Shelf-Life Prediction of Processed Milk by Solid-Phase Microextraction, Mass Spectrometry, and Multivariate Analysis

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A technique based on solid-phase microextraction, mass spectrometry, and multivariate analysis (SPME−MS−MVA) was used to predict the shelf life of pasteurized and homogenized reduced-fat milk and whole-fat chocolate milk sampled over a 7 month period. Using SPME−MS−MVA, which is essentially a mass spectrometry-based electronic-nose instrument, volatile bacterial metabolites were extracted from milk with SPME (Carboxen-PDMS) and injected into a GC capillary column at elevated temperature. Mass fragmentation profiles from the unresolved milk volatile components were normalized to the intensity of a chlorobenzene internal standard mass peak (*m*/*z* 112) and subjected to MVA. Prediction models based on partial least-squares regression of mass intensity lists were able to predict the shelf life of samples to approximately ±1 day, with correlation coefficients greater than 0.98 for the two types of milk samples. Using principal component analysis techniques, the procedure was also useful for classifying samples that were rendered unpalatable by nonmicrobial sources (contamination by copper and sanitizer) as well as by bacteria.

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

INTRODUCTION

Traditionally, the "shelf life" of milk—the period between processing and the time when milk becomes unacceptable to consumers because of taste or odor—has been determined by bacterial counts and sensory evaluation. The shelf life of pasteurized processed milk is generally 14−18 days, but can be much less if the milk supply is contaminated with chemicals or microbes (e.g., psychrotrophic bacteria). If pasteurized milk with an abnormally short shelf life could be detected prior to leaving the processing plant, there would be less chance that processed milk with off-flavors would reach consumers' tables.

Standard microbiological methods, including the Moseley keeping-quality test, have proven to be of little value as predictors of shelf life (Bishop and White, 1986). One problem with the Moseley keeping-quality test is the extended time period required to perform testing. Another problem is the relatively poor correlation between microbial counts and actual shelf life (Urbach and Milne, 1988; Vallejo-Cordoba and Nakai, 1994).

As a result of these problems, dairy chemists and microbiologists are increasingly investigating techniques that measure chemical changes produced by bacteria rather than measuring total numbers of bacteria. The most popular of these techniques is electrical impedance (Bishop and White, 1986). A popular rapid technique for assessing the number of bacteria present in milk is adenosine triphosphate (ATP) measurements using firefly luciferase and cofactors to produce light (Bossuyt, 1982; Van Crombrugge et al., 1989). However, these tests correlate better with total bacterial counts than they do with actual product shelf life because they do not necessarily measure the direct cause of off-flavor formation (e.g., malodorous bacterial metabolites) and the end of shelf life.

One promising nonmicrobiological technique that has been shown to be a better predictor of shelf life than microbiological plate count testing (e.g., psychrotrophic bacterial counts or PCBs) is the determination of volatiles in milk by dynamic headspace capillary gas chromatography (DH-GC) followed by multivariate interpretation (principal component regression analysis) of gas chromatographic peak area data (Vallejo-Cordoba and Nakai, 1994). DH-GC/MS with multivariate analysis has also been successfully used to classify abused milk samples as to the cause of off-flavors—i.e., light-induced oxidation, copper-induced oxidation, and microbial spoilage (Marsili and Miller, 1998).

However, there are significant problems with the DH-GC method. It is a tedious procedure, and potential errors associated with tracking the large number of gas chromatographic peaks generated by volatiles in the milk are possible—even when automatic peak recognition software is applied. Furthermore, operation of DH-GC instrumentation and peak tracking, identification, and area quantitation require a skilled chromatographer. In addition, the time required to generate a chromatogram with acceptable peak resolution is approximately 1 h. Thus, only 8−10 samples can be analyzed per day. As a result of these drawbacks, the technique is not amenable to more routine quality control applications.

Therefore, there is a need for a simpler and more rapid test that would provide a direct measurement of milk flavor quality, thus resulting in reliable estimates of milk shelf life. It is important that the test be simple enough to be performed at the dairy plant by relatively untrained QC personnel and that it provide results quick enough that effective corrective measures can be taken when problem samples—i.e., samples with a

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significantly shortened shelf life—are detected. In addition, it would be advantageous to detect potential nonmicrobial spoilage problems and to determine the nature of the nonmicrobial off-flavors (e.g., lipid oxidation off-flavor development, off-flavors originating from sanitizer contamination, etc.).

