SPME−MS−MVA as an Electronic Nose for the Study of Off-Flavors in Milk

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A new technique using solid-phase microextraction, mass spectrometry, and multivariate analysis (SPME−MS−MVA) was developed for the study of off-flavors in milk. The analytical column of a GC/MS system was replaced with a 1-m deactivated fused-silica column, which served as a transfer line to deliver volatiles extracted from milk samples with a Carboxen-SPME fiber to the mass spectrometer. Mass fragmentation data resulting from the unresolved milk volatile components were subjected to MVA. Principal component analysis based on SPME−MS−MVA provided rapid differentiation of control reduced-fat milk (2% butterfat content) samples from reduced-fat milk samples abused by light, heat, copper, and microbial contamination. The three psychrotrophic bacteria studied included *Pseudomonas fluorescens*, *Pseudomonas aureofaciens*, and *Pseudomonas putrefaciens*. SPME−MS−MVA is rapid and offers significant advantages over commercial electronic nose instruments currently being used as quality assurance tools to differentiate normal-tasting food and beverage samples from those containing off-flavors and malodors.

Keywords: *SPME−MS−MVA; electronic nose; solid-phase microextraction; off-flavors, multivariate analysis; milk*

INTRODUCTION

Off-flavors occur in milk through a variety of mechanisms (Azzara and Campbell, 1992). Resolving the cause of off-flavor formation as quickly as possible is important for food processors to avoid unnecessary expensive product recalls and to prevent reoccurrence of the problem in the future. Determining whether the cause of an off-flavor problem is related to microbiological spoilage, packaging problems, chemical reactions occurring within food material itself (e.g., lipid oxidation caused by exposure to light or pro-oxidant metals), etc. is challenging.

Capillary gas chromatography with mass spectrometry (capillary GC/MS) coupled with olfactometry detection has proven to be a potent tool for determining chemicals responsible for off-flavors in foods and beverages. Several sample preparation techniques to isolate and concentrate the odor-active compounds in malodorous foods or beverages prior to injection into the GC have been used. A few examples include liquid/liquid extraction (Weurman, 1969), simultaneous steam distillation and liquid extraction (Nickerson and Likens, 1966), static headspace (SH) (Chialva, 1982), dynamic headspace (DH) (Werkhoff and Bretschneider, 1987), and direct thermal desorption (Grimm et al., 1997; Marsili et al., 1994).

Solid-phase microextraction (SPME), a solventless extraction technique developed in 1990 (Arthur and Pawliszyn, 1990), has been shown to be a simple, effective tool for detecting low levels of flavor compounds in foods and beverages (Harmon, 1997; Yang, 1994). The evaluation of SPME for the analysis of flavor compounds produced by bacteria has recently been reported (Vergnais et al., 1998).

New fibers developed for SPME have extended the usefulness of the technique for studying food off-flavors and malodors. A Carboxen−polydimethylsiloxane (PDMS) SPME fiber, for example, has recently been shown to detect parts per billion levels of dimethyl disulfide, pentanal, hexanal, and heptanal produced in light-irradiated milk (Marsili, 1999). In this work, the technique of using static headspace SPME followed by capillary GC/MS was shown to be as sensitive as DH−GC/MS, but with significantly better precision, fewer carry-over problems, and fewer extraneous background peaks compared to DH−GC/MS.

Another significant development for the study of flavors and off-flavors is the recent introduction of so-called "electronic nose" (e-nose) instruments that employ an array of chemical sensors based on conducting polymers, metal oxides, surface acoustic wave (SAW) devices, quartz crystal microbalances (QCMs), or combinations of these devices. For example, e-noses have been used for monitoring the quality of edible oils (Bazzo et al., 1998) and for distinguishing different heat treatments of milk (Sberveglieri et al., 1998).

While e-nose instruments are tools for visually comparing the aromas of samples, they do not provide the same type of specific detailed chemical information that is possible with GC/MS methods, and the technique is probably not sensitive enough for some analytes and some types of matrices. The primary advantage of e-noses as a quality assurance tool for the food industry is speed of analysis—in terms of both data generation and data interpretation. Rapid, meaningful data interpretation is possible with various chemometric (multivariate analysis) techniques. As another alternative, data systems offered by some instrument manufacturers incorporate artificial neural networks for interpretation of data. Neural networks are data processing algorithms based loosely on the structure of the human brain.

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Quality control models can be developed by "training" the e-nose, and then routine samples can be tested against the model, providing a "goodness of fit" approximation or a sample accept/reject answer.

The purpose of this work was to develop a new SPME-based instrumental approach for studying off-flavors. This work describes an alternative design for an e-nose instrument based on SPME to isolate/extract volatile organics from the food matrix, MS as a chemical sensor, and multivariate analysis (MVA) to decipher meaningful trends in the mass spectroscopy output. The technique is referred to as SPME−MS−MVA. Principal component analysis (PCA) of abused and control normal-tasting milk samples is used as an example to illustrate the capability of SPME−MS−MVA to discriminate types of off-flavor problems in milk.

