SPME
A Faster, Simpler Sample Prep Technology
Winter 1998

SPME for Food and Beverage Applications

Analysts in the food and beverage industry continue to develop new SPME applications for extraction of flavors and odors. The article on this page describes the extraction of off-flavors in milk, the result of exposure to light. The applications on page 2 describe the performance of SPME in extracting pyrazines from peanut butter, as well as natural and artificial flavors from hard candy.

Monitoring Light-Induced, Off-Flavor Chemicals in Milk by SPME

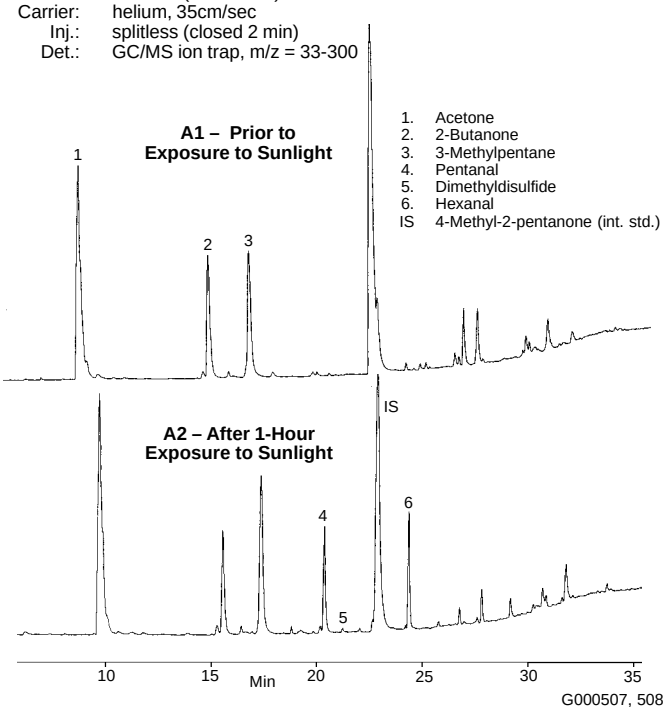
R. Marsili, Dean Foods Technical Center, Rockford, IL

Off-flavors can occur in milk by a variety of mechanisms. One of the most common causes of these off-flavors is exposure to light. It is estimated that exposure of milk samples to fluorescent lights in the supermarket dairy case is responsible for the development of off-flavors in some 80% of samples sold.

Although it provides the high sensitivity needed to monitor light-induced off-flavor reactions, dynamic headspace gas chromatography/mass spectrometry (DH-GC/MS) is time consuming and lacks precision.

Figure A. Milk Sample Off-Flavors by SPME-GC/MS

Sample:	3g of 2% milk + 10µL internal standard solution (20µg/mL 4-methyl-2-pentanone) (9mL GC vial)
SPME Fiber:	**PDMS/Carboxen, 75µm film**
Cat. No.:	**57318**
Extraction:	headspace, 15 min with constant stirring at 45°C
Desorption:	5 min, 250°C
Column:	**Supel-Q™ PLOT, 30m x 0.32mm ID**
Cat. No.:	**24242**
Oven:	70°C (2 min) to 140°C at 6°C/min (2 min hold) then to 220°C at 6°C/min (5 min hold)
Carrier:	helium, 35cm/sec
Inj.:	splitless (closed 2 min)
Det.:	GC/MS ion trap, m/z = 33-300



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The author used both DH-GC/MS and SPME-GC/MS to monitor milk samples in glass bottles exposed to sunlight for approximately one hour. An SPME fiber coated with 75µm polydimethylsiloxane (PDMS)/Carboxen™ was capable of extracting low ppb levels of pentanal, hexanal, and dimethyldisulfide – off-flavor byproducts. Figure A1 shows a chromatogram of 2% milk prior to exposure to sunlight; Figure A2 shows the same milk sample after exposure to sunlight for 1 hour.

SPME-GC/MS proved to be superior to the dynamic headspace method. SPME eliminates background, artifact, and carryover peaks which are observed in DH-GC chromatograms. SPME also is more accurate, faster, easier, and less expensive than DH. Furthermore, SPME-GC/MS provides sensitivities equivalent to DH-GC/MS.

Acknowledgment
All information and illustrations in this article were submitted by Ray Marsili, Dean Foods Technical Center, Rockford, IL 61108.

ACS Presentations

In addition to providing the information shown on this page, the author made the following detailed presentation at the American Chemical Society 216th National Meeting in Boston, MA, August 23-27, 1998. If you would like a copy of the paper, please use the postcard on page 4 of this newsletter.

