LabWorks APPNOTE

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Determination of the Efficacy of Rosemary Extract to Maintain Freshness in Plant-Based Meat Alternative Using Sensory-Directed Analysis

Megan Harper-Kerr[1], Nicole Kfoury[1], Tiffany Crawford[2], David Bolliet[2], Kristin Soave[2], Poulson Joseph[2]

[1]GERSTEL, Inc., 701 Digital Drive, Suite J, Linthicum, MD 21090, USA
[2]Kalsec, Inc., 3713 W. Main Street, Kalamazoo, MI 49006, USA

Keywords

Plant-Based Meat Alternative, Antioxidant, Rosemary, Oxidation, Off-odor, Dynamic Headspace, Sensory Directed Analysis, Gas Chromatography-Olfactometry/Mass Spectrometry, Olfactory Detection Port

Abstract

The plant-based protein market has been booming in the past few years. However, flavor remains the most significant barrier to overcome. Specifically, it is a challenge to maintain the flavor of plant-based meat alternative products as they age in storage. In this study, a sensory directed analysis (SDA) approach was employed to determine if adding rosemary extract, with antioxidant properties, could maintain freshness in plant-based meat alternatives in the form of a burger patty. The method utilized dynamic headspace (DHS) sample extraction and gas chromatographic separation paired with simultaneous olfactometry and mass spectrometric detection (GC-O/MS). The ability to smell individual sensory-active compounds and determine their identities is crucial in avoiding off-odors and creating the highest quality food products.

Introduction

As the demands for sustainability, animal welfare, and health consciousness continue to rise, developing new plant-based meat alternatives is necessary. While these plant-based products are environmentally friendly and provide a suitable protein choice for those who seek diverse options, they still present shelf-life and sensory challenges. Most plant-based meat alternatives have a reported shelf life of 7-65 days when stored unopened in the fridge [1]. While they have a longer shelf-life than traditional meats, plant-based meat alternatives still present off-odors and off-flavors within the "best if used by" dates. Many of these sensory challenges are influenced by processes and formulations that take place before producing a protein isolate, the plant source itself, and enzyme activity [2,3].

Rosemary is a known natural protectant in foods because it prevents oxidation and delays microbial growth to some extent [4]. A rosemary extract treated plant-based meat alternative patty was compared to a control to evaluate the extract's efficacy in extending the product's shelf-life and inhibiting spoilage. A sensory directed analysis (SDA) approach was used that combined the human nose and mass spectrometry to identify sensory-active compounds. The use of olfactory and MS detection enables the simultaneous determination of sensory-active regions of the chromatogram and mass spectral identification of associated compounds. Quadrupole-time-of-flight (Q-TOF) mass spectrometry enabled the collection of accurate mass data to further identify compounds with similar retention times and mass spectra. As a result, SDA can be used to solve sensory-related challenges by determining the compounds responsible for producing off-odors in food products.

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This study used DHS as an automated, solventless means of extracting analytes from plant-based meat alternatives. The DHS extraction technique purges the headspace above a sample and concentrates the volatiles onto a sorbent-filled trap. Because DHS is a non-equilibrium technique, more volatiles are driven into the headspace, resulting in improved recovery and extremely low limits of detection. Additionally, TD Multidesorption Mode, selected in the GERSTEL Maestro software, can be used to stack multiple thermal desorption injections onto the inlet for increased analyte mass on column in areas of interest where no peak signal is initially seen.

Experimental

Instrumentation

GERSTEL MPS LabWorks Platform with Dynamic Headspace (DHS) and Olfactory Detection Port (ODP 4) on Agilent 8890/5977B GC-MSD and Agilent 7890B/7250 GC/Q-TOF.

Analysis Conditions LabWorks Platform

DHS	
Trap	Tenax® TA
Incubation	40 °C
Sampling	Sample 40 °C
	Trap 25 °C
	Volume 1000 mL (50 mL/min)
TDU 2	
Pneumatics mode	Splitless
Temperature	40 °C (0 min); 720 °C/min;
	280 °C (3 min)
CIS 4	
Liner	Glass bead-filled
Pneumatic mode	Solvent vent (50 mL/min), split 10:1
Temperature	-120 °C; 12 °C/s; 280 °C (3 min)

Analysis Conditions Agilent 8890 and 7890B GC

Pneumatics	He; P_i = 13.293 psi
	Constant flow = 1 mL/min
Column	30 m DB-5MS UI (Agilent)
	d_i = 0.25 mm d_f = 0.25 µm
Oven	40 °C (1 min); 10 °C/min;
	280 °C (2 min)

Analysis Conditions Agilent 5977B and 7250

Scan Parameters

MSD	Full scan, 40-350 amu
Q-TOF	20-500 amu, 5 spectra/s

Standard Preparation

Standards of 2-methyl-2-butenal, 2,5-dimethylpyrazine, 2,3,5-trimethylpyrazine, benzeneacetaldehyde, 2-ethyl-3,5-dimethylpyrazine, and 2E,4E-decadienal were prepared in methanol. One microliter of each standard was spiked onto the glass frit of a glass thermal desorption tube filled with Tenax® TA. Dry nitrogen was passed through the tube for 3 minutes at a flow rate of 50 mL/min to purge the solvent. The standards were analyzed using the same instrument conditions as the samples to confirm the identification of these compounds as off-odors due to aging.

