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Determination of Flavor and Off Flavor Compounds in Dairy Products using Stir Bar Sorptive Extraction (SBSE) and Thermal Desorption GC/MSD/PFPD

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Abstract

The analysis of flavor compounds in dairy products such milk, cream, yoghurt and cheese as well as their blends with several ingredients 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. In addition heating of the sample should be avoided since this would lead to reaction products which dramatically modifiy the flavor and taste of any dairy product. 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. [1], 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

Milk and dairy products contain a complex mix of compounds that contribute to the aroma and flavor profile characteristic for each product. Some of them are blended with natural ingredients, like fruit pulps, juices and essences, chocolate or herbs. Hundreds of compounds therefore 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 dairy product 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 product. Furthermore, when the product 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 dairy product, 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 product or can migrate into the product 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 dairy product itself.

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 dairy products consist of a water matrix with an emulsified or non-emulsified fat content in the percent range and can have additional compounds present at relatively high levels 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 not sensitive enough. 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, steam-distillation or sometimes liquid/liquid extraction with water immiscible liquids like pentane/ether or pentane/dichloromethane can be used. The fat content 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 dairy products. Compounds are recovered

Figure 1. Gerstel Twister.

by thermal desorption and are analyzed by GC/MS. This technique is highly reproducible and sensitive, and requires no solvents.

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. Cheese and yoghurt samples were diluted 1:1 with LC-grade water. One Gerstel Twister stir bar was added to the vial before capping with PTFE faced silicone crimp caps. Samples were stirred for 1 hr.
Stir bars were removed with forceps, rinsed briefly in distilled water, blotted dry and placed into clean glass thermal desorption tubes.

Analysis Conditions. Analytes were desorbed at 200°C for 5 minutes with a 50 mL/min gas flow and cold trapped in the CIS4 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 0.25µm DB-Wax column (J&W) except where noted in the figures.

Results and Discussion

Milk. Raw or gently pasteurized milk has a mild, but characteristic taste. More than 400 volatile compounds have been identified so far. Typical aroma compounds include dimethylsulfide, 2-methylbutanol, 4-cis-heptenal and 2-trans-nonenal.