The Application of Dynamic Headspace and Solid-Phase Microextraction Gas Chromatographic Techniques for Studying Off-Flavors in Milk

R.T. Marsili and N. Miller, Dean Foods Co., PO Box 7005, Rockford, IL 61108

The following SPME presentations also were presented at the ACS meeting:

SPME Method Development for Headspace Analysis of Volatile Flavor Compounds

D.D. Roberts and P. Pollien, Nestlé Research Center, Vers-Chez-les-Blanc, 1000 Lausanne 26, Switzerland

Analysis of Orange Soda Flavor Volatiles Using Headspace SPME and GC-MS

V. Barrett, Sunkist Growers Inc., 760 E. Sunkist St., Ontario, CA 91761

Solid Phase Microextraction of Volatiles from Foods Using a Porous Carbon Fiber

B. Page, G. Lacroix, and D. Weber, Food Research, Health Protection Branch, Health Canada, Ottawa, Canada K1A OL2.

Natural and Artificial Flavors in Hard Candy

SPME can be applied to the comparison of artificial flavoring and natural flavoring. Using a 1cm StableFlex™ SPME fiber coated with divinylbenzene/Carboxen™/polydimethylsiloxane (DVB/Carboxen/PDMS), we extracted the components of two hard candies with cherry flavoring. One sample was labeled as having artificial flavoring (Figure A1), and the other was labeled as having both natural and artificial flavorings (Figure A2).

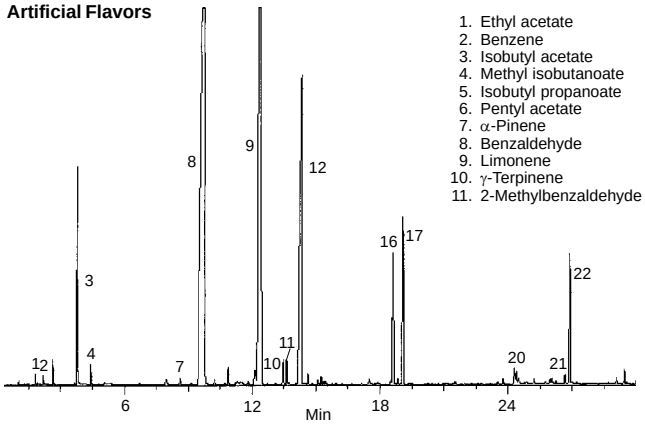
The two candies contained some common ingredients, primarily benzaldehydes. The candy with natural flavoring included a variety of terpinene-type compounds commonly found in citrus fruits. The artificially-flavored candy did not contain these compounds, except for a small amount of limonene. Identification of the components was not confirmed with standards, but was based on mass spectral determination.

The Carboxen portion of the fiber, layered under a DVB coating, extracts small analytes such as ethyl acetate, benzene, and other small esters. The DVB extracts larger analytes such as caryophyllene and BHT. The distinct characteristics of the multiple components of the fiber coating allowed extraction of a wide molecular weight range of compounds with one fiber.

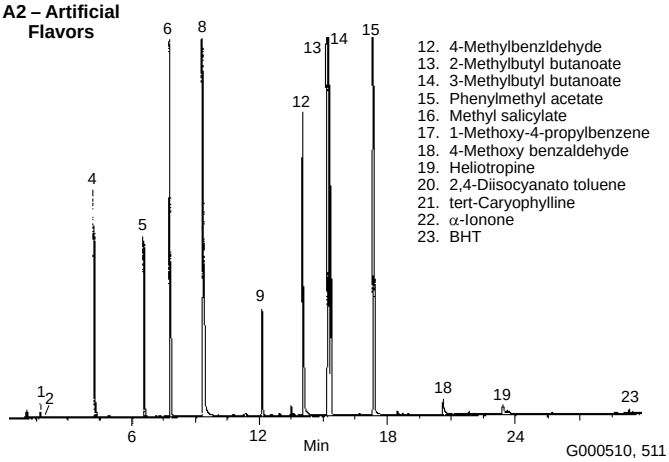
Figure A. Cherry Flavoring in Hard Candy

Sample:	0.5g candy in 5mL water in a 15mL vial
SPME Fiber:	**DVB-Carboxen-PDMS StableFlex**
Cat. No.:	**57328-U**
Extraction:	headspace, 30 min at 40°C
Desorption:	270°C for 5 min
Column:	**Meridian MDN-5, 30m x 0.25mm x 0.25µm film**
Cat. No.:	**24096**
Oven:	45°C (1.5 min) to 260°C at 4°C/min
Inj.:	split or splitless with 0.75mm liner, 270°C
Det.:	ion trap mass spectrometer, m/z = 33-400 at 0.6 sec/scan Selected ions used for quantitation.