MATERIALS AND METHODS

Sampling/Preparation of Abused Samples. Forty milk samples were analyzed by SPME−MS−MVA. Thirty-four samples were prepared from reduced-fat milk (2% butterfat) purchased from three different supermarkets. The sample profile and preparation of abused samples were as follows: 10 control samples (with 10 different code dates and prescreened by a sensory panel to ensure the absence of detectable malodors or off-flavors); 3 ultrahigh temperature (UHT) samples (different code dates); 7 light-abused samples (prepared from 7 different code dates of control samples by irradiation of milk in glass bottles with 200 ft-c of fluorescent light for 24 h stored at 3.7 °C); 8 samples abused by copper (prepared from 8 different code dates of control samples by the addition of 20 ppm of Cu added as cupric chloride and stored for 72 h at 3.7 °C prior to analysis); and 6 samples of microbially spoiled UHT milk. The 6 microbially spoiled UHT milk samples were prepared from 6 different code dates of control UHT milk. In addition to these 34 samples of reduced-fat milk, 6 samples of microbially spoiled homogenized whole-fat UHT milk (3.3% butterfat) were also prepared.

None of the control reduced-fat milk or UHT samples had a detectable malodor or off-flavor. Although none of the light-abused or copper-abused samples had a detectable odor, they all possessed an oxidized off-flavor. All of the UHT samples abused by microorganisms possessed a similar Cheddar cheese-like aroma.

Preparation of Microbially Spoiled Samples. Three different psychrotrophic bacteria were used to prepare the 12 microbially spoiled UHT milk samples: *Pseudomonas fluorescens* (ATCC 948), *Pseudomonas aureofaciens* (ATCC 17461), and *Pseudomonas putrefaciens* (isolated in-house from spoiled milk). The *P. fluorescens* and *P. aureofaciens* were purchased from the American Type Culture Collection (Rockville, MD). Different control samples were spiked with ∼2000 bacteria cells/mL of milk, and samples were incubated at 20 °C for 6 days. Two samples were prepared for each of the three different species of *Pseudomonas* bacteria using reduced-fat UHT milk, and two samples were prepared for each of the three different species of *Pseudomonas* bacteria using whole-fat UHT milk. Control samples of reduced-fat UHT milk and whole-fat UHT milk without added microorganisms were incubated along with the control samples spiked with *Pseudomonas* bacteria. Standard plate counts (SPCs) on the nonspiked control samples were conducted each of the 6 days. SPC results were negative for all nonspiked control samples.

Because the identities of off-flavor metabolites contributed by the microbially abused samples were unknown, the 12 microbially abused samples were analyzed by SPME−GC/MS. For this study, a 30 m × 0.25 mm FFAP capillary column was used (J&W Scientific, Folsom, CA). The film thickness of the column was 0.25 μm. The initial column temperature was 50 °C. The column was maintained at 50 °C for 2 min, then heated to 180 °C at a rate of 6 °C/min, and held at 180 °C for 6 min. The SPME extraction procedure for the SPME−GC/MS analysis of the microbially abused samples was identical to the SPME procedure used to perform SPME−MS−MVA. For SPME−GC/MS, the mass scan range was *m*/*z* 40−300.

SPME Analysis. A Saturn 3 GC/MS was used (Varian Analytical Systems, San Fernando, CA). The GC was equipped with a split/splitless model 1078 injector. The injector was operated in the splitless mode at a temperature of 250 °C. The SPME fiber used was 75 μm Carboxen−PDMS (Supelco, Bellefonte, PA). For thermal desorption, the SPME fiber remained in the injector for 2 min. Helium was used as the carrier gas. A 1 m × 0.25 mm uncoated fused silica line was used instead of an analytical column to transfer SPME-extracted volatiles to the MS. The temperature of this transfer line was held constant at 50 °C.

Optimization of SPME for the analysis of low molecular weight aldehydes in milk has been previously reported (Marsili, 1999). This previous work indicated that saturation of samples with salt did not significantly improve SPME sensitivity for pentanal and hexanal in milk. Therefore, salt addition was not considered for the present study.

Three grams of milk sample, 7 μL of internal standard solution (20 μg/mL 4-methyl-2-pentanone), and a microstirring bar (Fisher Chemical Co., catalog no. 09-312-102) were placed in a 9 mL glass GC vial (46 mm high and 20 mm in diameter) and capped with PTFE/Grey Butyl molded septa from Pierce Chemical Co. (Rockford, IL). Samples were analyzed in duplicate—once with the internal standard added and once with no added internal standard.

The setting on the SPME holder assembly scale was adjusted to 1.0 scale unit to ensure that the fiber was positioned in the headspace above the sample in exactly the same way from run to run. With the fiber exposed, the sample vial was placed in a 45 °C water bath, and the sample was stirred at high speed. After a 12 min exposure time, the fiber was retracted into the needle assembly and removed from the vial. The setting on the SPME holder assembly was changed to 3.4 scale units prior to injection into the GC injector port, which was fitted with a special insert for SPME analysis (Varian part no. 03-925330-00).