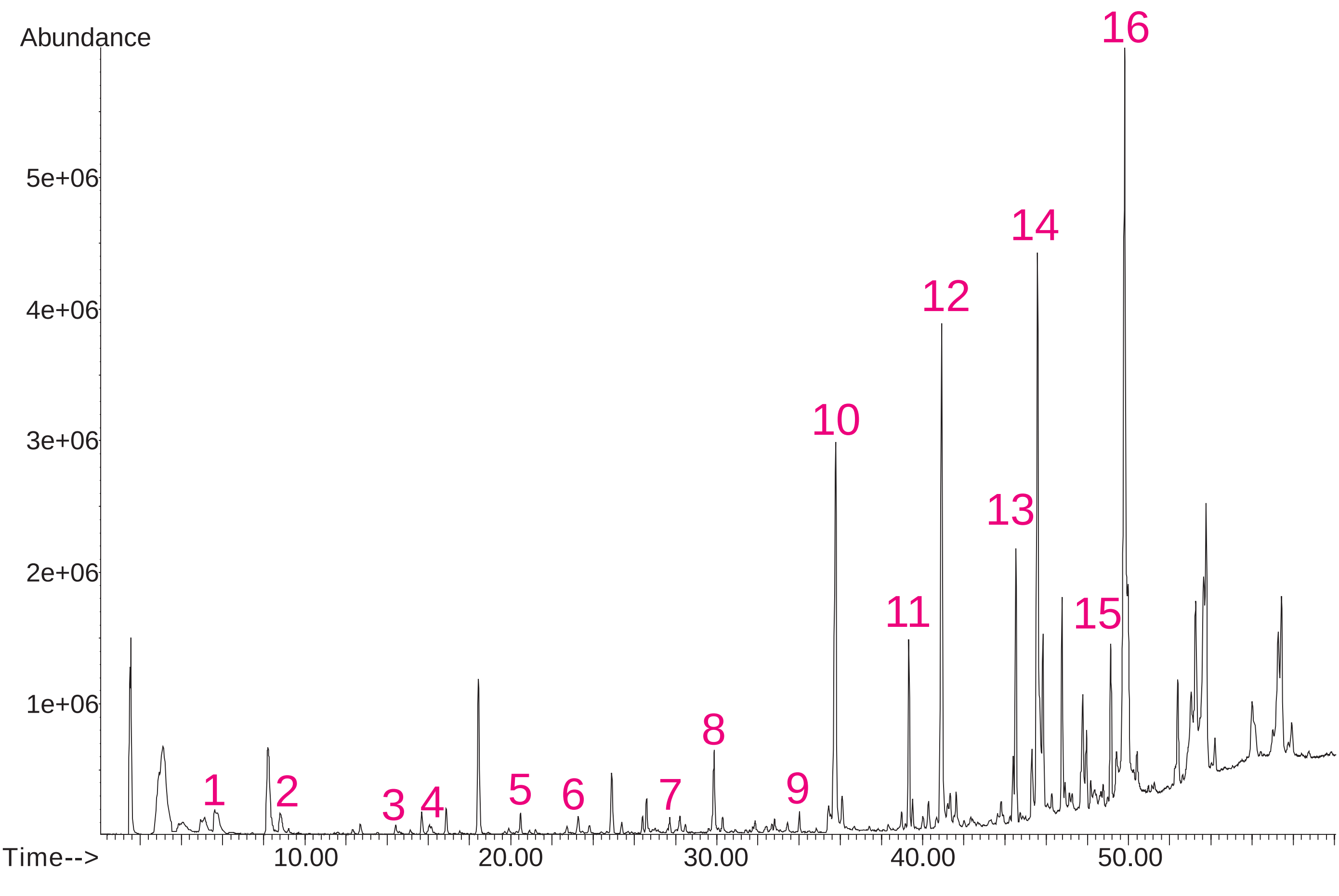

Figure 2. Milk (3.5 % fat), SPB-1, splitless.

Table I. Milk (3.5 % fat), list of compounds.

No.	Compound	No.	Compound
1	Acetone	9	2-Undecanone
2	2-Butanone	10	Capric Acid
3	Amyl Alcohol	11	δ-Decalactone
4	Butyric Acid	12	Lauric Acid
5	2-Heptanone	13	δ-Dodecalactone
6	Caproic Acid	14	Myristic Acid
7	2-Nonanone	15	Palmitic Acid
8	Caprylic Acid	16	δ-Tetradecalactone

Milk pasteurized at low temperatures (e.g. 73°C for 12 s) does not have a cooked flavor. That appears at higher temperatures mainly due to H_2S and methylketones formed by the thermal decarboxylation of β-ketoacids as well as lactones formed from γ- and δ-hydroxy fatty acids.

Figures 3 and 4 compare chromatograms of fresh and cooked milk, where figure 3 shows the increase of the lactone content (extracted ion chromatogram) and figure 4 the changes in the sulfur trace with an increase of H_2S (PFPD sulfur trace).