A1 – Natural and Artificial Flavors



A2 – Artificial Flavors



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Pyrazines in Peanut Butter

The analysis of semivolatile flavor compounds in fat matrices is difficult. The analytes tend to reside in the fat and are not removed easily. To drive enough of the analytes of interest into the headspace for traditional headspace analysis, the sample must be heated to more than 100°C. This can cause the formation of degradation byproducts and reduce analytical accuracy. SPME allows the use of lower temperatures to drive the analytes into the headspace because the SPME fiber concentrates the analytes.

Pyrazines, the primary flavor components in peanut butter, form when peanuts are roasted. We used a 1cm DVB/Carboxen/PDMS StableFlex SPME fiber to extract pyrazines from a peanut butter sample. We detected 21 pyrazines ranging in concentration from <1ppb to >500ppb (Figure B, peaks 6-26). The SPME fiber also extracted volatile components such as hexanal, dimethyldisulfide, and 1-methyl pyrrole (peaks 1-5). With a SUPELCOWAX™ 10 column, only two of the pyrazines co-eluted.

(contd. on next page)

Figure B. Peanut Butter

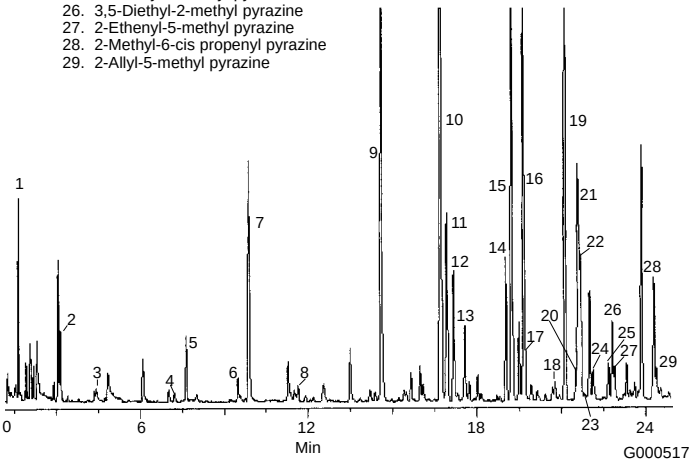
Sample:	5g peanut butter in 40mL vial
SPME Fiber:	**DVB-Carboxen-PDMS StableFlex**
Cat. No.:	**57328-U**
Extraction:	headspace, 30 min at 65°C in heating block
Desorption:	270°C for 5 min
Column:	**SUPELCOWAX 10, 30m x 0.25mm x 0.25µm film**
Cat. No.:	**24079**
Oven:	40°C (5 min) to 230°C at 4°C/min
Inj.:	splitless/split, closed 0.5 min, 270°C, with 0.75mm liner
Det.:	ion trap mass spectrometer, m/z = 30-350 at 0.6 sec/scan Selected ions used for quantitation.

Some Volatile Components in Peanut Butter

1. Carbon disulfide
2. 3-Methylbutanal
3. Pentanal
4. Dimethyl disulfide
5. Hexanal
6. 1-Methyl pyrrole
7. 4-Methyl-pentene-2-one
8. Heptanal

Pyrazines in Peanut Butter

9. 2-Methyl pyrazine
10. 2,5-Dimethyl pyrazine
11. 2,3-Dimethyl pyrazine
12. 2-Ethyl pyrazine
13. 2,6-Dimethyl pyrazine
14. 2-Ethyl-6-methyl pyrazine
15. 2-Ethyl-5-methyl pyrazine
16. Trimethyl pyrazine
17. 2-Ethyl-3-methyl pyrazine
18. 2,6-Diethyl pyrazine
19. 2-Ethyl-3,5-dimethyl pyrazine
20. 2,3-Diethyl pyrazine
21. 2-Methyl-5-isopropyl pyrazine
22. 3-Ethyl-2,5-dimethyl pyrazine
23. 5-Methyl-2-propyl pyrazine
24. 2-Methyl-5-propyl pyrazine
25. 2-Ethenyl-6-methyl pyrazine
26. 3,5-Diethyl-2-methyl pyrazine
27. 2-Ethenyl-5-methyl pyrazine
28. 2-Methyl-6-cis propenyl pyrazine
29. 2-Allyl-5-methyl pyrazine



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Pyrazines in Peanut Butter (contd.)

Some of the peak identifications were confirmed with standards, but most were based on mass spectral interpretation. Therefore, identification of all of the isomers may not be absolute.