MS Analysis. The Varian Saturn MS detector was used in the electron impact (EI) mode with a 1 s scan time and a 1 count peak threshold. The mass range used was *m*/*z* 50−150. The temperature of the ion trap manifold was 170 °C.

MVA Analysis. The software used for MVA was Pirouette from Infometrix, Inc. (Woodinville, WA). Two-dimensional PCA score plots were created with the Pirouette exploratory algorithm. Standardized PCA was performed on the data. The ability of SPME−MS−MVA to differentiate milk samples by type of abuse was determined by visual examination of class clustering in two-dimensional PCA plots.

RESULTS AND DISCUSSION

Significance of SPME as a Delivery System for Low Molecular Weight Volatiles to the MS. Low molecular weight aldehydes, ketones, and fatty acids are frequently responsible for off-flavors and malodors in foods and beverages. Although commercial SPME equipment has been available since 1993, the application of the technology to extremely low level detection of low molecular weight volatiles has been lacking until recently. Using PDMS fibers with small analytes is inadvisable because equilibrium is established rapidly and distribution constants are low, resulting in high minimum detection limits. Although increasing the thickness of the phase coating on the fiber is beneficial, a stronger adsorbing medium is required to capture analytes. Recently, Supelco has developed fibers coated with porous carbon to enable SPME to be used for the analysis of small analytes at trace levels.

SPME−GC/MS analysis of milk with light-induced off-flavors has been previously reported (Marsili, 1999). For this work, 75 μm Carboxen−PDMS fiber was found

to be suitable for the detection of pentanal and hexanal produced by the free radical-based reaction of light with polyunsaturated fatty acids in the butterfat triglycerides. No significant improvement in sensitivity was observed by the addition of salt. A comparison of DH−GC/MS and SPME−GC/MS was made with respect to linearity of standard calibration curves and for samples of milk exposed to 200 ft-c of fluorescent light for various time periods (0, 3, 6, 9, 12, 17, 24, and 48 h). Linear least-squares correlation coefficients for pentanal and hexanal were significantly better for the standard curves and for plots of parts per billion pentanal and parts per billion hexanal versus time of light exposure than they were for similar plots made by DH−GC/MS analysis. Furthermore, precision of replicates was substantially better for SPME than for DH.

This previous work also reported two other important advantages of SPME over DH as a technique for introducing volatiles into the MS detector: (1) No carry-over peaks from sample to sample were observed for SPME, whereas carry-over peaks from previous injections are frequently a problem for DH; and (2) no background peaks were observed for SPME. Artifact peaks originating from GC septa, thermal degradation of trapping media (e.g., Tenax), and other components of the DH instrumentation are frequently observed in DH−GC chromatograms. SPME was found to have approximately the same sensitivity as DH for pentanal and hexanal in reduced-fat milk and skim milk samples.

Compared to SH and DH, Carboxen−PDMS SPME is more efficient at extracting volatile fatty acids (VFAs) from milk. VFAs, important contributors to malodors and off-flavors in milk and other foods, are generated as metabolites by the growth of lipolytic psychrotrophic bacteria. Malodorous VFAs are too polar to detect at low levels using SH and DH as sample preparation/extraction tools.

SH is also a common technique for introducing volatiles into a GC or an e-nose instrument. However, unlike SPME and DH, SH as a sample preparation technique lacks one important feature: The ability to concentrate analytes. As a result, SH−GC is normally applicable for the analysis of analytes in the low parts per million range, whereas SPME can be extended to analytes in the parts per billion and even parts per trillion range. This is an important consideration because off-flavors in foods are often caused by volatiles present at extremely low levels.

Mass Spectra Anomalies Observed with SPME−MS. Volatiles from the SPME fiber were desorbed in the GC injection port (250 °C) onto a 1-m column of deactivated fused silica (0.25 mm i.d.). The purpose of the fused-silica column/transfer line is to transfer the volatiles to the MS—not to resolve chromatographic peaks.

In initial experiments, the transfer line was heated to 120 °C. Injection of volatiles from a control normal-tasting reduced-fat milk sample provided a "chromatogram" with one broad peak completely eluted after only 1 min. Examination of the mass spectrum for this peak, however, revealed an abnormally high mass value at *m/z* 77—a mass peak that is rarely observed in any of the volatiles normally found in milk samples. This experiment was repeated several times using different GC vials and septa and different control milk samples. Results consistently showed the anomalous *m/z* 77 peak.