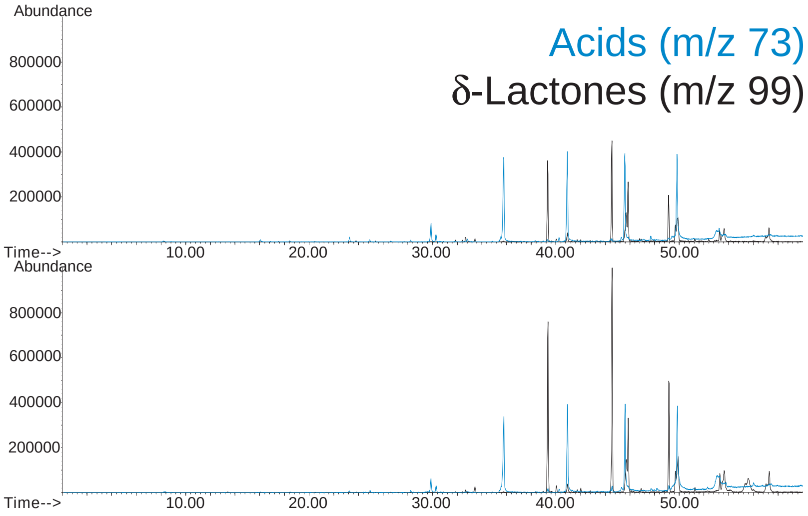

Figure 3. Comparison of fresh (top) and cooked milk (3.5 % fat), SPB-1, splitless, extracted ion chromatograms.

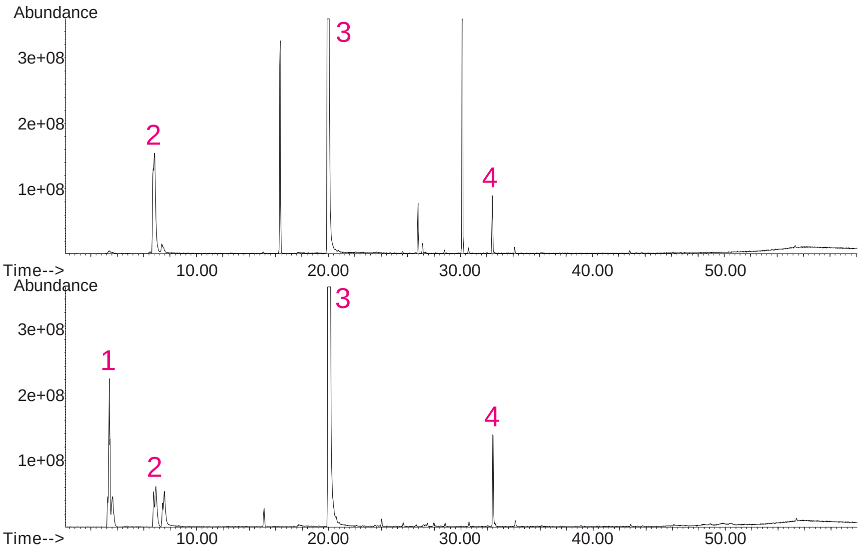

Figure 4. Comparison of fresh (top) and cooked milk (3.5 % fat), SPB-1, splitless, PFPD, sulfur-traces.

Table II. Comparison of fresh and cooked milk (3.5 % fat), list of sulfur compounds.

No.	Compound
1	Hydrogen Sulfide
2	Dimethyl Sulfide
3	Dimethyl Sulfone
4	Benzothiazole

