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Profiling Aged Artisanal Cheddar Cheese Using Secondary Electrospray Ionization Mass Spectrometry

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Supporting Information

ABSTRACT: A number of direct injection mass spectrometry methods that can sample foods nondestructively and without sample preparation are being developed with applications ranging from the rapid assessment of food safety to the verification of protected designations of origin. In this pilot study, secondary electrospray ionization mass spectrometry (SESI-MS) in positive- and negative-ion modes was used to collect volatile fingerprints of artisanal Cheddar cheeses aged for one to three years. SESI-MS fingerprints were found to change in an aging-dependent manner and can be used to descriptively and predictively categorize Cheddars by their aging period, identify volatile components that increase or decrease with aging, and robustly discriminate individual batches of artisanal cheese. From these results, it was concluded that SESI-MS volatile fingerprinting could be used by artisanal food producers to characterize their products during production and aging, providing useful data to help them maximize the value of each batch.

KEYWORDS: *SESI-MS, volatile fingerprinting, Cheddar cheese, artisanal food production*

INTRODUCTION

Artisanal food production is a growing sector of the United States' economy, in part because small-scale farms are turning to value-added products (e.g., cheeses, diced tomatoes, flour, jam, cured meats) to maintain profitability.[1,2] Since 1980, the number of annual applications for artisanal cheese production has increased more than 10-fold, and the trends indicate that the rate of new applications will continue to rise.[1] While cheese commands a higher price-per-pound than the milk that is used to produce it,[1,3] aged cheeses have larger profit margins.[3,4] However, aging artisanal cheeses is a high-risk, high-reward endeavor for cheesemakers, because only a fraction of batches will develop the proper flavor and texture profile during aging, and the rest can develop undesirable qualities if allowed to age too long. Therefore, cheesemakers strive to predict how long each batch of cheese should be aged to optimize its flavor and maximize its market price.

Direct injection mass spectrometry methods are being developed to rapidly analyze foods—often nondestructively and with no sample preparation—to assess food safety, quality, and region of origin.[5−13] A few of these methods have been applied to the characterization of cheese. The detection of plant oil adulterants in soft cheeses is possible using direct analysis in real time ionization high resolution mass spectrometry (DART-HRMS),[14] and proton transfer reaction (PTR)-MS has been used for a variety of cheese-related studies,[15−19] including investigations into the relationship between milk storage conditions[20] and cheese aging[21] on the volatiles of Trentingrana cheese. Zenobi, Chen, and their colleagues have demonstrated that neutral desorption-extractive electrospray ionization (ND-EESI) can robustly sample volatile and nonvolatile molecules within complex matrices[22,23] and have generated mass spectral fingerprints useful in discriminating different Swiss cheeses made from cow's milk.[24] Focusing on the analysis of fatty acids in the Swiss cheeses, their data indicate that negative-ion mode ND-EESI fingerprints can be used to distinguish cheeses of the same type but produced by different cheesemakers. From their work, it was hypothesized that acquiring additional information on the volatile bases would enhance the power of this approach, enabling the identity of subtle differences in cheeses of the same type that were aged for different periods of time.

In this pilot study, secondary electrospray ionization mass spectrometry (SESI-MS),[25−27] which is akin to ND-EESI-MS, was applied in both positive- and negative-ion modes to the characterization of artisanal Cheddar cheeses produced by a single cheesemaker and aged for one to three years. Chemometrics were applied to the SESI-MS Cheddar fingerprints to identify mass spectral characteristics that are correlated with the cheese aging process. From these data, it was concluded that SESI-MS volatile fingerprinting could be useful for artisanal food producers in characterizing their products during production and aging, providing useful data that could help them to maximize the value of each batch.