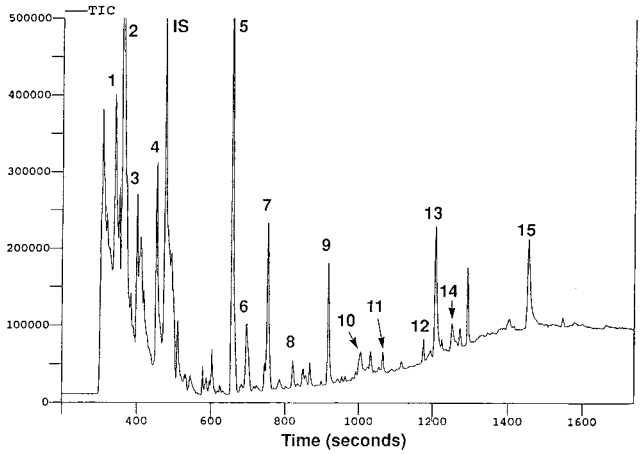

Figure 1. SPME−GC/MS chromatogram of a reduced-fat UHT milk sample contaminated with *P. putrefaciens*. Peaks: (1) methyl sulfide; (2) acetone; (3) 2-butanone; (4) 2-pentanone; (5) 2-heptanone; (6) 3-methylbutanol; (7) styrene; (8) 2-pentanol; (9) 2-nonanone; (10) acetic acid; (11) 2-nonanal; (12) 2-undecanone; (13) butryic acid; (14) furfuryl alcohol; (15) hexanoic acid; (IS) internal standard (4-methyl-2-pentanone).

The temperature of the transfer line was increased to 180 °C. A control milk sample was then analyzed. As in earlier experiments with the transfer line at 120 °C, a single peak resulted. The peak exhibited less tailing and less broadening. However, the anomalous *m/z* 77 peak was still present at a high-intensity level.

Next, the temperature of the transfer line was lowered to 50 °C, and another control milk sample was analyzed. This time two chromatographic peaks were observed. The mass spectrum of the second smaller peak consisted primarily of the *m/z* 77 peak. Library searches revealed the identity of the second peak to be fluorotrimethylsilane. Further experiments failed to reveal the source of the fluorotrimethylsilane contamination. Analyses of samples using different GC vials and different types of vial closures all showed the presence of fluorotrimethylsilane. It is possible that the contamination was present in all of the different types of vial closures or that it is a contaminant of the SPME fiber. Regardless of its source, it was possible to negate the mass contribution of the fluorotrimethylsilane contaminant by setting the transfer line temperature to 50 °C and subjecting only the mass spectrum of the initial peak to MVA.

SPME−GC/MS Analysis of Microbially Abused Samples. Figure 1 is an example of an SPME−GC/MS metabolite profile obtained for the reduced-fat UHT milk sample spiked with *P. putrefaciens*. This chromatogram illustrates the wide variety of malodorous chemicals, including VFAs, that can be extracted with the Carboxen−PDMS fiber. Table 1 lists the major metabolites found in the microbially abused samples. This information was used in developing Table 5.

Optimization of Mass Spectrometer Data. For SPME−MS−MVA, only masses from *m/z* 50 to 150 were measured. Most volatile chemicals contribute significant mass peaks below *m/z* 50. Therefore, monitoring masses below 50 amu would offer little or no improvement in the discrimination of volatiles. Masses above *m/z* 150 were ignored, because few volatiles with masses above this value would be extracted by the Carboxen−PDMS SPME technique.

Further improvement in discrimination was accomplished by excluding the major mass peaks contributed

Table 1. Relative Concentrations of Volatile Metabolites in Reduced-Fat UHT Milk and Whole-Fat UHT Milk Spiked with Three Different Species of *Pseudomonas* Bacteria As Determined by SPME−GC/MS

	relative concentration[a,b]					
metabolite	BA	BF	BP	WBA	WBF	WBP
dimethyl sulfide	1.1	6.4	11.0	7.6	1.6	6.1
dimethyl disulfide	0.4	2.4	0.6	ND	0.5	0.3
2-pentanone	3.9	8.4	8.1	3.5	4.5	3.4
2-heptanone	9.0	4.7	10.6	14.2	10.3	14.8
2-nonanone	1.4	0.9	2.4	3.6	3.5	5.6
2-undecanone	0.2	0.1	0.3	0.5	0.4	0.6
3-methylbutanol	ND	6.6	2.2	ND	1.9	ND
2-pentanol	0.3	0.9	0.6	0.3	0.8	0.8
2-nonanol	0.2	0.3	0.4	ND	ND	ND
furfuryl alcohol	0.3	0.4	0.5	1.6	2.4	2.2
acetic acid	0.6	1.8	0.7	1.1	1.8	3.0
butyric acid	3.1	0.5	3.5	12.2	2.9	1.4
hexanoic acid	2.3	0.1	3.1	10.2	2.7	1.4
octanoic acid	1.6	ND	0.5	9.5	7.3	2.7

[a] Peak area of metabolite divided by peak area of internal standard multiplied by 10, with 7 μL of internal standard solution (20 μg/mL 4-methyl-2-pentanone) added to 3 mL of milk sample prior to SPME. [b] BA, BF, and BP refer to reduced-fat UHT milk spiked with *P. aureofaciens*, *P. fluorescens*, and *P. putrefaciens*, respectively. WBA, WBF, and WBP refer to whole-fat UHT milk spiked with *P. aueofaciens*, *P. fluorescens*, and *P. putrefaciens*, respectively.