The objective of this research was to develop an analytical system for shelf-life prediction of pasteurized milk that meets these requirements for an ideal method. Like the Vallejo-Cordoba and Nakai procedure, prediction of shelf life is based on measurement of volatiles and semivolatiles present in milk after a preincubation period. However, the analytical system presented in this work is much simpler to implement. In essence, the analytical system applied here is an electronic nose, with solid-phase microextraction (SPME) used to extract/concentrate volatiles from the milk, MS used as a chemical sensor, and multivariate analysis (MVA) used as the tool to decipher meaningful trends in the mass spectroscopy output. The technique is referred to as SPME−MS−MVA and has been previously reported (Marsili, 1999b). In SPME−MS−MVA, a mass intensity list representing all the volatiles in the milk sample is the basis for shelf-life prediction—not GC peak area data as in the case of DH-GC.

MATERIALS AND METHODS

Sampling. All samples were commercially pasteurized and homogenized reduced-fat milk (2% milkfat) or chocolate milk (3.5% milkfat) free of off-flavors at the time of manufacture. Samples were packaged in either pint or half-pint high-density polyethylene (HDPE) contoured bottles with screw caps. Thirty samples of reduced-fat milk and thirty samples of chocolate milk were sampled consecutively from the production line at a dairy plant the day of processing. This sampling scheme was conducted on six occasions over a 7 month period.

Samples were immediately taken from the dairy plant and refrigerated in a walk-in cooler at 7.2 ± 0.5 °C until the end of shelf life. During refrigerated storage, two bottles of reduced-fat milk and two bottles of chocolate milk were removed for testing at predetermined intervals—three times weekly in the initial stage of refrigerated storage and then daily when a decline in flavor quality was observed. One sample from each pair was subjected to organoleptic evaluation, and one sample was placed in a 19 ± 1 °C incubator for 16 h. After 16 h, the preincubated sample was subjected to SPME−MS−MVA.

Sensory Evaluation. Four judges experienced in tasting dairy products were used for sensory evaluation of milk samples. The method used for sensory scoring was based on a 10-point scale according to the scoring guide of the American Dairy Science Association. Shelf life was ended when a score of 5 or lower was recorded by three of the four judges, and the day before was considered the end of shelf life.

SPME Analysis. A Saturn 3 gas chromatograph/mass spectrometer was used (Varian Analytical Systems, San Fernando, CA). The gas chromatograph was equipped with a split/splitless model 1078 injector. The injector was operated in the split mode (6:1 split ratio) at a temperature of 275 °C. The SPME fiber used was 75 μm Carboxen/PDMS (Supelco, Bellefonte, PA). For thermal desorption, the SPME fiber remained in the injector for 3 min. Helium was used as the carrier gas. A 30 m × 0.25 mm i.d. DB-5 fused-silica capillary column with a film thickness of 1 μm (J&W Scientific, Folsom, CA) was used, and the flow rate of the helium carrier gas was 1.0 mL/min. The following column temperature programming sequence was used: An initial temperature of 150 °C was maintained for 4 min, increased to 180 °C at a rate of 15 °C/min, and held at 180 °C for an additional 2 min. All milk volatile peaks eluted within 7 min, with many components coeluting. The objective was to transfer extracted volatiles from the SPME fiber to the mass spectrometer in a relatively short time period, rather than waiting approximately 1 h for a high-resolution chromatographic run. With this approach, more samples per hour can be tested. If a sample with unacceptable shelf life is discovered and more details about specific volatiles are desired, the sample can easily be retested to improve peak resolution by using a column temperature sequence that starts at a lower temperature (e.g., 50 °C) and uses more gradual temperature ramps. Since the same analytical column can be used for both approaches, no time is lost to column changeover and mass spectrometer shutdown.