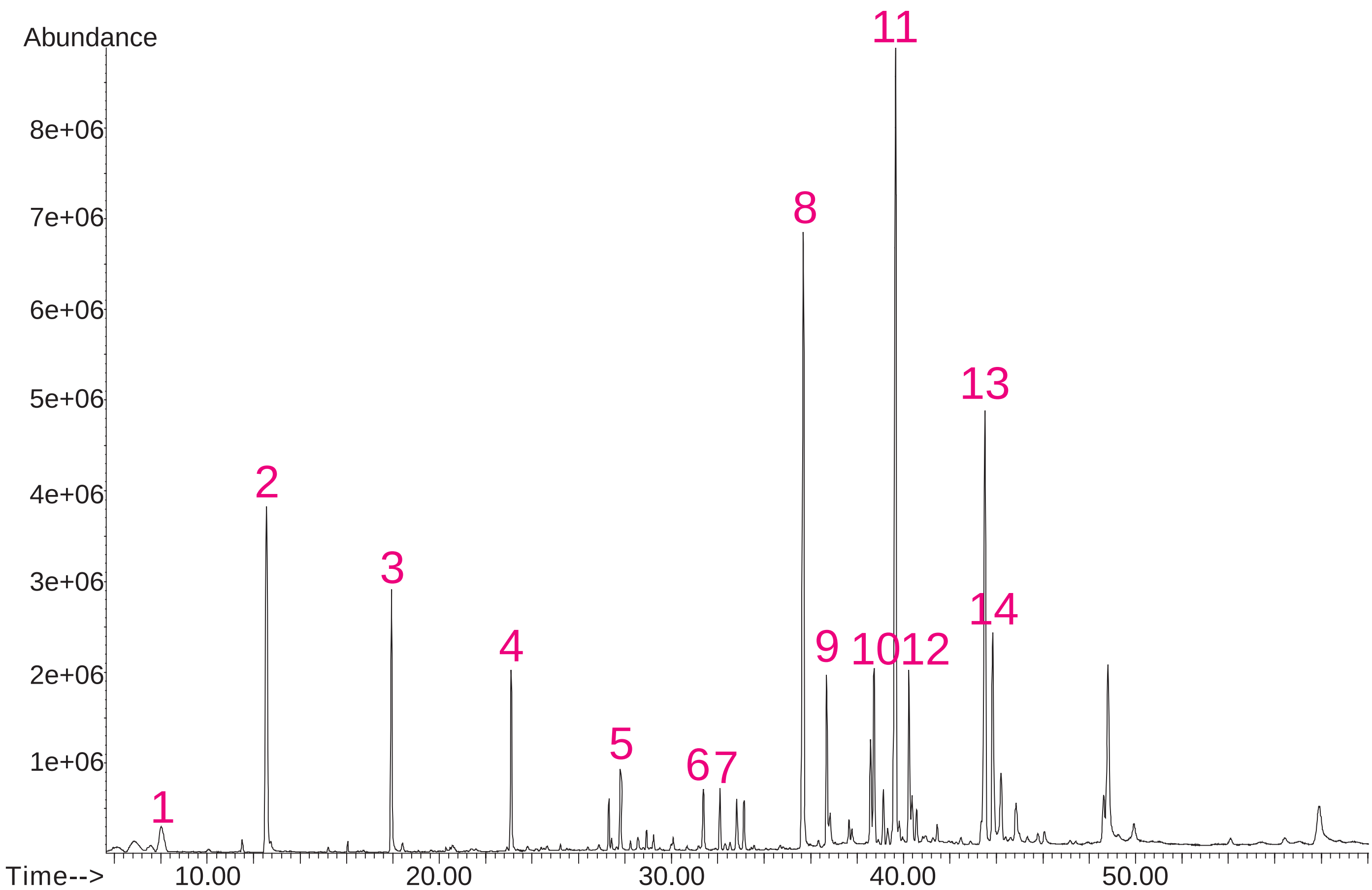

Figure 5. Condensed milk, splitless.

Table III. Condensed milk, list of compounds.

No.	Compound	No.	Compound
1	2-Pentanone	8	δ-Decalactone
2	2-Heptanone	9	Capric Acid
3	2-Nonanone	10	γ-Decalactone
4	2-Undecanone	11	δ-Dodecalactone
5	2-Tridecanone	12	Lauric Acid
6	δ-Octalactone	13	δ-Tetradecalactone
7	2-Pentadecanone	14	Myristic Acid

Condensed milk. During the concentration of milk reactions similar to heated milk occur, but to a greater extent. Ketones, lactones and also Maillard-products now determine the typical aroma.

A typical contaminant entering dairy products from the packaging material is styrene. Figure 6 shows extracted ion chromatograms (m/z 94) of two differently packed condensed milks: one came in a polystyrene package, the other one in a polyethylene package. The styrene content found in the polystyrene-packed condensed milk was more than 100 times higher than in the polyethylene-packed milk.