Table 2. Mass Fragmentation Patterns of Some Volatiles Commonly Found in Control (Nonabused) Milk

compound	major fragments, mass (%)
acetone[a]	42 (30), 43 (72), 44 (100), 57 (8), 58 (38)
2-butanone[a]	40 (17), 41 (10), 42 (24), 43 (64), 44 (100), 55 (5), 57 (20), 58 (5), 71 (3), 72 (40)
limonene	42 (9), 53 (20), 67 (51), 81 (100), 94 (42), 107 (31), 121 (22), 136 (38)
toluene	41 (14), 43 (26), 45 (17), 50 (13), 51 (12), 62 (7), 63 (15), 65 (19), 89 (6), 91 (100)
xylene isomers	41 (25), 42 (14), 43 (43), 44 (7), 45 (17), 50 (14), 51 (9), 53 (7), 55 (17), 57 (9), 63 (11), 65 (9), 78 (9), 79 (11), 91 (100), 95 (17), 103 (7), 105 (25), 106 (33)
styrene	40 (16), 41 (16), 43 (10), 50 (29), 51 (45), 52 (12), 62 (7), 63 (15), 74 (10), 77 (35), 78 (89), 102 (11), 103 (59), 104 (100)
chloroform	40 (15), 43 (37), 44 (13), 47 (44), 48 (22), 49 (14), 83 (100), 85 (61)
trimethylbenzenes	40 (12), 41 (31), 42 (14), 43 (38), 50 (9), 51 (13), 55 (15), 57 (11), 77 (21), 78 (25), 91 (18), 103 (12), 105 (100)

[a] Volatiles normally present in highest concentration in control milk samples.

by volatiles normally present in milk at higher concentrations (Badings and Neeter, 1980; Badings et al., 1985). These chemicals and their primary mass fragments are listed in Table 2.

Table 3 lists important volatiles that are known to contribute off-flavors to milk, the common mechanism(s) of formation, and references that describe the mechanism of formation in more detail. Table 4 lists the mass fragmentation patterns for these important contributors to milk off-flavor.

Two peaks were observed in all SPME−MS−MVA "chromatograms". Masses comprising the smaller fluorotrimethylsilane peak were ignored. The larger initial peak contained masses of ion fragments from headspace volatiles of the sample. For this peak, masses from 8 to 80 s were averaged, whereas masses from 0 to 7 s and from 81 to 100 s were subtracted as background. The resulting mass intensity list provided the data used for PCA.

An improvement in the ability of MVA to discriminate between control and abused samples was achieved by performing transformations of the mass data as described below.

Optimization of Multivariate Analysis Parameters. On the basis of consideration of Tables 1 and 3 and examination of numerous two-dimensional PCA plots using various combinations of masses, the intensities of 30 different mass peaks from each of the 40 samples were selected for PCA. The 30 masses used to construct the PCA plots in Figures 2 and 3 are listed in Table 5.

Two-dimensional PCA plots of mass intensities for control and abused samples provided tighter groupings of similar types of samples when data transformations were performed prior to PCA. Multiplying the mass intensity values for masses 94, 127, and 142 by 10 improved PCA discrimination. The intensities of masses 94, 127, and 142 were higher in all light-abused samples. The advantages of performing this type of data transformation have been previously discussed (Marsili and Miller, 1998).

Employing an internal standard (4-methyl-2-pentanone) calibration technique also significantly improved PCA discrimination. 4-Methyl-2-pentanone has been used previously as an internal standard for the DH−GC/MS analysis of volatiles in milk (Marsili and Miller, 1998).

No significant *m*/*z* 100 peak was observed in the mass spectra of control or abused milk samples that did not contain added internal standard. Therefore, 4-methyl-2-pentanone internal standard was added in known concentrations to each sample, and the samples were reanalyzed. The intensity values of all mass peaks for a sample were then divided by the intensity of the *m*/*z* 100 peak for the sample. This approach to internal standard normalization significantly improved groupings of similar sample types in PCA plots.

All samples were analyzed with and without added internal standard to determine if PCA discrimination was improved by internal standard normalization and to be sure no unsuspecting volatile appeared that contributed a mass intensity at *m*/*z* 100.

PCA of Milk Samples. Figures 2 and 3 show PCA plots for all 40 milk samples: 10 controls (C), 3 UHT controls (U), 7 light-abused samples (L), 8 samples abused by copper (M), and 12 UHT samples spoiled by three different species of *Pseudomonas* microorganisms (BA, BF, BP, WBA, WBF, and WBP). "A," "F," and "P" refer to the species of *Pseudomonas* (*aureofaciens*, *fluorescens*, and *putrefaciens*, respectively), and "W" refers to whole-fat UHT milk samples. The data transformation described above for the *m*/*z* 94, 127, and 142 peaks was performed prior to PCA. Whereas mass intensity data were collected from *m*/*z* 50 to 150 for each sample, only the 30 masses in Table 5 were used in preparing the PCA plots in Figures 2 and 3.