MATERIALS AND METHODS

Cheese Production and Sampling. Cheddar cheese samples aged for one, two, and three years were obtained from Shelburne Farms in Shelburne, Vermont, on November 11, 2010, after the cheeses had met the flavor qualifications for their respective ages (Table 1). In total, eight 2-lb blocks of Cheddar cheese were analyzed,

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Table 1. Cheddar Cheese Production, Aging, And Flavor Profiles

aging period, flavor	batch	milk collection/cheese production date	cow herd feed
one year, sharp	1	14 Jul 2009	pasture, silage, grain
	2	17 Jul 2009	pasture, silage, grain
	3	03 Jun 2009	pasture, silage, grain
two years, extra sharp	1	18 Jul 2008	pasture, silage, grain
	2	14 Aug 2008	pasture, silage, grain
	3	15 Jul 2008	pasture, silage, grain
three years, extra extra sharp	1	24 Sep 2007	silage, grain
	2	23 Sep 2007	silage, grain

including blocks from three batches of one-year-aged, three batches of two-year-aged, and two batches of three-year-aged Cheddar. Table 1 provides the date each block was produced, which is also the date the aging process began for each batch of cheese, and the herd's feed at the time the milk was collected. The cheese blocks were stored in vacuum-sealed packaging shielded from light at 4 °C until testing. The cheese samples were prepared for analysis by cutting off ∼50 g of each 2-lb block and removing the exterior 1 cm of cheese. The remaining 30 g piece was cut into approximately 1 cm × 1 cm × 1 cm cubes (1 g each), which were stored at −20 °C until the day of analysis (3 days maximum). Five grams of Cheddar in a 100 mL sample bottle were used for each SESI-MS analysis. Two technical replicates of each block were tested per day over a period of three consecutive days for a total of six SESI-MS measurements for each block in positive-ion mode. Additional samples were prepared for negative-ion mode SESI-MS analysis following the same procedure. In total, 48 positive-ion and 48 negative-ion SESI-MS spectra were collected.

Secondary Electrospray Ionization-Mass Spectrometry (SESI-MS). *Tuning and Standardization.* SESI-MS fingerprints were collected as previously described on a modified SCIEX API 3000 triple quadrupole mass spectrometer (for a schematic of the SESI modifications and sampling apparatus, see ref 25). To control for potential fluctuations in ionization efficiency, we used mixtures of semivolatile compounds (Table 2) for tuning the SESI operation voltage to maximize the match score between replicate spectra of the standards mixtures at the beginning and periodically throughout sample testing. For instrument tuning only, the declustering potential (DP), focusing potential (FP), and entrance potential (EP) were set at 50, 375, and 10 V, respectively, in positive-ion mode, and −50, −300, and −10 V in negative-ion mode to induce in-source fragmentation of the standard molecules. Generating multiple fragment ions from the standards mixtures provided more coverage over the mass range that was tested and created overlapping groups of ions whose relative intensities were more dependent on the operation voltage and less dependent on the gas-phase concentration of any single compound to compensate for small changes in the relative concentrations of the three individual standards in each mixture. Table 2 provides the mass-to-charge ratios (m/z) for the molecular and fragment ions that were monitored for each component of the standards mixtures, which had a minimum of 10% relative intensity in positive-ion mode and 5% relative intensity in negative-ion mode. The intensities of these fragments, relative to each other and to background peaks (m/z = 129, 149, 185 in positive-ion mode, and m/z = 42, 61, 79, 123, 185 in negative-ion mode), were recorded to measure intraday and interday variability in the SESI-MS fingerprint. Interday replicates of the positive-ion mode standards spectra had an average match score of 964 (±20), which is statistically indistinguishable from the intraday variation (969 ± 24).