Three milliliters of milk sample, 5 μL of internal standard solution (10 μg/mL chlorobenzene), and a micro stirring bar (Fisher Chemical Co., cat. no. 09-312-102) were placed in a 6 mL glass GC vial (38 mm high and 22 mm in diameter) and capped with 20 mm PTFE/silicone septa, 20 mm diameter (Wheaton Scientific Products, cat. no. 224173). The stock chlorobenzene solution that was used to prepare the 10 μg/mL working internal standard solution was purchased as a 5000 μg/mL in methanol standard solution from Supelco (cat. no. 4-0006). Clogging of the 10 μL syringe after internal standard addition to milk was common. This was prevented by rinsing the syringe with distilled water after each addition of internal standard to milk.

The setting on the SPME holder assembly scale was adjusted to 0.8 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 50 °C water bath (fiber exposure started immediately with the sample at 19 °C), and the sample was stirred at 350 rpm. An excessive stirring speed—i.e., greater than 1000 rpm—sometimes caused milk to splash onto the fiber. When fibers contaminated with small amounts of milk were injected, unusual peaks from the thermal decomposition of lactose were observed. Examples included 2-furanmethanol, 5-methyl-2(3*H*)-furanone, 5-hydroxymethyl-2-furancarboxaldehyde, and 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one.

After a 20 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 4.0 scale units prior to injection into the gas chromatograph injector port, which was fitted with a special insert for SPME analysis (Varian, cat. no. 03-925330-00). Multiple SPME setups initiated at 10 min intervals significantly increased the number of samples that could be run per hour.

MS Analysis. The Varian Saturn mass spectrometer 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 180 °C.

A mass intensity list was obtained for each sample by averaging the masses between 100 and 500 s. The mass intensities were then normalized by dividing by the intensity of the major mass peak for the chlorobenzene internal standard (*m*/*z* 112). For PLS calculations, these normalized mass ratios from *m*/*z* 50 to *m*/*z* 150 were used as independent variables, and the shelf life determined by sensory analysis was used as the dependent variable. Internal standard normalization of mass intensity data and generation of a normalized mass intensity file in a ".csv" file format suitable for conversion to a Pirouette spreadsheet format were accomplished automatically by a program ("Listfile") written in the Varian Saturn system's application-specific programming language called Procedure Language. From within the Procedure Language, the user may program specific actions to accomplish tasks that would otherwise have to be performed manually.

MVA. The software used for MVA was Pirouette from Infometrix, Inc. (Woodinville, WA). Prediction of shelf life was based on the partial least-squares (PLS) method. Correlation between the predicted shelf life and actual shelf life of samples was optimum when the following PLS parameters were used: exclusion of masses 59, 73, 77, and 150; autoscale data preprocessing; log 10 and SNV data transformation; and 17 model factors. (Note: An explanation of these parameters can

Table 1. Metabolite Changes Observed in Reduced-Fat Milk during Shelf Life

reduced-fat milk series	metabolite changes observed from the beginning to the end of shelf life[a]
1	⇑: dimethyl sulfide; ↑: C4 through C12 fatty acids; ↓: 2-butanone
2	⇑: 2-heptanone, pentanal, ethyl acetate; ↑: butyric acid, dimethyl sulfide; ↓: 2-butanone
3	⇑: 2-heptanone, pyrrolidine[b], furfuraldehyde; ↑: dimethyl sulfide, pentanal, hexanal; ↓: 2-butanone
4	⇑: dimethyl sulfide, unknown1; ↑: butyric acid; ↓: pentanal, hexanal
5	⇑: ethyl acetate, pentanal, hexanoic acid; ↑: 2-heptanone, hexanal, butyric acid, isobutyric acid; ⇓: 2-butanone
6	⇑: unknown2, 2-methylbutanal, 3-hydroxy-2-butanone; ↑: dimethyl sulfide, methyl butyrate, methyl caproate, hexanal, 2-heptanone, C4−C10 fatty acids

[a] ↑ = slight increase in concentration. ⇑ = significant increase in concentration. ↓ = slight decrease in concentration. ⇓ = significant decrease in concentration. [b] tentative identification by mass spectrometry.

be found in the Pirouette manual.) Masses 73 and 77 were excluded because these are significant mass peaks that appear in extraneous background compounds—e.g., hexamethylcyclotrisiloxane, octamethylcyclotetrasiloxane, and decamethylcylcopentasiloxane (from GC septa and degradation of the GC column liquid phase) and fluorotrimethylsilane (a component of the SPME fiber).