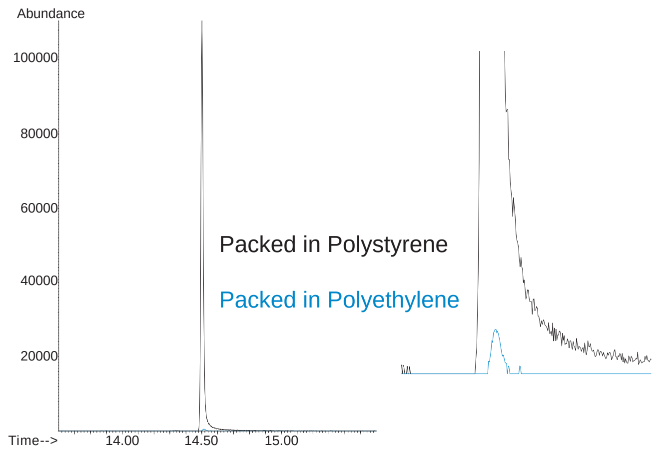

Figure 6. Comparison of differently packed condensed milk, splitless.

Cheese. A very large number of compounds contribute to the aroma of cheese depending on the variety and the way it is processed.

Cream cheese has a creamy aroma caused by lactones and carbonyl compounds like in cooked milk products but with a higher content of fatty acids.