Sample Preparation

Control and rosemary extract treated plant-based meat alternative burger patties were prepared by Kalsec, Inc., shipped frozen to GERSTEL, Inc., and stored in the freezer until analysis. The patties were formulated with textured pea protein, water, methylcellulose, salt, pea flour, coconut oil, and yeast to contain fat and protein contents similar to those found in other plant-based meat alternative retail products. At the start of the study, the patties were transferred to the refrigerator for 0, 7, 14, 21, and 28 days before cooking. A control and treated patty were evaluated at each time point. The patties were cooked on an electric grill for 4 minutes to reach an internal temperature of at least 73.9 °C. Then, the patties equilibrated at room temperature for 8 minutes. Two panelists tasted the control and treated patties side-by-side to determine sensory differences. The control and treated patties stored to day 28 were unsafe for consumption due to increased microbial activity and were not evaluated. Immediately after tasting, the samples were prepared for GC-O/MS.

A 2 g sample of each patty was broken into smaller chunks and weighed into a 20 mL screw-capped vial for DHS extraction.

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Sample Introduction

The patties were incubated at 40 °C for 2 minutes and then extracted for 20 minutes at 50 mL/min helium flow for a total trap volume of 1000 mL. The analytes were trapped at 25 °C on a Tenax® TA packed tube. The tubes were desorbed at 280 °C for 3 minutes with a 50 mL/min helium flow, and analytes were trapped in the CIS 4 inlet using a glass bead-filled liner at -120 °C. When desorption was complete, samples were transferred to the column in split mode (10:1) by rapidly heating the inlet to 280 °C.

Olfactometry

When GC-O analysis was performed, the column effluent was split 2:1 between the ODP 4 and MS, respectively. The ODP transfer line was heated to 250 °C. The mixing chamber was heated to 150 °C and purged with humidified nitrogen to prevent olfactory fatigue and nasal dehydration.

GC/Q-TOF

Fractions were collected on Tenax® TA tubes at the ODP during specific retention time regions. The tubes were shipped to Kalsec, Inc. and analyzed on an Agilent GC/Q-TOF system for accurate mass data. The resulting data was processed using Agilent Unknowns Analysis with Suremass deconvolution, which is available in the MassHunter Quantitative Analysis software. Compound identification was determined using NIST library matching, retention index values, and the accurate mass Formula Calculator, which is available in the MassHunter Qualitative Analysis software.

Results and Discussion

A sensory evaluation was conducted on each sample to compare the odors of interest in the control and treated patties on days 0, 7, 14, 21, and 28 of storage, as presented in Table 1. The treated sample maintained the aroma of the day 0 sample throughout the 28-day storage period, with only slight oxidation notes detected as the sample aged. On the other hand, the control sample exhibited more pungent, nutty, oily, and paint odors across the 28-day storage period, which suppressed the original green pea/beany, oaty aroma found in the fresh, day 0 sample. The samples were extracted using DHS to correlate the sensory characteristics in Table 1 to an identifiable compound. The Thermal Extractor was used to smell the total odor released from the sorbent tubes to confirm that DHS successfully extracted the odors of interest.

Table 1: Sensory characteristics of control and rosemary extract treated patties obtained from two panelists listed in order of decreasing intensity.

	Control	Rosemary Extract Treated
Day 0	Grainy/oat, green peas/beany	Grainy/oat, green peas/beany
Day 7	Grainy/oat, nutty, oily, oxidized, slight green peas/beany	Grainy/oat, green peas/beany, slight nutty
Day 14	Nutty, oxidized, oily, slight paint, grainy, slight green peas/beany	Grainy/oat, green peas/beany, slight nutty/oxidized
Day 21	Nutty, oxidized, oily, paint, slight grainy	Grainy/oat, slight green peas/beany, slight nutty/oxidized
Day 28	N/A	N/A

Figure 1 shows the TE setup where the Tenax® TA tube was heated with no nitrogen flow to desorb the volatiles from the sorbent. Then, the flow was applied to allow the analyst to smell the total odor profile of the extract. The DHS extractions were found to be representative of the odors detected in each of the patties throughout the 28-day storage period.

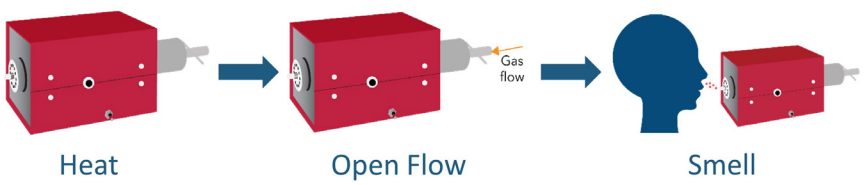

Figure 1: TE setup for smelling the total odor of the DHS extracts.