SPME−MS−MVA shelf−life prediction models were developed for reduced-fat milk and chocolate milk samples of known shelf life. Mass intensity lists were determined for 84 samples of reduced-fat milk and 73 samples of chocolate milk. For the reduced-fat milk, 64 samples were used to develop a PLS calibration model, and 20 samples (a "model validation subset") were randomly selected from the set of 84 total samples to evaluate how well the PLS model for reduced-fat milk could predict shelf life. For chocolate milk, 53 samples were used to develop a PLS calibration model, and 20 samples (a model validation subset) were randomly selected from the 73 total samples to evaluate how well the PLS model for chocolate milk could predict shelf life.

In another set of experiments, two-dimensional principal component analysis (PCA) scores plots were used to determine whether samples of microbially spoiled milk and nonmicrobially abused milk could be grouped into different classes as a means of identifying the cause of off-flavor formation in samples with reduced shelf life. Prior to PCA being conducted, the data were preprocessed using the autoscale mode and transformed using the normalize mode. The ability of SPME−MS−MVA to differentiate milk samples by the type of abuse was determined by visual examination of class clustering shown in two-dimensional PCA scores plots.

RESULTS AND DISCUSSION

Evaluation of SPME Fibers. Preliminary experiments were conducted to judge which types of SPME fibers were good candidates for extracting volatile metabolites from milk. Several samples of fresh reduced-fat milk and reduced-fat milk past shelf life were tested with various types of SPME fibers using conventional GC/MS techniques and a DB-5 column. For most volatile metabolites, results showed that the 75 μm Carboxen/PDMS fiber was able to extract more different types of volatiles and/or higher levels of volatiles than other fibers. Other fibers evaluated were 100 μm PMDS, 70 μm Carbowax/DVB StableFlex, and 50/30 μm DVB/Carboxen/PDMS StableFlex. One exception to this was that the 70 μm Carbowax/DVB StableFlex extracted greater quantities of volatile acids than the 75 μm Carboxen/PDMS fiber. However, the Carbowax/DVB StableFlex fiber did a poor job extracting most other metabolites, and the 75 μm Carboxen/PDMS fiber was still found to extract these acids when they were present at exceptionally low levels. In fact, a major advantage of using SPME with Carboxen/PDMS fibers is the higher sensitivity of this extraction technique compared to dynamic headspace and static headspace techniques for free fatty acids (Marsili, 1999a). Optimization of the SPME method using 75 μm Carboxen/PDMS fibers for milk volatiles has been previously reported (Marsili, 1999a,b).

Reasons for Poor Correlation between Shelf Life (Off-Flavors) and Bacteria Counts. Numerous attempts at predicting shelf life based on the number of microorganisms present in pasteurized milk have shown mediocre correlation (Bishop, 1989). It is well-known that some milks with a standard plate count of 10^6 cfu/mL are perfectly palatable whereas others have become unpalatable (Urbach and Milne, 1988). Very low correlations were produced by the linear and quadratic relationships of PBCs to the shelf life of pasteurized milk in experiments by Vallejo-Cordoba and Nakai (1994). Regression statistics for the best model based on Vallejo-Cordoba and Nakai PBC data had a multiple R of 0.57, which is comparable to previously reported values (Phillips and Griffiths, 1985). Rapid tests for estimating levels of total bacteria are a valuable tool for estimating the overall sanitation integrity of a dairy processing plant but are not very accurate for estimating milk shelf life.

Poor correlation of PBCs to shelf life is likely due to two factors. First, spoilage is not always related to the number of organisms present. The type of bacteria present rather than the actual numbers determines the types and the extent of off-flavor development and consequently the end of shelf life (Bishop and White, 1986). Second, poor flavor and shelf life can also be attributed to the presence of microbial enzymes and metabolic products from organisms present before pasteurization (Patel and Blankenagel, 1972). In some cases, pasteurization can kill microorganisms, but not inactivate microbial enzymes.