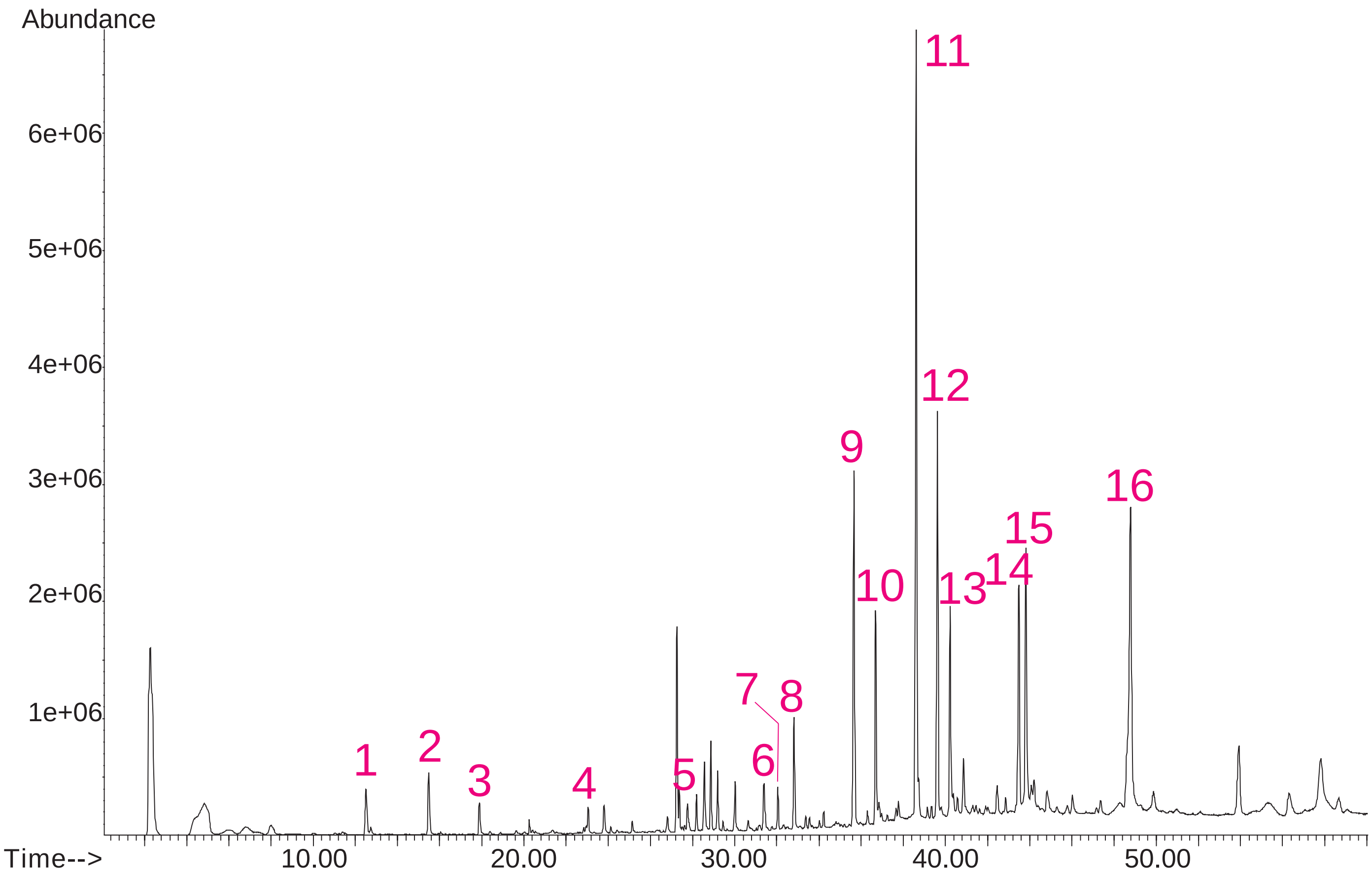

Figure 7. Cream cheese, splitless.

Table IV. Cream cheese, list of compounds.

No.	Compound	No.	Compound
1	2-Heptanone	9	δ-Decalactone
2	Acetoin	10	Capric Acid
3	2-Nonanone	11	Diethylphthalate
4	2-Undecanone	12	δ-Dodecalactone
5	2-Tridecanone	13	Lauric Acid
6	δ-Octalactone	14	δ-Tetradecalactone
7	2-Pentadecanone	15	Myristic Acid
8	Caprylic Acid	16	Palmitic Acid

The addition of herbs or herbal extracts to cream cheese provides a typical aroma mainly caused by some sulfur compounds also found in garlic and onions. Figure 8 illustrates the usefulness of a sulfur specific detector (PFPD) operated in parallel to an MSD to detect those compounds in such a complex matrix. The upper trace shows the total ion chromatogram of a herb-flavored cream cheese which does not differ too much from the plain cream cheese, whereas the simultaneously recorded sulfur trace allows one to locate the low concentrated sulfur compounds like a needle in a haystack.