Figure 2 is a PCA plot of the data normalized to the *m*/*z* 100 peak contributed by the internal standard. Figure 3 is a magnified view of the region in Figure 2 where the C, M, and L samples appear in a tightly clustered unresolved grouping.

Figure 2 shows significant separation of samples with microbial off-flavors from control, copper-abused, light-abused, and UHT samples. All microbially abused samples had a similar, strong cheese-like aroma, whereas

Table 3. Some Volatile Compounds Reported in Abused Milk with Off-Flavors

compound	possible abuse agent	reference
3-methylbutanal	microbiological contamination, Cu oxidation	Azzara and Campbell, 1992; Marsili and Miller, 1998
pentanal	light abuse, Cu oxidation	Cadwallader and Howard, 1998; Marsili and Miller, 1998
hexanal	light abuse, Cu oxidation	Cadwallader and Howard, 1998; Marsili and Miller, 1998
heptanal	light abuse, Cu oxidation	Forss et al., 1955a, 1955b; Marsili and Miller, 1998
octanal	Cu oxidation	Forss et al., 1955a, 1955b; Marsili and Miller, 1998
nonanal	Cu oxidation	Forss et al., 1955a, 1955b; Marsili and Miller, 1998
2-octenal	Cu oxidation	Forss et al., 1955a, 1955b; Marsili and Miller, 1998
2-nonenal	Cu oxidation	Forss et al., 1955a, 1955b; Marsili and Miller, 1998
2-pentanone	high heat, microbiological contamination	Hawke, 1966
2-heptanone	high heat, microbiological contamination	Hawke, 1966
2-nonanone	high heat, microbiological contamination	Hawke, 1966; Milton et al., 1979
2-undecanone	high heat, microbiological contamination	Hawke, 1966
1-octen-3-one	Cu oxidation, light abuse	Cadwallader and Howard, 1998; Marsili and Miller, 1998
acetic acid	microbiological contamination	Azzara and Campbell, 1992
butanoic acid	microbiological contamination; lipases	Azzara and Campbell, 1992
hexanoic acid	microbiological contamination; lipases	Azzara and Campbell, 1992
octanoic acid	microbiological contamination; lipases	Azzara and Campbell, 1992
3-methylbutanol	microbiological contamination	Hawke, 1966
2-pentanol	microbiological contamination	Hawke, 1966
dimethyl sulfide	microbiological contamination	Milton et al., 1979
dimethyl disulfide	microbiological contamination, light abuse	Jung et al., 1998; Milton et al., 1979

Table 4. Mass Fragmentation Patterns of Some Chemicals Produced in Milk Abused by Light, Copper, Heat, and/or Bacteria