Furthermore, volatiles produced as bacterial metabolites or from active microbial enzymes are not the only causes of off-flavors in milk and shortened shelf life. For example, chemical contaminants can generate significant off-flavor formation. Contamination of milk with relatively low levels of copper, for example, can result in formation of oxidation off-flavors during shelf life. Another nonmicrobiological cause of off-flavors that sometimes occurs in milk samples that first come off the processing line is contamination by sanitizer from processing lines that have not been properly flushed. For example, one popular sanitizer, Matrixx (Ecolab, Inc., St. Paul, MN), which has hydrogen peroxide (6.9%), peroxyacetic acid (4.4%), and octanoic acid (3.4%) as active ingredients, has been observed to cause off-flavors in samples of pasteurized, processed milk. As shown below, testing by SPME−MS−MVA is able to detect and identify these nonmicrobiological sources of off-flavors and decreased shelf life.

Partial Least−Squares Analysis to Predict Shelf Life. Each sample tested by SPME−MS−MVA was also

Table 2. Actual[a] Shelf Life and Predicted[b] Shelf Life of Homogenized, Pasteurized Reduced-Fat Milk and Whole-Fat Chocolate Milk (Shelf Life in Days)

reduced-fat milk			whole-fat chocolate milk		
actual	predicted	error[c]	actual	predicted	error[c]
15	15.3	0.3	12	10.3	−1.7
18	18.2	0.2	7	5.6	−1.4
14	14.7	0.7	17	16.9	−0.1
8	7.4	−0.6	13	10.9	−2.1
5	5.4	0.4	11	9.4	−1.6
1	1.8	0.8	7	8.1	1.1
14	13.6	−0.4	0	−0.6	−0.6
11	10.3	−0.7	13	13.6	0.6
10	10.2	0.2	12	12.7	0.7
7	6.4	−0.6	6	5.4	−0.6
4	5.0	1.0	1	1.8	0.8
3	4.2	1.2	0	2.0	2.0
2	2.3	0.3	18	17.1	−0.9
0	0.7	0.7	14	14.2	0.2
10	12.8	2.8	11	10.4	−0.6
7	7.3	0.3	7	7.1	0.1
3	3.5	0.5	5	3.2	−1.8
2	2.6	0.6	3	2.8	−0.2
5	4.9	−0.1	4	4.3	0.3
0	0.0	0.0	2	0.9	−1.1
	av error:[d] ± 0.62			av error:[d] ± 0.88	
	error range: −0.7 to ±2.8			error range: −2.1 to ±2.0	
	R^2: 0.9801			R^2: 0.9832	

[a] Determined by sensory a panel. [b] Predicted from SPME−MS−MVA data using PLS prediction models. [c] Error = predicted − actual. [d] Av error = $(\sum|\text{error}|)/n$, where $n = 20$.

analyzed by a more time-consuming SPME−GC/MS procedure (Marsili, 1999a) to be sure that the volatiles being measured were typical microbial metabolites. This approach showed that the off-flavors produced in samples over shelf life were, indeed, typical psychrotropic bacterial metabolites. A list of metabolites that were observed to increase over shelf life in reduced-fat samples appears in Table 1. It is important to note that different types of bacteria are likely causing spoilage in the six sets of samples tested over the 7-month period. This is indicated by the differences in metabolites produced, as well as the differences in malodors observed in end-of-shelf-life samples. Therefore, SPME−MS−MVA is capable of accurate shelf-life prediction even when different spoilage microorganisms are involved.

SPME−GC/MS chromatograms of the chocolate milk samples were significantly more complex than the chromatograms of the reduced-fat milk samples. The chocolate milk chromatograms showed that a greater variety and higher concentrations of metabolites formed over shelf life, with many peaks coeluting.

Table 2 compares actual shelf life (determined by sensory evaluation) to predicted shelf life for the 20 PLS model validation subset samples for reduced-fat milk and the 20 PLS model validation subset samples for chocolate milk. On average, the SPME−MS−MVA PLS model for reduced-fat milk predicted the shelf life with an accuracy of ±0.62 day, with a correlation coefficient of 0.9801 and a range of −0.7 to +2.8 days. On average, the SPME−MS−MVA PLS model for chocolate milk predicted the shelf life with an accuracy of ±0.88 day, with a correlation coefficient of 0.9832 and a range of −2.1 to +2.0 days.