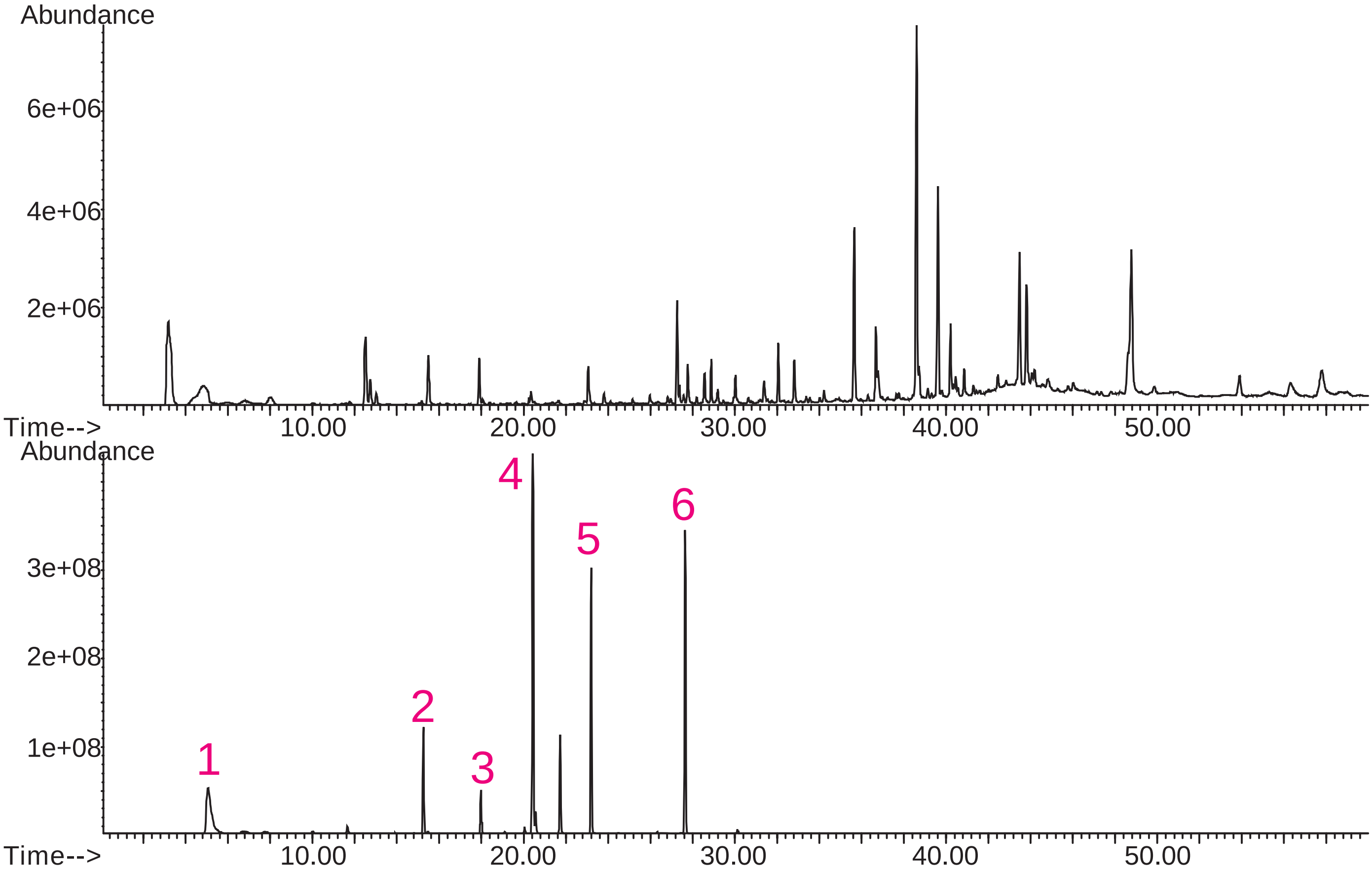

Figure 8. Herb-flavored cream cheese, total ion chromatogram (top) and sulfur trace (PFPD), splitless.

Table V. Herb-flavored cream cheese, list of sulfur compounds.

No.	Compound	No.	Compound
1	Hydrogen Sulfide	4	Diallyl Disulfide
2	Methyl Allyl Disulfide	5	Methyl Allyl Trisulfide
3	Dimethyl Trisulfide	6	Diallyl Trisulfide

Yoghurt. Yoghurt is another dairy product which often is blended with fruit and fruit ingredients and several aroma substances. Here the analysis of the fruit flavor, not the components from the plain yoghurt, is of importance. Figure 9 shows the chromatogram of a strawberry-flavored yoghurt.