Cheddar SESI-MS Volatile Fingerprinting. Cheddar samples were allowed to warm to room temperature prior to analysis. The cheese volatiles were introduced into the mass spectrometer by displacing the sample bottle headspace with CO_2 (99.99%; 2 L/min) for 2 min. Formic acid (0.1% (v/v)) and methanol (5% (v/v)) in water were used as an electrospray solution in positive-ion mode, and ammonium hydroxide (0.03% (v/v)) and methanol (25% (v/v)) in water were used for negative-ion mode, which provided the optimal ionization efficiency and stability in our system. The electrospray solutions were delivered at a flow rate of 5 nL/s through a nonconductive silica capillary (40 μm ID) with a sharpened needle tip. The DP, FP, and EP were set at 5, 350, and 2 V, respectively, in positive-ion mode and −5, −300, and −2 V in negative-ion mode to minimize in-source fragmentation for volatile fingerprints of the Cheddar samples. The operation voltage of the electrospray was ∼2.2 kV in positive-ion mode, and approximately −2.2 kV in negative-ion mode. Spectra were collected over 2 min as an accumulation of 40 scans over 20−200 m/z in single quadrupole mode. Deionized water (30 mL) at room temperature was used as the blank. The system was flushed with CO_2 between samples to prevent carryover. Tandem mass spectrometry (MS/MS) fragmentation spectra were collected for a selection of positive-ion mode peaks as an accumulation of 10 scans using 50, 375, and 10 V for DP, FP, and EP, respectively, 25−40 eV collision energy, and N_2 collision gas.

Data Analysis. Analyst 1.4.2 software (Applied Biosystems) was used for data collection and raw data processing. SESI-MS spectra were blank-subtracted and normalized to the peak of highest intensity. Spectral matching was calculated using Spearman's rank correlation analyses for the Cheddar SESI-MS fingerprints, and with NIST MS search V 2.0 software (National Institute of Standards and Technology) for the standards mixtures and MS/MS fragmentation spectra. Presence/absence analyses on a year-by-year basis were performed with the criterion that a SESI-MS peak had to have a signal-to-noise (S/N) greater than 2 in at least 50% of all of the samples in an aging category (i.e., at least 9 replicates for 1- and 2-year-aged Cheddars, at least 6 replicates for 3-year-aged Cheddars) to be considered as present. Presence/absence analyses on a batch-to-batch basis was performed with the criterion that a SESI-MS peak had to have a S/N > 2 in at least 3 (50%) of the replicates for a batch.

JMP version 10, SAS version 10 (SAS Institute Inc., Cary, NC), and MATLAB version 8.2 (MathWorks Inc.) were used for statistical analyses in this study. Principal component analyses (PCA) were performed using all experimental replicates as observations, and the

Table 2. SESI-MS Tuning Mixtures

compound	CAS ID	conc. (v/v)	conc. (μM)	M_w (g/mol)	molecular and fragment ions (m/z)
Positive-Ion Mode Standards in Water					
pyrimidine	289-95-2	5×10^{-6}	63	80	81, 54
indole	120-72-9		17	117	118, 91
2-aminoacetophenone	551-93-9	1×10^{-5}	82	135	136, 118, 91
Negative-Ion Mode Standards in 1% (v/v) Methanol in Water					
hexanoic acid	142-62-1	1×10^{-4}	798	116	115
heptanoic acid	111-14-8	1×10^{-4}	705	130	129, 115
octanoic acid	124-07-2	1×10^{-4}	631	144	143, 129, 115

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absolute intensities of the SESI-MS peaks 20−200 m/z with S/N > 2 as variables. The data were mean centered, and the range was scaled prior to PCA analysis; then, the statistical significance of the separations in PCA were calculated on a pairwise basis using two-sided Student's t tests of the principal component scores. The loading scores in principal components 1−3 were analyzed for each SESI-MS peak, and peaks with at least one loading score with an absolute intensity ≥0.7 (|LS| ≥ 0.7) were considered significant drivers of the PCA separation. To identify SESI-MS fingerprint peaks that can be used to predict the age classification of Cheddar cheeses, we performed partial least-squares-discriminant analysis (PLS-DA) of the normalized SESI-MS volatile fingerprints using all of the data for model building. In addition, PLS-DA leave-one-out cross validation was performed for the eight batch groups using all 48 samples. The correct classification rate was calculated by dividing the number of correctly classified samples by the total number of samples.