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Figures 2-6 show the stacked view of control (top) and rosemary extract treated (bottom) patties on days 0, 7, 14, 21, and 28, respectively. The chromatograms in red are overlaid with the olfactory regions in yellow with important odor regions enclosed in blue rectangles. The odor regions detected at the ODP indicative of freshness or aging with retention times and identified compounds are listed in Tables 2 and 3 for the control and treated patties, respectively. Compounds identified in the day 0 samples, including 2-methylpropanal, 3-methylbutanal, hexanal, and heptanal, are responsible for the taste of the fresh patties. On day 7, only one oxidation note was detected at the ODP in both patties and was identified as 2E,4E-decadienal. Overall, there were minimal odor differences between the control and treated patties through day 7.

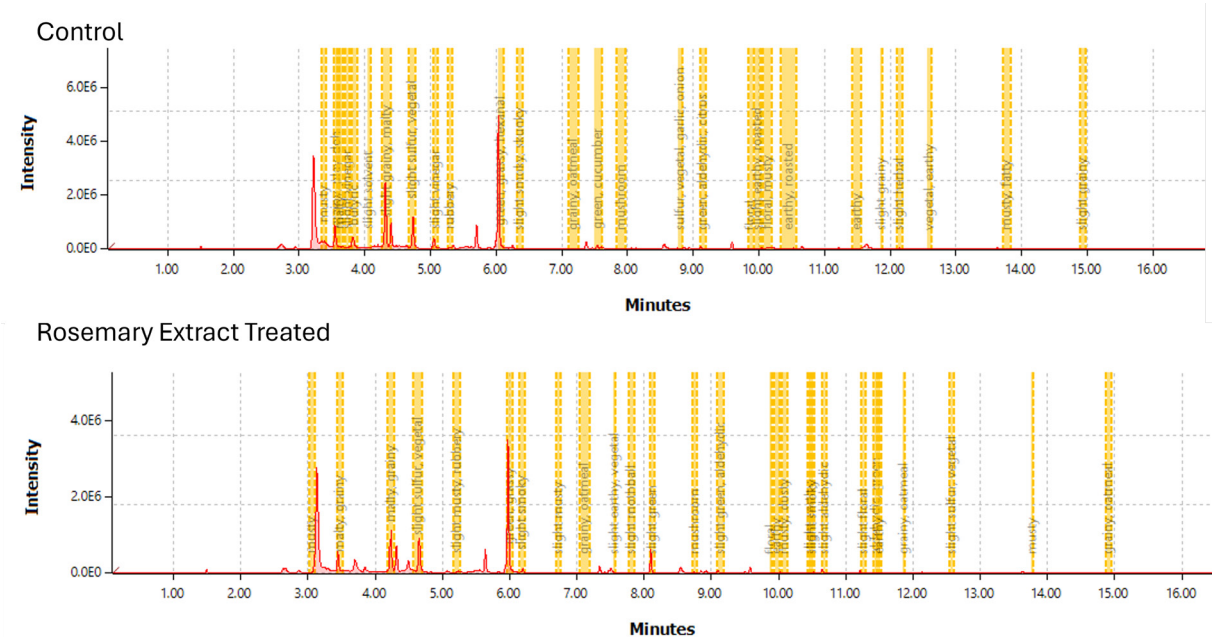

Figure 2: Stacked view of total ion chromatograms of control (top) and rosemary extract treated (bottom) patties on day 0.

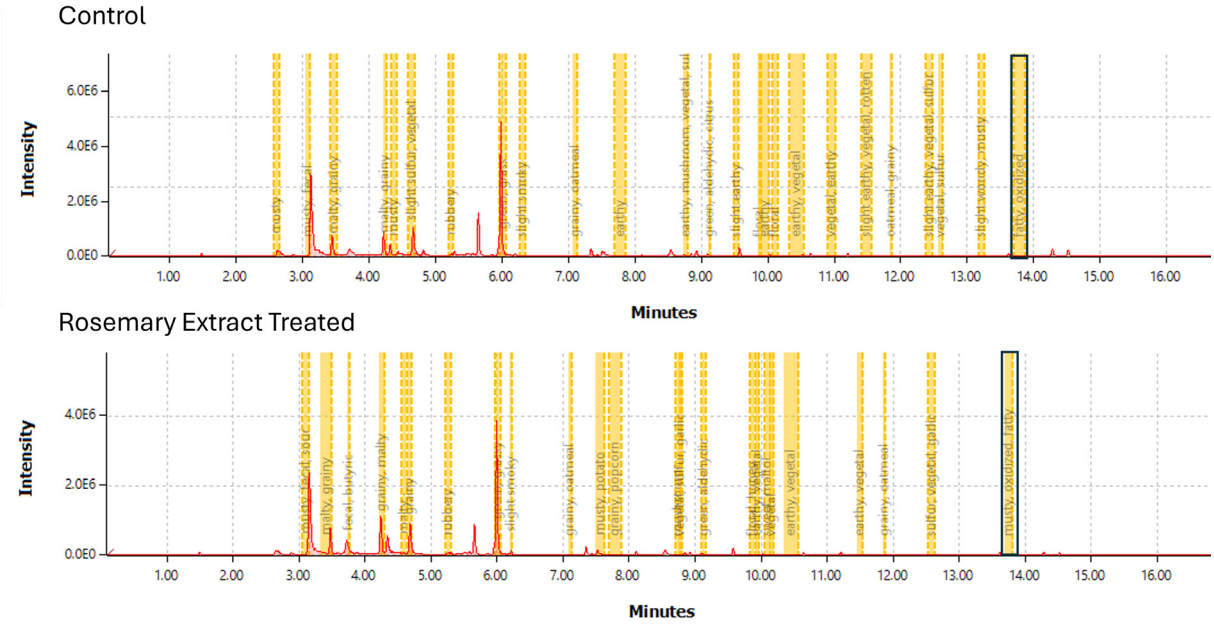

Figure 3: Stacked view of total ion chromatograms of control (top) and rosemary extract treated (bottom) patties on day 7.