Figure 1 shows a plot of predicted shelf life versus actual shelf life (based on sensory testing) for the 64 samples used to prepare the PLS model for reduced-fat milk, and Figure 2 shows the same type of plot for the 53 samples used to prepare the PLS model for chocolate milk. Table 3 shows PLS statistics for PLS models and PLS predictions of model validation subsets.

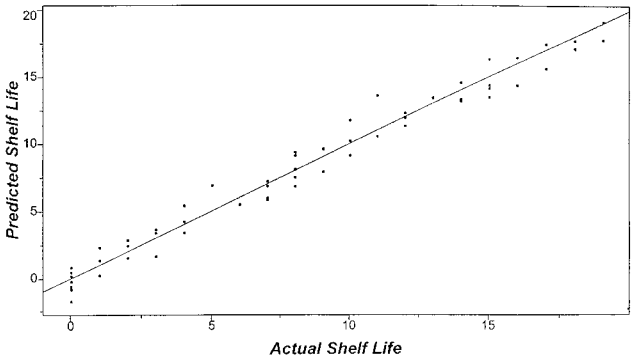

Figure 1. PLS plot of predicted shelf life (based on SPME−MS mass intensity data) versus actual shelf life (based on sensory testing) for the 64 samples used to prepare the PLS model for reduced-fat milk.

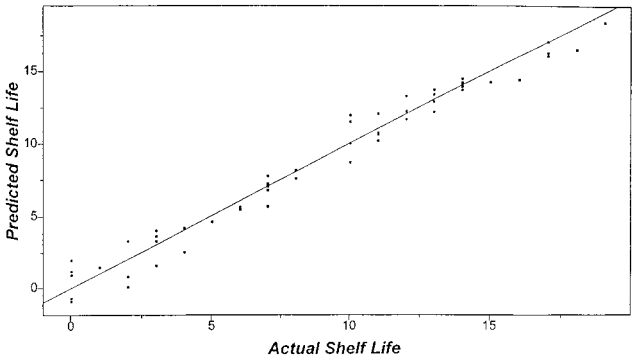

Figure 2. PLS plot of predicted shelf life (based on SPME−MS mass intensity data) versus actual shelf life (based on sensory testing) for the 53 samples used to prepare the PLS model for chocolate milk.

Principal Component Analysis to Determine Cause of Off-Flavor. Another experiment was designed to see what type of shelf-life prediction would be made for samples with off-flavors derived from nonmicrobiological causes and whether PCA plots could be applied as a tool to help identify causes of off-flavor. Using two-dimensional PCA plots of SPME−MS−MVA data to classify milk samples as to the cause of off-flavor has been previously reported (Marsili, 1999b).

Reduced-fat milk samples were spiked (aseptically with a 5 mL glass syringe through a pinhole in the top of the cap) with either copper (C) or Matrixx sanitizer (S), incubated at 19 °C for 16 h, and then analyzed by SPME−MS−MVA to generate normalized mass intensity data. Fresh reduced-fat milk (F) was used as a control, and bacteria-abused milks (B) were obtained by sampling unopened bottles with 0 days of shelf life. All samples were packaged in half-pint HDPE bottles and were from the same day's production. Also, like the copper and sanitizer samples, the control and microbially abused samples were incubated at 19 °C for 16 h before SPME−MS−MVA was conducted.

The C samples were spiked with copper sulfate at a concentration of 5 ppm copper in milk, and the S samples were spiked with Matrixx at a concentration of 1300 ppm in milk. These levels of abuse agents were selected because sensory testing determined that this was the threshold taste levels for these off-flavor-causing agents. Reduced-fat milk with 5 ppm copper was judged organoleptically to have a shelf life of 1 day. The milk spiked with 1300 ppm Matrixx was judged to

Table 3. PLS Error Analysis for the Calibration Model and Model Validation Subset for Reduced-Fat and Chocolate Milks (Shelf Life in Days as Dependent Variable)

	reduced-fat milk		chocolate milk	
	calibration model	model validation subset	calibration model	model validation subset
PRESS[a]	53.4204	25.5115	43.1876	20.6472
SEC[b] (days)	1.0441		0.9689	
SEP[c] (days)		1.1294		1.0161
R^2	0.9882	0.9801	0.9861	0.9832
no. of factors	15	7	7	16
slope	0.9766	0.9492	0.9724	0.9688
intercept (days)	0.2014	0.0750	0.2406	0.2033
n	64	20	53	20

[a] PRESS = prediction residual error sum of squares. [b] SEC = standard error of calibration = $(\text{PRESS}/n)^{1/2}$. [c] SEP = standard error of prediction = $[\text{PRESS}/(n - k)]^{1/2}$, where k = number of factors.