Two-sided nonparametric Mann−Whitney U-tests were carried out to identify SESI-MS fingerprint peaks that are significantly different between pairs of aging categories using a 5% significance level cutoff. The U-test z-scores were used as criteria for categorizing peaks as increasing or decreasing on a year-to-year basis. Increasing peaks have at least one z-score that exceeds the 5% cutoff (≤−1.96) for any two-year comparison, and all other z-scores for that peak must also be negative (i.e., must be less than zero; Table S2 in the Supporting Information). Decreasing peaks have at least one z-score that exceeds the 5% cutoff (≥1.96) for any two-year comparison and all other z-scores for that peak must also be positive (i.e., must be >0).

RESULTS AND DISCUSSION

SESI-MS Fingerprinting of Aged Artisanal Cheddar. *SESI-MS Volatile Fingerprints are Descriptive of Cheddar Cheese Aging.* The SESI-MS volatile fingerprints (m/z = 20−200) of one-, two-, and three-year-aged artisanal Cheddar cheeses were collected in both positive- and negative-ion modes (Figure 1) and were comprised of cheese volatiles that are Brønsted−Lowry bases and acids, respectively. Although there are volatile molecules that can be protonated or deprotonated depending on the electrospray solution, and therefore would be represented in both the positive- and negative-ion mode spectra, the information in these two spectra were considered to be complementary and have been combined into a single volatile fingerprint for each Cheddar sample. By visual inspection, the volatile fingerprints of aged Cheddar appear quite similar year-to-year, as the majority of the dominant SESI-MS peaks are present in all three ages. To quantify the similarity of the volatile fingerprints, the average Spearman's rank correlation coefficient (ρ) was calculated for the total SESI-MS fingerprint (positive- and negative-ion mode spectra) of all of the Cheddar samples. The fingerprints are well correlated within each aging period (ρ > 0.70; Table S1 in the Supporting Information), demonstrating that the SESI-MS fingerprints for the Cheddar samples are similar and reproducible.

It was hypothesized that the SESI-MS fingerprint of each aged Cheddar is unique and indicative of its aging period. A qualitative analysis of the spectral fingerprints indicates that 39% of the total peaks are uniquely present or uniquely absent in one of the aging categories (Figure 2a; Table S2 in the Supporting Information), and the unique peaks are consistently found in every batch of cheese tested in each category (Figure 2b-d; Table S2 in the Supporting Information). On the basis of these observations, it was posited that principal component analysis (PCA) could be used to cluster the Cheddar samples by their SESI-MS fingerprints (i.e., combined positive- and negative-ion mode spectra; Figure 3). Indeed, using all of the