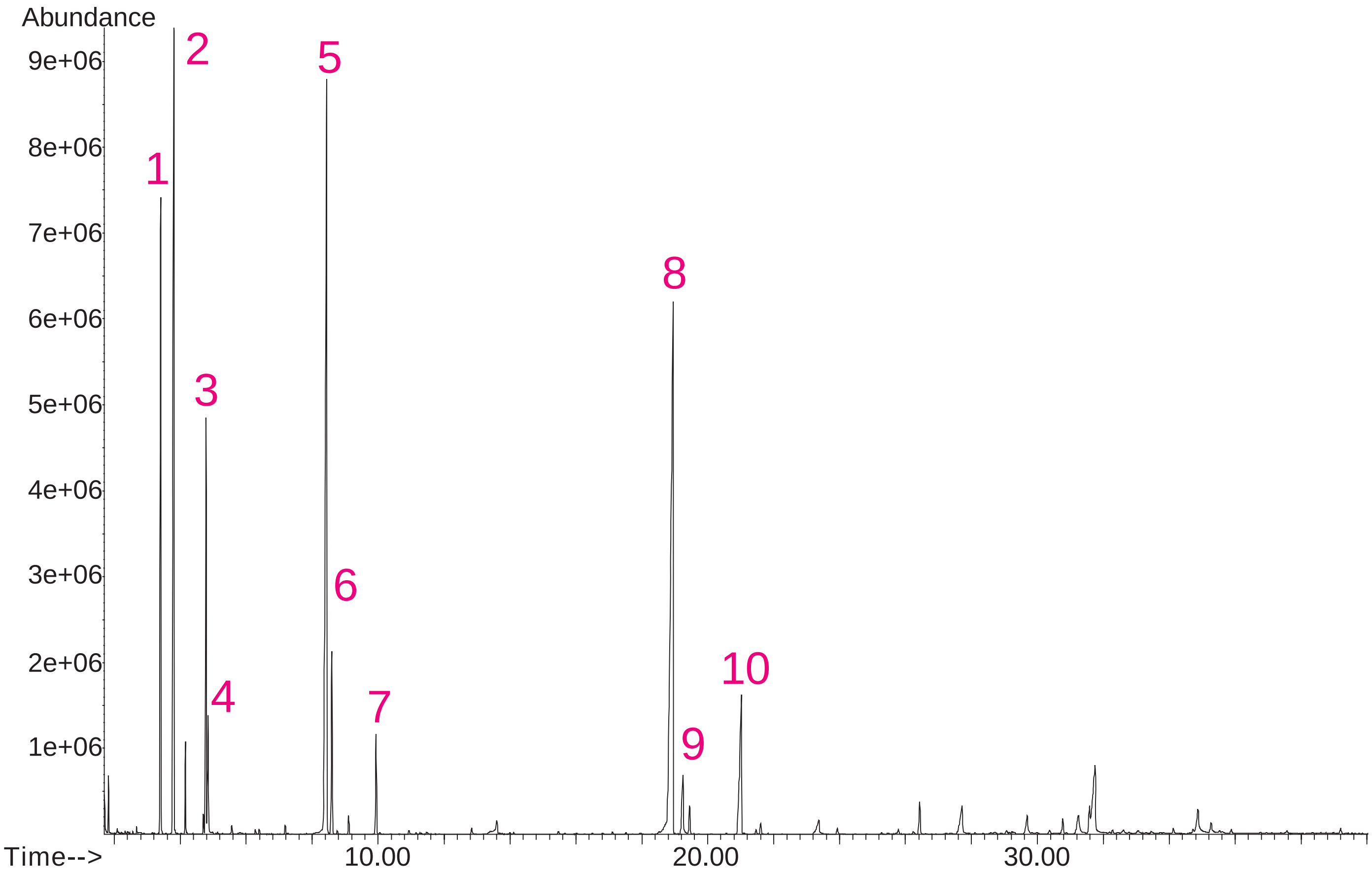

Figure 9. Strawberry yoghurt, split 1:20.

Table VI. Strawberry yoghurt, list of compounds.

No.	Compound	No.	Compound
1	Methyl-2-methyl Butyrate	6	cis-3-Hexenyl Acetate
2	Ethyl Butyrate	7	Isoamyl Butyrate
3	Ethyl-3-methyl Butyrate	8	Methyl Cinnamate
4	cis-3-Hexenol	9	Vanillin
5	Ethyl Caproate	10	γ-Decalactone

Conclusions

Stir bar sorptive extraction (SBSE) is an extremely powerful technique for flavor profiling of different types of dairy products 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”.

References

[1] E. Baltussen, P. Sandra, F. David and C. Cramers, J. *Microcol. Sep.* **1999**, 11, 737.

Literature

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