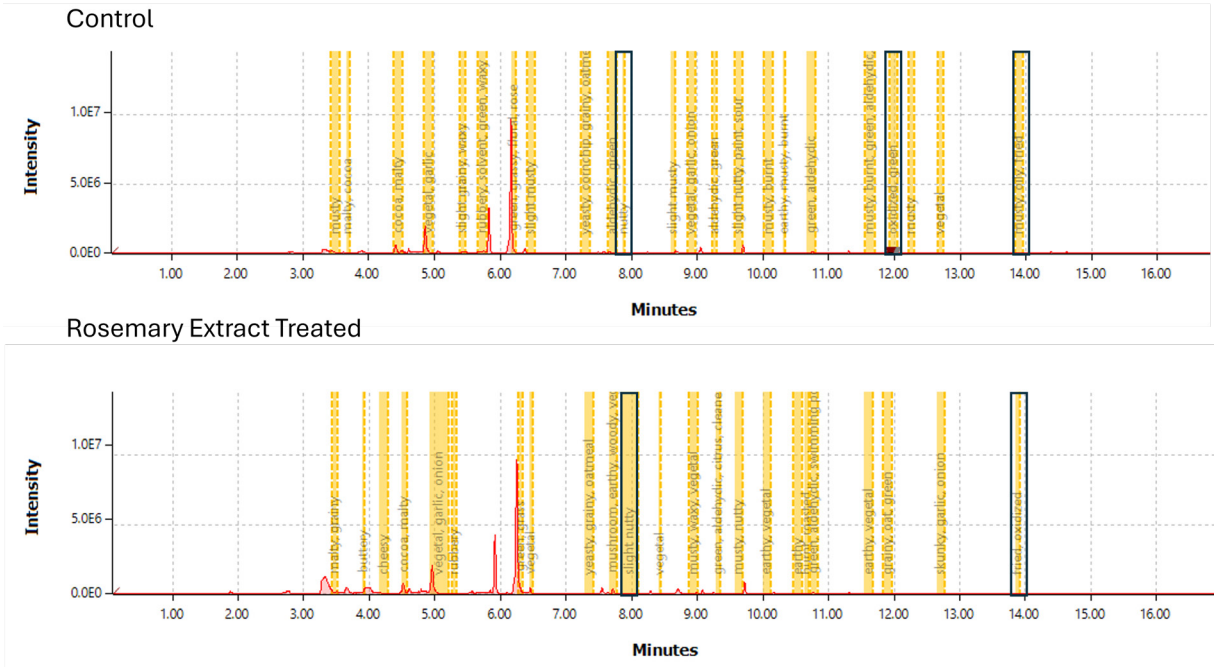

Figure 4: Stacked view of total ion chromatograms of control (top) and rosemary extract treated (bottom) patties on day 14.