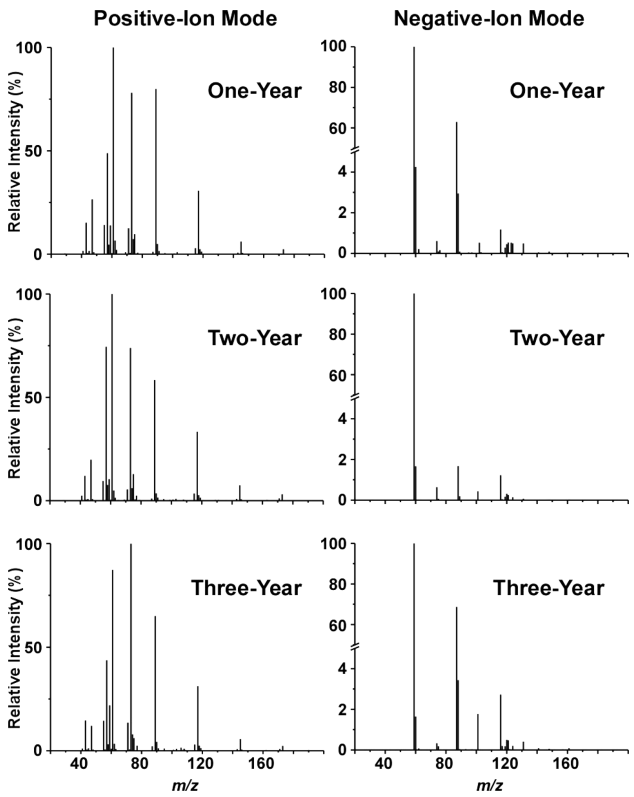

Figure 1. SESI-MS fingerprints from one-, two-, and three-year-aged Shelburne Farms Cheddar cheese in positive-ion mode (left column) and negative-ion mode (right column). The spectra are averages of 18 replicates for 1- and 2-year-aged Cheddar, and 12 replicates for 3-year-aged Cheddar, and normalized to the peak of greatest intensity in each spectrum.

SESI-MS peaks as variables and the 48 cheese samples as observations, it was found that all of the samples within an aging category clustered together and could be separated from the other aged Cheddars using the first three principal components (p < 0.0001; Table S3 in the Supporting Information). Partial least-squares discriminant analysis (PLS-DA) of the SESI-MS fingerprints was used to build a model for classifying the Cheddars by their aging periods and to identify any peaks that contributed significantly to the separation (i.e., VIP > 1.5; Table S2 in the Supporting Information). Surprisingly, it is not the peaks that are unique to an aging category that are responsible for the majority of the separation. Of the 35 peaks uniquely present or uniquely absent in the three aging groups, only 8 peaks (23%) have PCA loadings with an absolute value greater than 0.7, and 16 peaks (46%) have VIP scores greater than 1.5 (Table S2 in the Supporting Information). Looking at the data from the perspective of the 20 significant peaks in the PCA (loading absolute values ≥0.7) and the 27 peaks significant in the PLS-DA, 40% and 59% are uniquely present or uniquely absent in an aging category, respectively (Table S2 in the Supporting Information). Therefore, a large portion of the separation is generated by peaks that are shared by all of the aged Cheddars.

It was hypothesized that the shared SESI-MS peaks contribute to the statistical separation of the Cheddars due to nuances in peak intensity between the aging categories. The Mann−Whitney U-test was utilized on a peak-by-peak basis to identify SESI-MS peaks that are significantly different between each group (Figures S1 and S2 and Table S2 in the Supporting

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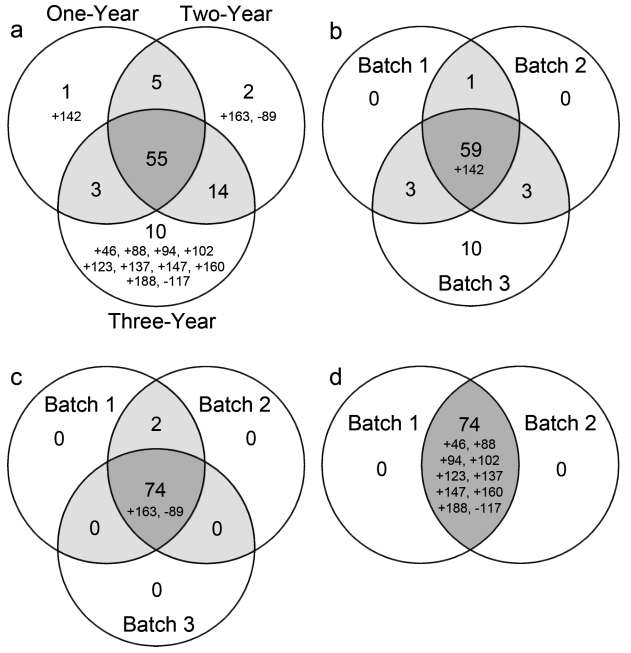

Figure 2. Number of SESI-MS peaks shared among artisanal Cheddar cheeses aged for 1−3 years. (a) Peaks shared by one-, two-, and three-year-aged Cheddars; (b) 1-year-aged Cheddar, batches 1, 2, and 3; (c) 2-year-aged Cheddar, batches 1, 2, and 3; and (d) 3-year-aged Cheddar, batches 1 and 2. Peaks were included if they were detected in at least half of the replicates for a given aging group. The mass-to-charge ratios (m/z) of peaks that are unique to a given age are listed.