Quantifying analytes in a food matrix can be difficult. We achieved our quantitation of methyl pyrazine and the dimethyl pyrazines (Table 1) by determining the area counts of a selected ion for each pyrazine in a known weight of peanut butter. We spiked a second vial containing a known weight of peanut butter with a known amount of each pyrazine standard (in this case 188ppb with respect to the weight of peanut butter). Using the following formula, we determined the amount of pyrazines in the peanut butter:

$$\text{Area counts (spiked p. butter)} - \text{Area counts (unspiked p. butter)} = \text{Area counts (spiked pyrazine)}$$

$$\text{Area counts (spiked pyrazine)} = 188\text{ng/g for each pyrazine}$$

$$\text{Pryazines in ppb} = \frac{188 \times \text{area counts (unspiked peanut butter)}}{\text{area counts (spiked pyrazine)}}$$

Table 1. Quantitation of Pyrazines (ppb)

2-Methyl pyrazine	158
2,5-Dimethyl pyrazine	526
2,3-Diemthyl pyrazine	47
2,6-Dimethyl pyrazine	16

The values listed in Table 1 are similar to those reported in the journal article listed below.

Reference
Ku, K.L., Lee, R.S., Young, C.T. and Chiou, R.Y.Y. *Roasted Peanut Flavor and Related Compositional Characteristics of Peanut Kernels of Spring and Fall Crops Grown in Taiwan.* J. Agric. Food Chem. (1998) **46:** 3220-3224.

Seminars and Exhibits

Water Quality Conference

San Diego, CA, Nov. 1-3

We will highlight a new odor screening method for geosmin and methyl isoborenol at 5ppt concentration.

Northeastern Association of Forensic Sciences

Newport, RI, Nov. 5-6

We will discuss the use of SPME for direct derivatization of amphetamines from urine in street drug screening.

Eastern Analytical Symposium

Somerset, NJ, Nov. 14-16

We will provide an in-depth comparison of artificial and natural flavors in foods.

Helpful Hints for Using SPME!

To learn how to minimize problems when using SPME, request our newest bulletin:

Bulletin 923 – *Solid Phase Microextraction: Theory and Optimization of Conditions*

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SPME Inlet Guide	**57356-U**	$22

P000047

The sampling stand does not include SPME fiber holder or vials.

P000048

SPME Sampling Stands

Support the SPME fiber holder during sample extraction. Aluminum construction allows even heating of up to eight vials. Available for 4mL or 15mL vials. The vial puck holds eight 15mL vials, and can be used with either sampling stand.

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SPME Vial Puck for 15mL vials	**57358-U**	$75

For our complete selection of SPME fibers and accessories, see the Supelco catalog, or call us.

New! SPME Books

Solid Phase Microextraction: Theory and Practice
Janusz Pawliszyn, 1997, 241 pp.

Describes the operating principles and construction of SPME devices, theory, method development, and applications.

Solid Phase Microextraction: Theory and Practice	**26591-U**	$59.95

Techniques for Analyzing Food Aroma
Ray Marsili (Ed.), 1997, 371 pp.

Discusses the analytical methods for food flavors and aromas, showing how to select appropriate techniques for resolving the problems of major food trends. Includes a chapter by Dr. Alan Harmon, McCormick Co., discussing the application of SPME to food analysis.

Techniques for Analyzing Food Aroma	**26589-U**	$150.00

New Literature from Supelco

SPME: Solventless Sample Preparation for Monitoring Flavor Compounds by Capillary GC (Bulletin 869)
Solid Phase Microextraction: Theory and Optimization of Conditions (Bulletin 923)
SPME Sampling Stand (496037)
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We Need Your Help!!

We would like to know what types of applications you want to see in our SPME reference literature. If you would like to share your experiences in SPME, please respond in the comment area on the clip-and-mail card below.

[1]US patent no. 5,691,206;. European patent #0523092. Technology licensed exclusively to Supelco.
[2]Requires an SPME fiber holder (see the Supelco catalog).
[3]Use with AutoSampler requires Varian SPME upgrade kit (available from Varian).

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Please send the following new literature:
- __ Publication 498342 - *The Application of Dynamic Headspace and Solid-Phase Microextraction Gas Chromatographic Techniques for Studying Off-Flavors in Milk* (R.T. Marsili and N. Miller)
- __ Bulletin 869 - *SPME: Solventless Sample Preparation for Monitoring Flavor Compounds by Capillary GC*
- __ Bulletin 923 - *Solid Phase Microextraction: Theory and Optimization of Conditions*
- __ Publication 496037 - *SPME Sampling Stand*
- __ Publication 494044 - Updated list of more than 250 SPME journal references

Please send information about the following products:
- __ Carboxen/DVB/PDMS fibers
- __ SPME Inlet Guide
- __ SPME fiber assemblies for Merlin Microseal Systems

Comments/ Suggestion for Applications:

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