metabolite	major fragments, mass (%)
3-methylbutanal	40 (10), 41 (100), 42 (21), 43 (63), 44 (58), 45 (14), 57 (23), 58 (51), 69 (40), 85 (5), 86 (13)
pentanal	40 (19), 41 (79), 42 (19), 43 (53), 44 (100), 45 (16), 57 (34), 58 (47), 69 (10), 86 (6)
hexanal	40 (20), 41 (100), 42 (19), 43 (66), 44 (65), 45 (15), 55 (25), 56 (56), 57 (43), 67 (15), 71 (10), 72 (15), 82 (11), 83 (9)
heptanal	40 (10), 41 (100), 42 (48), 43 (77), 44 (58), 45 (16), 53 (8), 54 (9), 55 (68), 57 (38), 67 (14), 68 (17), 69 (12), 70 (56), 71 (17), 81 (32), 86 (10), 96 (7), 97 (34)
octanal	40 (11), 41 (100), 42 (30), 43 (67), 44 (43), 45 (13), 53 (8), 54 (7), 55 (44), 56 (58), 57 (48), 67 (31), 68 (20), 69 (43), 72 (7), 81 (21), 82 (19), 84 (19), 95 (8)
nonanal	40 (11), 41 (100), 42 (22), 43 (50), 44 (31), 45 (10), 50 (5), 51 (9), 53 (8), 54 (11), 55 (44), 56 (42), 57 (66), 67 (32), 68 (22), 69 (30), 70 (34), 71 (11), 77 (12), 81 (29), 82 (21), 83 (13), 95 (16), 96 (14), 98 (9), 105 (11)
2-octenal	41 (100), 55 (85), 70 (75), 83 (50), 97 (10), 98 (8)
2-nonenal	40 (49), 41 (100), 42 (30), 43 (66), 44 (28), 53 (15), 54 (14), 55 (64), 57 (30), 69 (31), 70 (30), 81 (23), 83 (27), 96 (8)
2-pentanone	40 (13), 41 (54), 42 (25), 43 (83), 44 (100), 58 (20), 71 (20), 86 (35), 87 (25)
2-heptanone	41 (26), 42 (15), 43 (100), 50 (3), 51 (3), 53 (6), 55 (7), 58 (55), 67 (13), 71 (14), 72 (7), 85 (4), 91 (7), 93 (7), 94 (4), 99 (4), 115 (9), 121 (2), 136 (2)
2-nonanone	40 (25), 41 (44), 42 (14), 43 (100), 44 (13), 45 (8), 55 (14), 57 (25), 58 (61), 59 (17), 71 (16), 127 (3), 142 (5)
2-undecanone	40 (32), 41 (55), 42 (17), 43 (100), 44 (29), 45 (21), 55 (22), 57 (28), 58 (50), 71 (22), 85 (10), 95 (5), 97 (4), 170 (11)
1-octen-3-one	41 (34), 42 (24), 43 (28), 52(21), 53 (41), 55 (100), 56 (31), 57 (49), 70 (52), 83 (11), 97 (24), 126 (19)
acetic acid	42 (11), 43 (100), 45 (16), 60 (40)
butanoic acid	40 (18), 41 (61), 42 (57), 43 (43), 44 (13), 45 (39), 55 (26), 60 (100), 71 (15), 73 (41), 89 (15)
hexanoic acid	40 (26), 41 (88), 42 (44), 43 (65), 44 (43), 45 (47), 50 (4), 51 (5), 53 (7), 55 (39), 56 (21), 57 (27), 60 (100), 61 (13), 69 (12), 71 (11), 73 (51), 77 (4), 87 (16), 99 (7), 105 (6), 117 (5)
octanoic acid	40 (49), 41 (100), 42 (33), 43 (99), 44 (86), 45 (43), 55 (55), 56 (23), 57 (45), 60 (55), 69 (23), 71 (20), 73 (43), 85 (14), 101 (12), 105 (9)
3-methylbutanol	41 (100), 42 (65), 43 (54), 45 (16), 53 (7), 55 (86), 56 (21), 57 (25), 69 (11), 70 (52), 71 (30)
2-pentanol	41 (11), 43 (24), 44 (10), 45 (100), 55 (26), 71 (30), 73 (7)
furfuryl alcohol	41 (100), 42 (73), 51 (18), 53 (61), 55 (18), 69 (47), 81 (92), 97 (39), 98 (84)
dimethyl sulfide	41 (13), 43 (8), 44 (45), 45 (84), 46 (44), 47 (92), 61 (35), 62 (100), 63 (58)
dimethyl disulfide	41 (12), 43 (38), 45 (100), 46 (38), 47 (27), 48 (10), 58 (9), 61 (17), 64 (13), 79 (44), 93 (7), 94 (88)

Table 5. Mass Fragments Used for PCA

53	68	84	98
55	69	85	101
56	70	86	114
57	71	87	115
60	73	89	127
62	79	94	142
66	81	95	
67	82	96	

all other abused samples had no detectable aroma; however, they did possess oxidized off-flavors. Therefore, it is reasonable that the malodorous microbially abused samples would be well separated from the control and nonmicrobially abused samples and that the odorless nonmicrobially abused samples would tend to cluster together. Figure 3 shows that control, copper-abused, and light-abused samples can be resolved—even though they did not possess a detectable odor.

CONCLUSION

The technique using SPME−MS−MVA appears to be a viable alternative to current commercial e-nose instruments. With multiple SPME setups, it could be possible to analyze a sample every 3 min using the same GC/MS system. As discussed, SPME offers significant advantages over other techniques currently used to introduce sample volatiles to the chemical sensor device of the e-nose. Also, SPME is easy to use and maintain and does not require expensive ancillary equipment. In addition, automated SPME instrumentation is commercially available.

Using a mass spectrometer as a chemical sensor is advantageous because it is sensitive and robust, does not suffer from memory effects, and is not poisoned by low levels of moisture injected from SPME extractions. Furthermore, unlike the relatively new chemical sensor technologies currently used in commercial e-nose in-