Table 4: Actual[a] and Predicted[b] Shelf Life of Fresh (F), Sanitizer-Contaminated (S), Copper-Contaminated (C), and Bacteria-Spoiled[c] (B) Pasteurized and Homogenized Reduced-Fat Milk

	shelf life in days		
sample	actual	predicted	error[d]
F1	20	19.7	−0.3
F2	20	20.1	0.1
F3	20	22.0	2.0
F4	20	18.6	−1.4
F5	20	18.2	−1.8
S1	0	10.6	10.6
S2	0	0.1	0.1
S3	0	6.4	6.4
S4	0	0.0	0.0
S5	0	6.3	6.3
C1	1	−2.4	−1.4
C2	1	8.9	7.9
C3	1	9.4	8.4
C4	1	11.3	10.3
C5	1	12.7	11.7
B1	0	0.2	0.2
B2	0	0.0	0.0
B3	0	0.6	0.6
B4	0	0.1	0.1
B5	0	0.5	0.5

[a] Determined by a sensory panel. [b] Predicted from SPME−MS−MVA data using the same PLS prediction model as was used for reduced-fat milk in Table 2. [c] Tested at the end of shelf life (after 20 days of incubation at 7.2 °C). [d] Error = predicted − actual.

have a shelf life of 0 days. The actual shelf life and predicted shelf life of these samples appear in Table 4, and a PCA two-dimensional plot of SPME−MS−MVA data appears in Figure 3.

This experiment showed that while SPME−MS−MVA was not able to accurately predict shelf life for copper- and sanitizer-abused samples, the technique does provide strong indication that there is a shelf-life problem for these samples. Furthermore, PCA can be used to distinguish copper- and sanitizer-abused samples from control samples and microbially abused samples. To improve prediction accuracy for copper- and sanitizer-abused samples, a number of these types of samples could be included in future PLS modeling experiments.

CONCLUSION

The technique using SPME−MS−MVA as an electronic-nose system appears to give more accurate predictions of milk shelf life than microbiological plating methods that have been previously reported in the literature and is significantly faster and easier to implement than the DH-GC approach reported by Vallejo-Cordoba and Nakai (1994). Furthermore, SPME−MS−MVA has been shown to be useful for identifying samples with nonmicrobiological induced off-flavors and for determining the cause of off-flavors even when nonmicrobiological agents are involved.

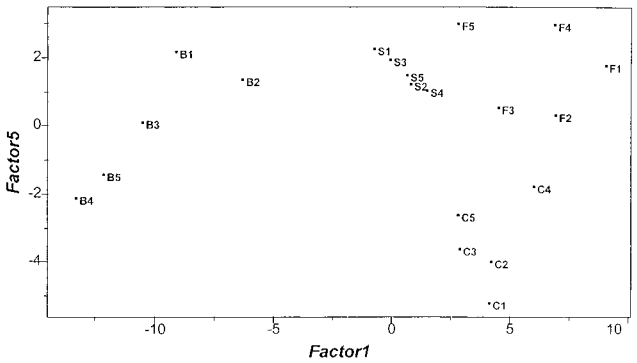

Figure 3. PCA scores plot for fresh (F), sanitizer-contaminated (S), copper-contaminated (C), and bacteria-spoiled (B) reduced-fat milk samples. F samples had a shelf life of 20 days, S samples had a shelf life of 0 days, and C samples had a shelf life of 1 day. B samples, which had a shelf life of 0 days, were tested at the end of shelf life (after 20 days of incubation at 7.2 °C). Shelf-life evaluation was done by a sensory panel. All samples were from the same day's production.

