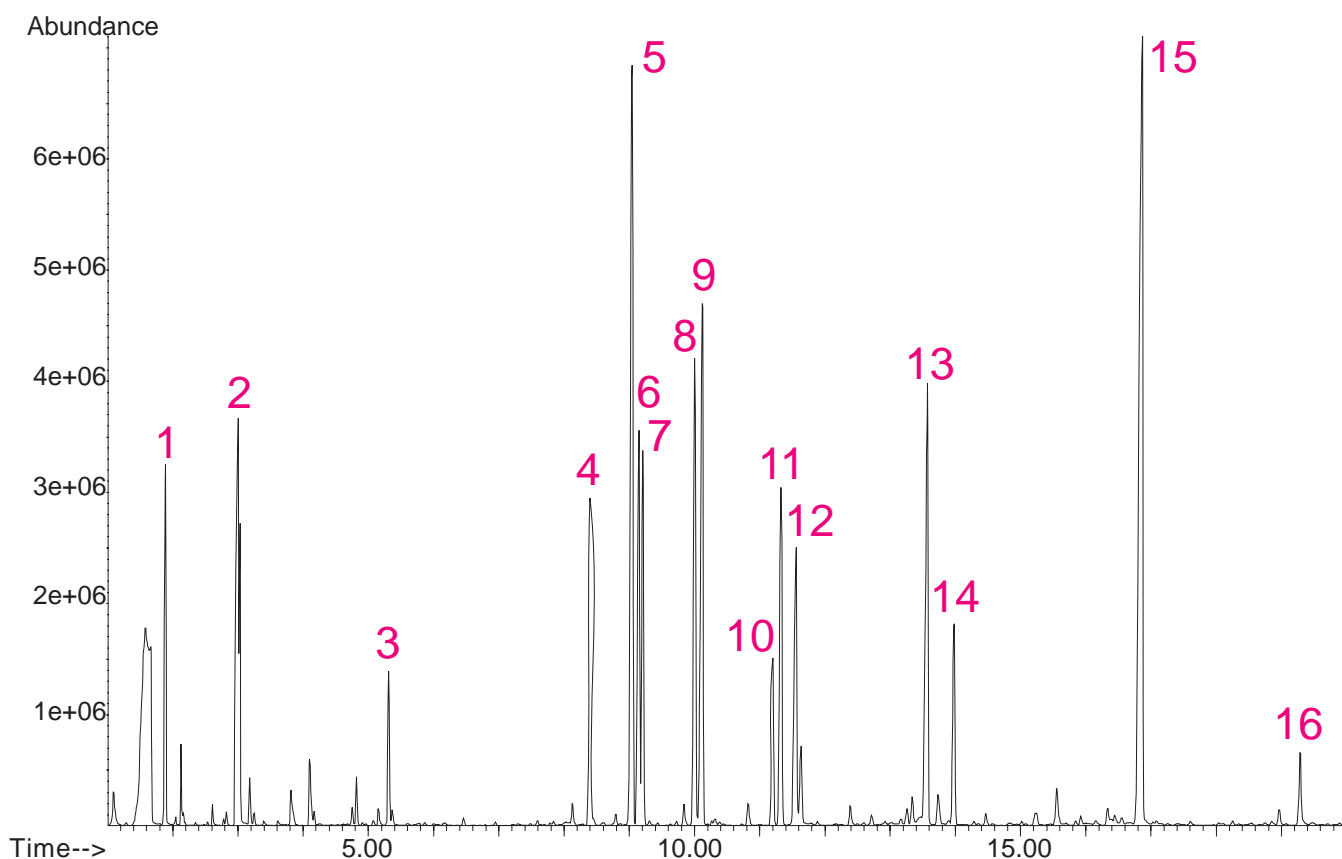


Figure 15. Vermouth, split 1:20.

Table XI. Vermouth, list of compounds.

No.	Compound	No.	Compound
1	Ethyl Acetate	9	Artemisia Ketone
2	Isoamyl Alcohol	10	Linalool
3	Isoamyl Acetate	11	Thujone
4	Ethyl Caproate	12	Phenylethyl Alcohol
5	p-Cymene	13	Diethyl Succinate
6	Limonene	14	Ethyl Caprylate
7	1,8-Cineole	15	Thymol
8	γ -Terpinene	16	Vanillin

CONCLUSIONS

Stir bar sorptive extraction (SBSE) is an extremely powerful technique for flavor profiling of different types of beverages since it combines ease of use, ruggedness, precision, speed and sensitivity.

In addition the absence of any organic solvents involved in sample preparation and analysis makes this methodology totally "environmentally friendly".

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LITERATURE

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GERSTEL GmbH & Co. KG

Eberhard-Gerstel-Platz 1
45473 Mülheim an der Ruhr
Germany

- +49 (0) 208 - 7 65 03-0
- +49 (0) 208 - 7 65 03 33
- gerstel@gerstel.com
- www.gerstel.com

GERSTEL Worldwide

GERSTEL, Inc.

701 Digital Drive, Suite J
Linthicum, MD 21090
USA

- +1 (410) 247 5885
- +1 (410) 247 5887
- sales@gerstelus.com
- www.gerstelus.com

GERSTEL AG

Wassergrabe 27
CH-6210 Sursee
Switzerland

- +41 (41) 9 21 97 23
- gerstelag@ch.gerstel.com
- www.gerstel.ch

GERSTEL K.K.

1-3-1 Nakane, Meguro-ku
Tokyo 152-0031
SMBC Toritsudai Ekimae Bldg 4F
Japan

- +81 3 5731 5321
- +81 3 5731 5322
- info@gerstel.co.jp
- www.gerstel.co.jp

GERSTEL LLP

Level 25, North Tower
One Raffles Quay
Singapore 048583

- +65 6622 5486
- +65 6622 5999
- SEA@gerstel.com
- www.gerstel.com

GERSTEL Brasil

Av. Pascoal da Rocha Falcão, 367
04785-000 São Paulo - SP Brasil

- +55 (11)5665-8931
- +55 (11)5666-9084
- gerstel-brasil@gerstel.com
- www.gerstel.com.br

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