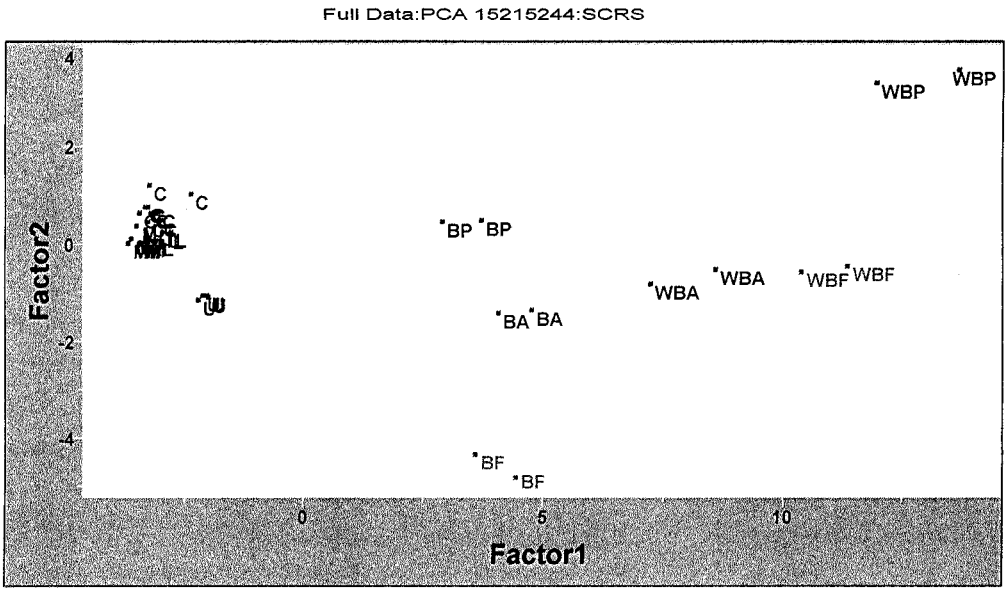

Figure 2. PCA plot of mass intensity data of 30 masses for 40 samples. Mass intensities were normalized by dividing by the intensity of the *m*/*z* 100 peak from the internal standard.

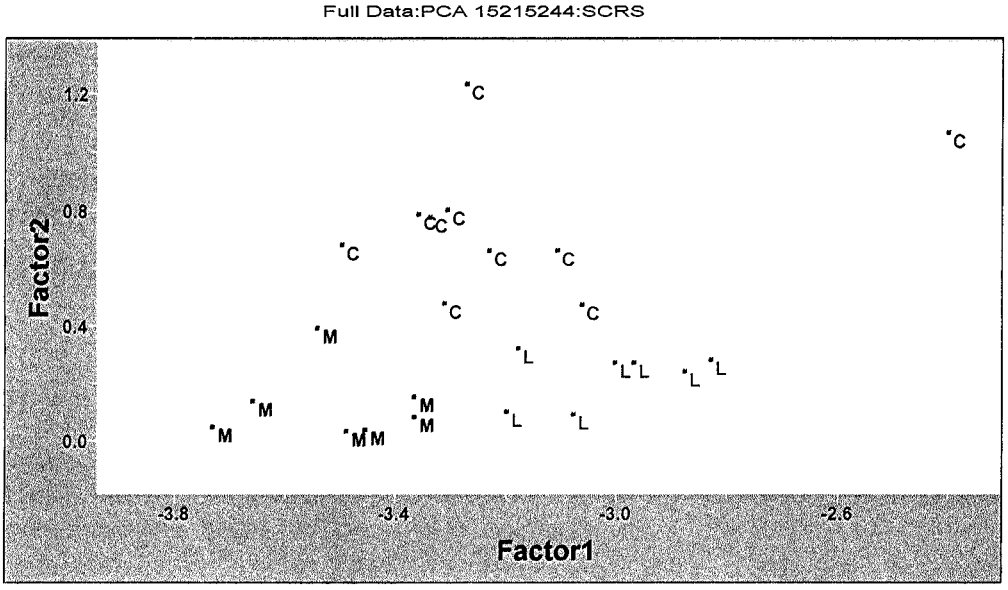

Figure 3. Magnified view of Figure 2, showing region of PCA plot of control, light-abused, and copper-abused samples.

struments, reliable easy-to-use benchtop MS detectors have been in routine use for decades and have a proven track record.

Even though MS detectors are more stable than current chemical sensors used in commercial e-nose instruments, they do degrade over time. Our laboratory has analyzed milk samples by SPME−MS−MVA for over one year and has not needed to recalibrate the method. However, future studies will include a second internal standard—one with higher mass ions (because degraded MS performance would most likely show up by a decrease of higher mass ions). Inclusion of a second internal standard may improve the long-term performance of the technique and reduce the frequency of recalibrations.

SPME−MS−MVA can be easily and quickly converted to SPME−GC/MS (perhaps equipped with an olfactory detector) simply by replacing the 1-m fused-silica transfer line with an appropriate coated capillary GC column. Researchers can then perform more detailed traditional analyses, including identification and quantification of specific odor active GC peaks. This approach can be extremely helpful in determining what masses to monitor (as well as what masses to exclude) for specific e-nose applications. Another advantage of using SPME as a way of introducing volatiles to the e-nose detector is that different fibers can be selected for different applications. For example, a PDMS fiber works better for extracting high molecular weight volatiles/semivolatiles such as terpenes and sesquiterpines than does the Carboxen−PDMS fiber. The ability to "tune" the SPME−MS−MVA method to such an extent is not possible with current e-nose instruments.

Studies in our laboratory are in progress to develop and validate classification models that would allow determination of the type of abuse suffered by milk samples that drew customer complaints. The MVA techniques of KNN and SIMCA modeling will be used for this purpose (Marsili and Miller, 1998). Identification of the cause of specific malodors and off-flavors could

be helpful in suggesting corrective actions to implement to prevent or reduce further off-flavor incidents.

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