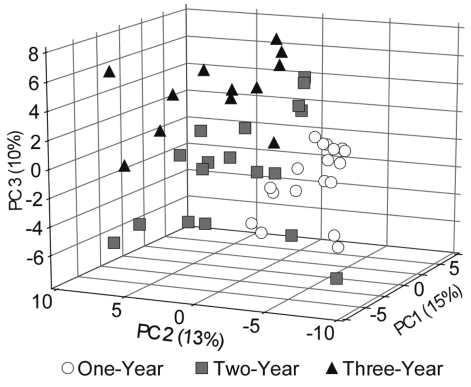

Figure 3. Principal component analysis of the SESI-MS volatile fingerprints of 1-, 2-, and 3-year-aged Cheddar cheeses. A total of 48 samples are included: 18 replicates of 1-, 18 replicates of 2-, and 12 replicates of 3-year-aged Cheddar.

Information). Of the 55 peaks in common with samples from all three Cheddar aging periods, 35 peaks (64%) have z-scores that exceed the 5% significance cutoff (≥1.96 or ≤−1.96) in at least one year-to-year comparison, eight of which also had significant loadings in the PCA and 11 of which have VIP > 1.5 in the PLS-DA (Table S2 in the Supporting Information). These data confirm that the presence and absence of SESI-MS peaks are not solely responsible for the characterization of aged Cheddars by their volatile profile. To utilize the differences in shared peaks, it is necessary to robustly measure the peak intensity (absolute or relative) in each sample day to day, and these data confirm that SESI-MS has this capability.

Peaks in the SESI-MS Volatile Fingerprint Correlate to Cheddar Cheese Aging. SESI-MS volatile fingerprints have utility beyond descriptively or predictively categorizing the Cheddar cheeses by aging period and can also be used to explore the impact of aging on the subtleties of the volatile profile. As Cheddars are aged, certain flavored components increase in intensity (e.g., sulfurous compounds) and others decrease (e.g., fresh milk-related flavor compounds);[28] therefore, it was hypothesized that a subset of SESI-MS peaks would trend over time. Of the 77 peaks in positive- or negative-ion modes that were different between any two ages of Cheddar (i.e., Mann−Whitney z-scores ≥1.96 or ≤−1.96; Figures S1 and S2 and Table S2 in the Supporting Information), we identified 26 peaks that were increased during the aging process and 11 peaks that were decreased (Table 3). For the majority of peaks, increasing intensity also correlated with increasing prevalence in the batches that were tested (or vice versa for decreasing peaks); that is, peaks that increase in intensity with age are also more likely to be measured in samples of three-year-aged Cheddars. One peak that increases with age, m/z = 95, may represent known aging-related flavor compounds in Cheddar (e.g., dimethyl disulfide (94 g/mol) and dimethyl sulfone (94 g/mol));[28] however, many of the aging-correlated SESI-MS peaks that were observed are likely not related to flavor. The flavorless compounds may still be valuable to cheesemakers, as they can be used as markers of the aging process.[29] All of the increasing and decreasing peaks that were observed contribute to the classification of Cheddars by aging period using PLS-DA (i.e., VIP > 0.8) and one-third of these peaks make very strong contributions to the classification (i.e., VIP > 1.5) and/or to the descriptive categorization of the Cheddars by PCA (i.e., |LS| ≥ 0.7; Table S2 in the Supporting Information).