Over a 7-month period, SPME−MS−MVA has been shown to be an accurate technique for predicting the shelf life of reduced-fat milk and chocolate milk. Despite the fact that during the testing period significant changes occurred with the mass spectrometer (replacement of the turbomolecular pump and replacement of the electron multiplier) and the fact that several different Carboxen/PDMS fibers were used, internal standard normalization with chlorobenzene allowed accurate prediction over the 7-month period. Long-term sensor stability has been a problem with some commercial electronic-nose instruments in the past (Marsili, 1999b).

Using Carboxen/PDMS SPME fibers to extract volatiles offers impressive advantages over SH and DH sampling techniques. It does not require expensive ancillary instrumentation and is far more efficient than either SH or DH at extracting volatile fatty acids (VFAs) from milk. VFAs, important contributors to malodors and off-flavors in milk, 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. Other advantages of SPME over SH and DH have been reported in earlier work (Marsili, 1999a,b).

The analytical system reported in this work has strong potential applications in the dairy industry for shelf-life prediction. Results confirmed previous findings that techniques based on metabolic activity (production

of bacterial metabolites) correlate better with sensory quality and shelf life than bacterial counts. Although sensory analysis was required for developing PLS models, once the PLS models were developed, the objective evaluation of unknown samples was accomplished without the need for time-consuming sensory evaluation.

We are currently investigating automation of the SPME extraction step. With a SPME autoinjector and minor test modifications, it would be possible to analyze one sample every 5−7 min. The only labor required by the QC technician would be to pipet 3 mL of milk into a GC vial.

LITERATURE CITED

Bishop, J. R.; White, C. H.; Firstenberg-Eden, R. Rapid impedimetric method for determining the potential shelf life of pasteurized whole milk. *J. Food Prot.* **1984**, *47*, 471−475.

Bishop, J. R.; White, C. H. Assessment of dairy product quality and potential shelf life—a review. *J. Food Prot.* **1986**, *49*, 739−753.

Bishop, J. R. A simple shelf life estimation method as an integral part of a total dairy quality assurance program. *Dairy, Food Environ. Sanitation* **1989**, *12*, 698−701.

Bossuyt, R. A 5-minute ATP platform test for judging the bacterial quality of raw milk. *Neth. Milk Dairy J.* **1982**, *36*, 355−364.

Marsili, R. T.; Miller, N. Determination of the cause of off-flavors in milk by dynamic headspace GC/MS and multivariate data analysis. In *Food Flavor Formation, Analysis, and Packaging Influences*; Mussinan, C., Contis, E., Ho, C.-T., Parliament, T., Spanier, A., Shaidi, F., Eds.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1998; pp 159−171.

Marsili, R. T. Comparison of solid-phase microextraction and dynamic headspace methods for the gas chromatographic-mass spectrometric analysis of light-induced lipid oxidation products in milk. *J. Chromatogr. Sci.* **1999a**, *37*, 17−22.

Marsili, R. T. SPME-MS-MVA as an electronic nose for the study of off-flavors in milk. *J. Agric. Food Chem.* **1999b**, *47*, 648−654.

Patel, G. B.; Blankenagel, G. Bacterial counts of raw milk and flavour of the milk after pasteurization and storage. *J. Milk Food Technol.* **1972**, *35*, 203−206.

Phillips, J. D.; Griffiths, M. W. Bioluminescence and impedimetric methods for assessing shelf life of pasteurized milk and cream. *Food Microbiol.* **1985**, *2*, 39−51.

Urbach, G.; Milne, T. The concentration of volatiles in pasteurized milk as a function of storage time and storage temperature—a possible indicator of keeping quality. *Aust. J. Dairy Technol.* **1988**, *43*, 53−58.

Vallejo-Cordoba, B.; Nakai, S. Keeping-quality assessment of pasteurized milk by multivariate analysis of dynamic headspace gas chromatographic data. 1. Shelf life prediction by principal component regression. *J. Agric. Food Chem.* **1994**, *42*, 989−993.

Van Crombrugge, J.; Waes, G.; and Reybroeck W. ATP−F test for estimation of bacteriological quality of raw milk. *Milk Dairy J.* **1989**, *43*, 347−354.

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