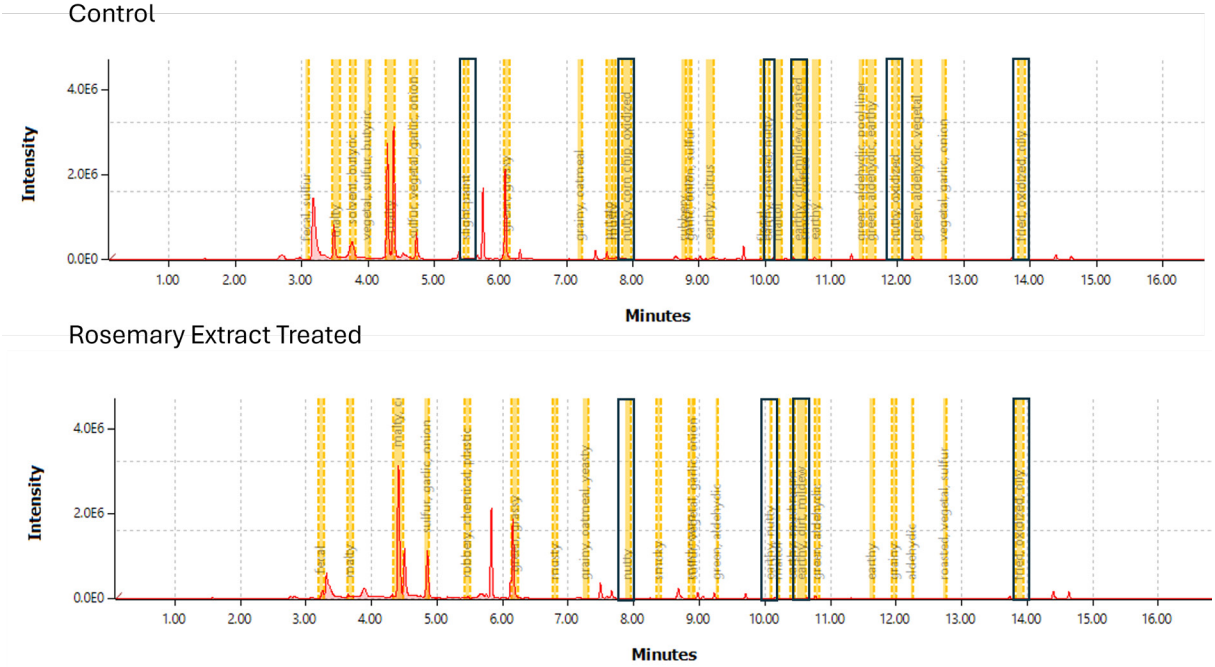

Figure 5: Stacked view of total ion chromatograms of control (top) and rosemary extract treated (bottom) patties on day 21.

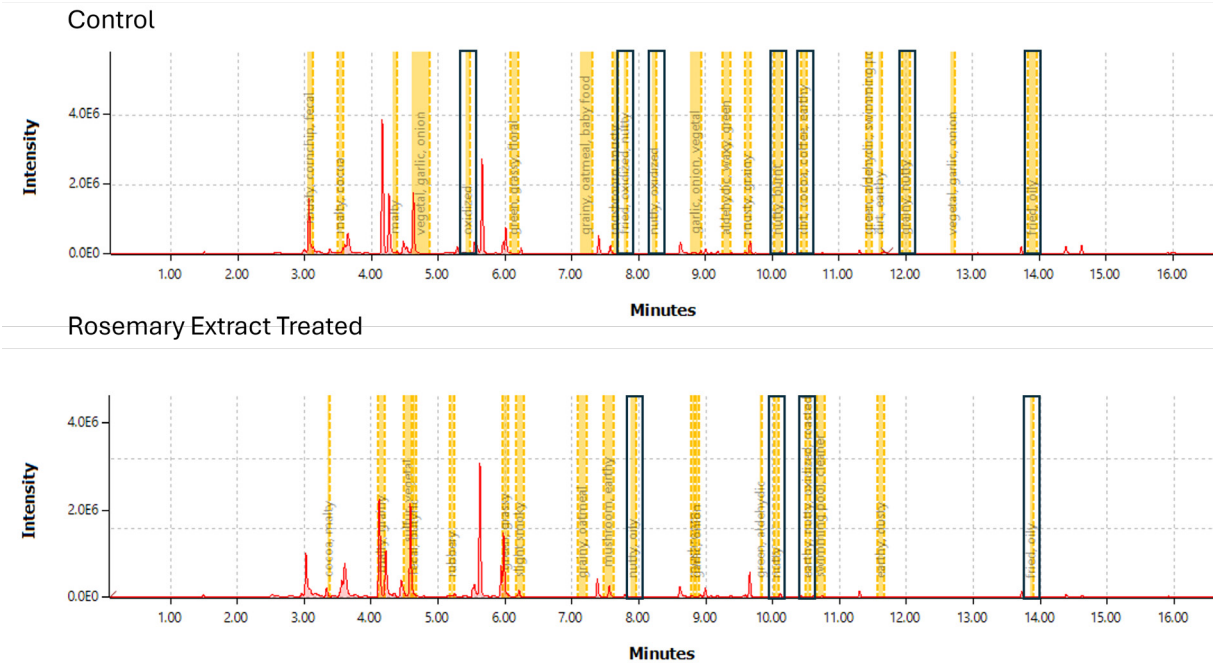

Figure 6: Stacked view of total ion chromatograms of control (top) and rosemary extract treated (bottom) patties on day 28.

Table 2: Key retention time odor regions, sensory characteristics, and identified compounds in the control patties.

RT (min)	Day 0	Day 7	Day 14	Day 21	Day 28	Compound
3.45	malty, grainy	malty, grainy	musty	malty	malty, cocoa	2-Methylpropanal
4.29	malty, grainy	malty, grainy	malty, cocoa	malty	malty	3-Methylbutanal
5.29	rubbery	rubbery	rubbery, solvent, green, waxy	slight paint	oxidized	n.d.
6.03	green, grassy	green, grassy	green, grassy	green, grassy	green, grassy, floral	Hexanal
7.19	grainy, oatmeal	grainy, oatmeal	grainy, oatmeal, yeasty, corn chip	grainy, oatmeal	grainy, oatmeal, baby food	n.d.
7.62	green, cucumber	n.d.	aldehydic, green	musty	mushroom, musty	Heptanal
7.85	mushroom	earthy	nutty	nutty, corn chip, oxidized	fried, nutty, oxi-dized	2,5-Dimethylpyrazine
8.28	n.d.	n.d.	n.d.	n.d.	nutty, oxidized	n.d.
9.21	green, aldehydic, citrus	green, aldehydic, citrus	green, aldehydic	earthy, citrus	green, aldehydic, waxy	Octanal
9.96	earthy, roasted	earthy	burnt, musty	earthy, roasted, nutty	nutty, burnt	n.d.
10.42	earthy, roasted	earthy, vegetal	earthy, musty, burnt	earthy, roasted, mildew, dirt	dirt, cocoa, earthy, coffee	2-Ethyl-3,5-dimethylpyrazine
10.83	n.d.	n.d.	green, aldehydic	earthy	n.d.	Nonanal
11.95	n.d.	n.d.	green, oxidized	nutty, oxidized	grainy, nutty	n.d.
12.24	n.d.	n.d.	musty	green, vegetal, aldehydic	n.d.	Decanal
13.85	musty	oxidized, fried	musty, oily, fried	oxidized, fried, oily	oxidized, fried, oily	2E,4E-Decadienal