There are an additional 40 peaks that have different intensities between two aging periods but do not demonstrate an increasing or decreasing trend (e.g., m/z = 101; Figure S1 and Table S2 in the Supporting Information). In some cases, the peak intensity variance is too great among these eight batches of Cheddar to confirm trends, and repeated longitudinal samples on several batches of Cheddar may be required to identify additional aging-related peaks. In other cases, it is postulated that several different compounds of the same nominal mass with unique aging-associated processes are being reported as a single peak in the SESI-MS fingerprints. MS/MS was performed on 29 positive-ion peaks (12% of the total peaks), and match scores between the fragmentation spectra indicate that the SESI-MS peaks represent the same compound(s) in >90% of the cases (Table S2 in the Supporting Information). However, the fragmentation spectra of the higher mass peaks do indicate mixtures of isobaric compounds (data not shown), and in two cases (m/z = 95 and 115), the relative abundances of the isobars appear to change year-to-year, which is reflected in the relatively low year-to-year match scores for m/z = 115 (Table S2 in the Supporting Information). Cheesemakers may find it beneficial to obtain additional information on isobaric compounds, as some will be flavored, and the nonflavored compounds may enhance or abrogate the sensory experience of the flavor volatiles of aged Cheddar.[30] Performing high resolution mass spectrometry (HRMS) or tandem mass spectrometry (MS/MS) will facilitate monitoring of additional aging-related compounds while preserving the nondestructive, rapid-analysis characteristics of SESI.[14,31]

Batches of Artisanal Cheddar Cheese Can Be Discriminated Using SESI-MS Fingerprints. The batches of aged Cheddars that were analyzed by SESI-MS are highly

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Table 3. SESI-MS Peaks That Increase or Decrease in Intensity (+) or Prevalence (Shading) During Cheddar Aging

Increasing Trends

m/z [a]	1-Year	2-Year	3-Year
70	+	++	+++++
74	+++++	++++	+++++
77	++	+++++	+++++
95	+++	++++	+++++
106	+	+	+++++
107	++	+++	+++++
108	+	+++	+++++
-87	++++	++++	+++++
-88	++++	++++	+++++
-115	+++	++++	+++++
-116	++	++++	+++++
78		++++	+++++
88	++	++++	+++++
102		++++	+++++
120	++	+++	+++++
132		+++	+++++
134		+++	+++++
160		+++	+++++
162	+	+++	+++++
188		+++	+++++
190		+++	+++++
46		++	+++++
94		++	+++++
133		++	+++++
161			+++++
196		++	+++++

Decreasing Trends

m/z [a]	1-Year	2-Year	3-Year
47	+++++	++++	+++
48	+++++	++++	++++
63	+++++	++++	+++
69	+++++	+++	++
-124	+++++	+++	+++
29	+++++	+	
93	+++++	+++++	+
142	+++++	++	
187	+++++	+++++	
-102	+++++		
-103	+++++		

Peak Prevalence [b]
= 100% of batches
= ≥ 50% of batches
= < 50% of batches
= 0 batches

Normalized Peak Intensity [c]
+ 1-20%
++ 21-40%
+++ 41-60%
++++ 61-80%
+++++ 81-100%

[a]Peaks indicated in bold have statistically significant increases or decreases in all pairwise comparisons between years (Table S2, Supporting Information). [b]Peak prevalence was determined on a batch-by-batch basis using batch criteria described in the Materials and Methods. [c]Mean absolute peak intensities, normalized to 3-year peaks for increasing trends and 1-year peaks for decreasing trends.