n.d = not detected

Table 3: Key retention time odor regions, sensory characteristics, and identified compounds in the rosemary extract treated patties.

RT (min)	Day 0	Day 7	Day 14	Day 21	Day 28	Compound
3.45	malty, grainy	malty, grainy	malty, grainy	malty	malty, cocoa	2-Methylpropanal
4.29	malty, grainy	malty, grainy	malty, cocoa	malty, grainy	malty, grainy	3-Methylbutanal
5.29	musty, rubbery	rubbery	rubbery	rubbery, chemical, plastic	rubbery	n.d.
6.03	green, grassy	green, grassy	green, grassy	green, grassy	green, grassy	Hexanal
7.19	grainy, oatmeal	grainy, oatmeal	grainy, oatmeal, yeasty	grainy, oatmeal, yeasty	grainy, oatmeal	n.d.
7.62	vegetal, earthy	musty, potato	vegetal, earthy, mushroom, woody	mushroom, musty, earthy	mushroom, earthy	Heptanal
7.85	mothball	grainy, popcorn	slight nutty	earthy, nutty	nutty, oily	2,5-Dimethylpyrazine
9.11	green, aldehyde, citrus	green, aldehydic	green, aldehyde, citrus, cleaner	green, aldehydic, cleaner	n.d.	Octanal
9.96	earthy	earthy, vegetal	earthy, vegetal	earthy,nutty	nutty	n.d.
10.42	earthy	earthy, vegetal	earthy	earthy, dirt, mildew	earthy, nutty, oxidized, roasted	2-Ethyl-3,5-dimethylpyrazine
10.83	aldehydic	aldehydic	green, aldehydic, swimming pool	green, aldehydic	swimming pool, cleaner	Nonanal
11.85	grainy, oatmeal	grainy, oatmeal	grainy, oat, green	grainy	n.d.	n.d.
13.85	musty	musty, oxidized, fatty	oxidized, fried	oxidized, fried, oily	fried, oily	2E,4E-Decadienal

n.d = not detected

In the sensory evaluations on days 14 and 21, the control patties had increased nutty, oxidized, oily, and painty notes, which suppressed the green and oaty notes of the fresh sample. On the other hand, the treated patties remained relatively unchanged and only presented slight nutty and oxidation notes. These differences are shown in table 1 as the odor characteristics are listed in order of decreasing intensity. Day 28 samples were not tested on the sensory panel but were evaluated at the ODP. After 28 days of aging, seven odor regions in the control and four in the treated samples were detected at the ODP with odors matching the nutty and oxidation notes determined in the sensory panel. At 7.85 minutes, good match factors were seen for isomers 2,(5 or 6)-dimethylpyrazine, and at 10.42 minutes, good match factors were seen for the isomers 2-ethyl-3,(5 or 6)-dimethylpyrazine and 2,6-diethylpyrazine. Additional characterization is required to determine the correct isomer identification. The odor region at 9.96 minutes was tentatively identified as benzeneacetaldehyde. However, a standard of benzeneacetaldehyde was analyzed, and the odor at the ODP was characterized as floral and honey, which does not match the earthy, nutty notes detected. For identification, additional separation would be needed to isolate the compound of interest from the benzeneacetaldehyde peak.

After 28 days, four odor regions remained unidentified, as seen in tables 2 and 3, due to a lack of peak signal in the chromatogram. The odor region detected at 7.19 minutes matched that of the fresh patties. Therefore, further compound identification was not necessary for this specific study. Three additional DHS extractions of the 28-day-old control patty were performed for the remaining three unidentified odor regions. TD Multidesorption Mode, in the GERSTEL Maestro software, was used to increase analyte mass on column. The odor regions detected at 8.28 and 11.95 minutes still did not correlate with a detectable peak. This indicates that these compounds have extremely low odor thresholds, allowing the analysts to smell them at the ODP at concentrations well below the instrument detection limit. Additional extractions would be neces-

sary to introduce the analyte mass on column required for identification. The compound at 5.29 minutes was identified as 2-methyl-2-butenal. Figure 7 shows the peak signal obtained from one versus three DHS extractions. A standard of 2-methyl-2-butenal was analyzed, and the odor, retention time, and mass spectrum matched that of the sample, confirming its identity.

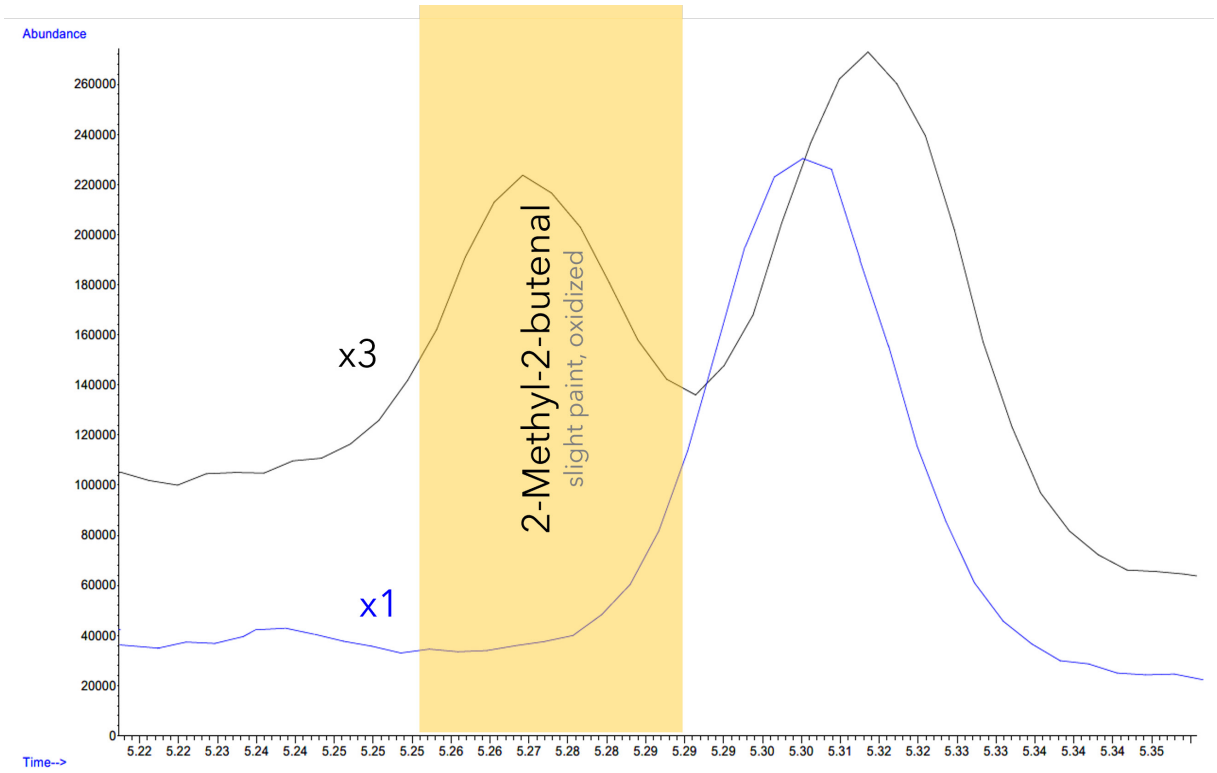

Figure 7: TD Multidesorption mode for identification of 2-methyl-2-butenal.

Two additional pyrazines were identified at 9.24 and 9.26 minutes as 2-ethyl-(5 or 6)-methylpyrazine and 2,3,5-trimethylpyrazine, respectively. Although these pyrazines did not correlate with detectable odors at the ODP, they may have contributed to synergistic effects with the other pyrazines present and were therefore considered relevant to the study. To identify the four corresponding pyrazines identified at retention times 7.85, 9.24, 9.26, and 10.42 minutes with higher mass accuracy, the ODP 4's fraction collecting capabilities were utilized to collect and concentrate these regions onto Tenax® TA tubes. The tubes were shipped to Kalsec, Inc. and analyzed by GC/Q-TOF to confirm compound identification with higher mass accuracy. Table 4 lists the calculated mass errors for the pyrazines identified, which were all below 10 ppm. One pyrazine corresponded to each peak except for that at 10.42 minutes. In this case, 2-ethyl-3,5-dimethylpyrazine and 2,6-diethylpyrazine were both possible matches. These two compounds have slightly different aroma descriptors of potato, cocoa, roasted, nutty or nutty, hazelnut, respectively, as reported by Good Scents [5-6]. The reported odors in Tables 2 and 3 at 10.42 minutes were dirt, cocoa, roasted, mildew, oxidized, and coffee, which closely match the Good Scents report for 2-ethyl-3,5-dimethylpyrazine. As a final confirmation step, standards of 2,5-dimethylpyrazine, 2,3,5-trimethylpyrazine, and 2-ethyl-2,5-dimethylpyrazine were analyzed to verify retention time, mass spectra, and their respective odors at the ODP. A standard of 2-ethyl-5-methylpyrazine was not analyzed as it was discontinued.

Table 4: Absolute and calculated molecular ion mass errors for pyrazine compounds.