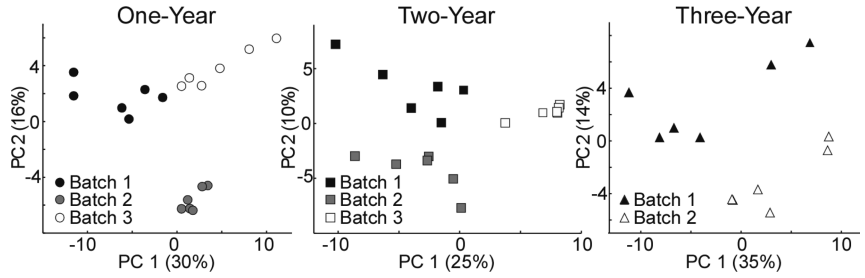

Figure 4. Principal component analysis (PCA) of the SESI-MS volatile fingerprints of batches of aged artisanal Cheddar cheeses. The two PCs providing the best separation for each age category are displayed. (a) Three batches of 1-year-aged Cheddar cheese ($p < 0.0001$). (b) Three batches of 2-year-aged Cheddar cheese ($p < 0.0001$). (c) Two batches of 3-year-aged Cheddar cheese ($p < 0.005$). Six replicates are included for each batch.

similar in their volatile fingerprint within each aging period (Figure 3; Table S1 in the Supporting Information) as well as in their flavor profiles, which met the age characteristics set forth by the cheesemakers (Table 1). However, subtle differences in the SESI-MS fingerprints of batches of the same age (Table S2 and Figure S3 in the Supporting Information) were observed, which were hypothesized to be statistically significant. Using PCA to evaluate the SESI-MS fingerprints within each age category, we observed that the six technical replicates of each batch cluster together and are statistically different from other batches of the same age (Figure 4; Table S4 in the Supporting Information). Whether the SESI-MS fingerprint could be used to identify the Cheddar samples by batch was also tested. PLS-DA leave-one-out cross validation was performed for all 48 samples in eight batch groups and obtained 100% correct batch assignments, confirming that the batches have unique volatile profiles and that SESI-MS fingerprints have the sensitivity and reproducibility necessary to discriminate highly similar samples with complex matrices. It must be noted that by testing only one sample per batch in this pilot study, it cannot be concluded whether the discrimination that was observed is on a batch-to-batch or sample-to-sample basis. However, other results from our lab indicate that SESI-MS volatile fingerprinting data can be used to correctly classify independent samples from closely related specimens.[32−35] From these previous studies and the observations presented here, it is posited that batch fingerprinting of Cheddar would be possible. Batch-to-batch variation is inherent to artisanal food production, and several variables in the milk and in the cheesemaking process impact the development of the Cheddar's flavor.[28] The cheesemakers at Shelburne Farms measure salinity, moisture, and pH and assess the overall milk quality and composition to predict the optimal aging time for each batch of Cheddar, balancing the time required to develop favorable flavor characteristics while diminishing less desirable ones. Prospectively monitoring volatile fingerprints for individual batches, and correlating early and final aging volatile fingerprints using rapid mass spectral fingerprinting techniques

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such as SESI-MS may improve cheesemakers' ability to accurately forecast the aging time for every batch.

Through this pilot-scale study of the volatiles associated with aged artisanal Cheddar, it has been observed that SESI-MS is a robust and sensitive method for characterizing volatile mixtures arising from complex matrices. SESI-MS volatile fingerprints contain a wealth of data, and when coupled with chemometrics, the fingerprints can be mined for a variety of information on the samples' characteristics. The data suggest that SESI-MS can be employed to descriptively and predictively categorize artisanal Cheddar cheeses by their aging period, measure batch-to-batch differences in volatile profiles, and identify volatile components that are correlated with aging. Because SESI-MS is a rapid, nondestructive method for volatiles analysis, it is proposed that this technology is well-suited for quality analysis in food production and could be extended to the production of other artisanal aged products with complex flavor profiles, such as wines, spirits, and cured meats.

■ ASSOCIATED CONTENT

Supporting Information

Spearman's rank correlations, SESI-MS fingerprint peak table and summary of statistical analyses, principal component analyses t-tests, Mann-Whitney U-test z-score plots, and Venn diagrams for SESI-MS peaks shared among Cheddar batches. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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