RT (min)	Compound	Absolute m/z	Calculated m/z	Mass error (ppm)
7.85	2,5-Dimethylpyrazine	108.0681	108.0687	-5.6
9.24	2-Ethyl-5-methyl pyrazine	122.0834	122.0843	-7.4
9.26	2,3,5-Trimethylpyrazine	122.0834	122.0843	-7.4
10.42	2-Ethyl-3,5-dimethyl pyrazine	136.0990	136.1000	-7.6
10.42	2,6-Diethylpyrazine	136.0990	136.1000	-7.3

Several aldehydes and pyrazines were identified in the plant-based meat alternative samples, with many contributing to the detected off-odors. Aldehydes are typical lipid oxidation markers that contribute to the presence of off-odors and off-flavors in food products. Furthermore, pyrazines are common products of Maillard reactions promoted by high temperatures and storage [4]. The peak areas for the identified compounds of interest were examined to determine differences over time in storage and between control and treated samples. Aldehyde and pyrazine production in the control patties increased in peak area. Interestingly, 2E,4E-decadienal, and the four pyrazines remained stable over time in the treated patties. The stacked view of total ion chromatograms for the control and treated patties on days 0 and 28 are shown in figures 8 and 9, respectively. The peak areas increased in the control patties, particularly after day 14. An example of this trend is shown for 2,5-dimethylpyrazine in figure 10, and the corresponding data is listed in table 5 with peak areas normalized to the control on day 28.

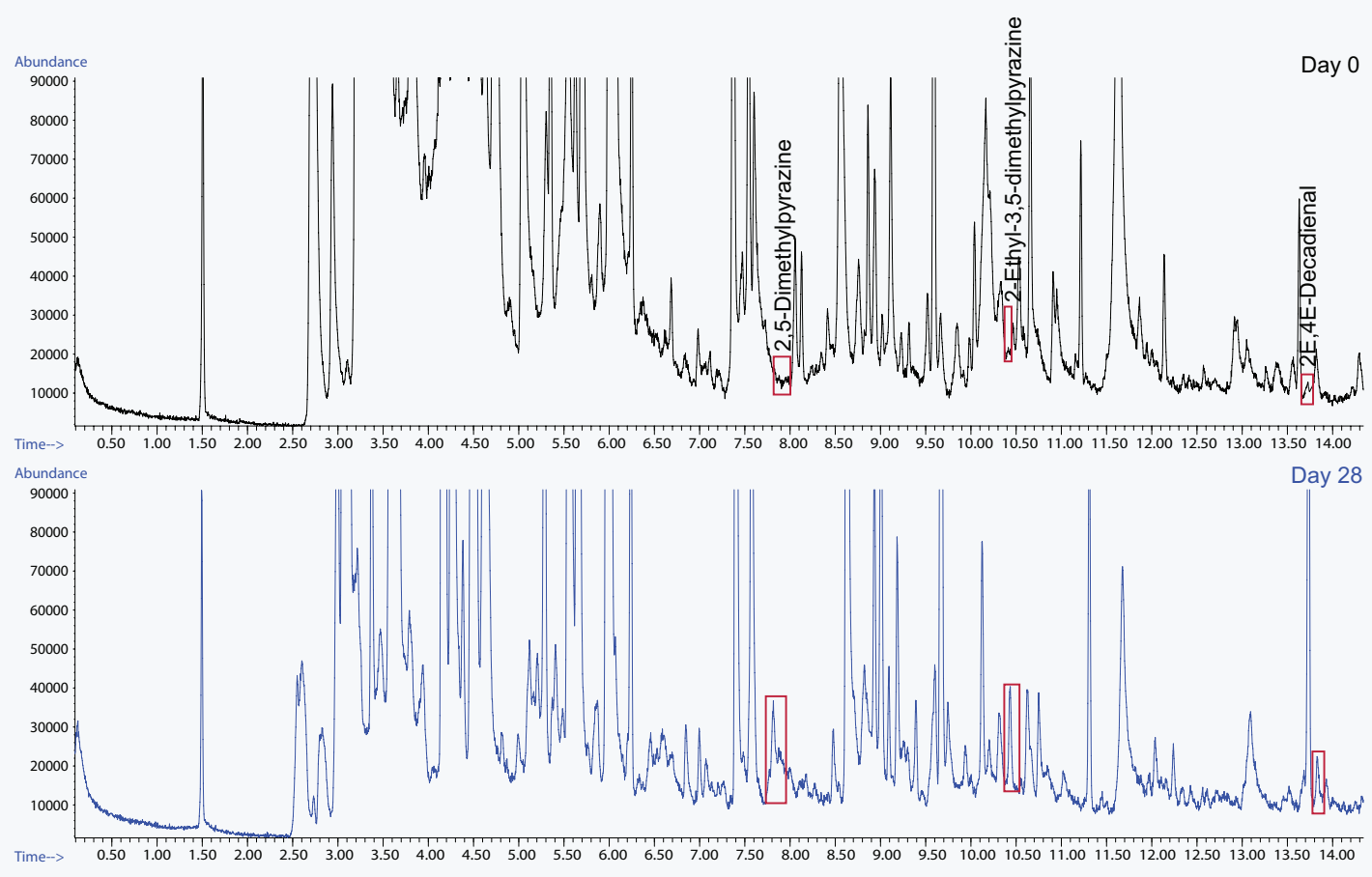

Figure 8: Stacked view of TICs of control patties on day 0 (black) and day 28 (blue).

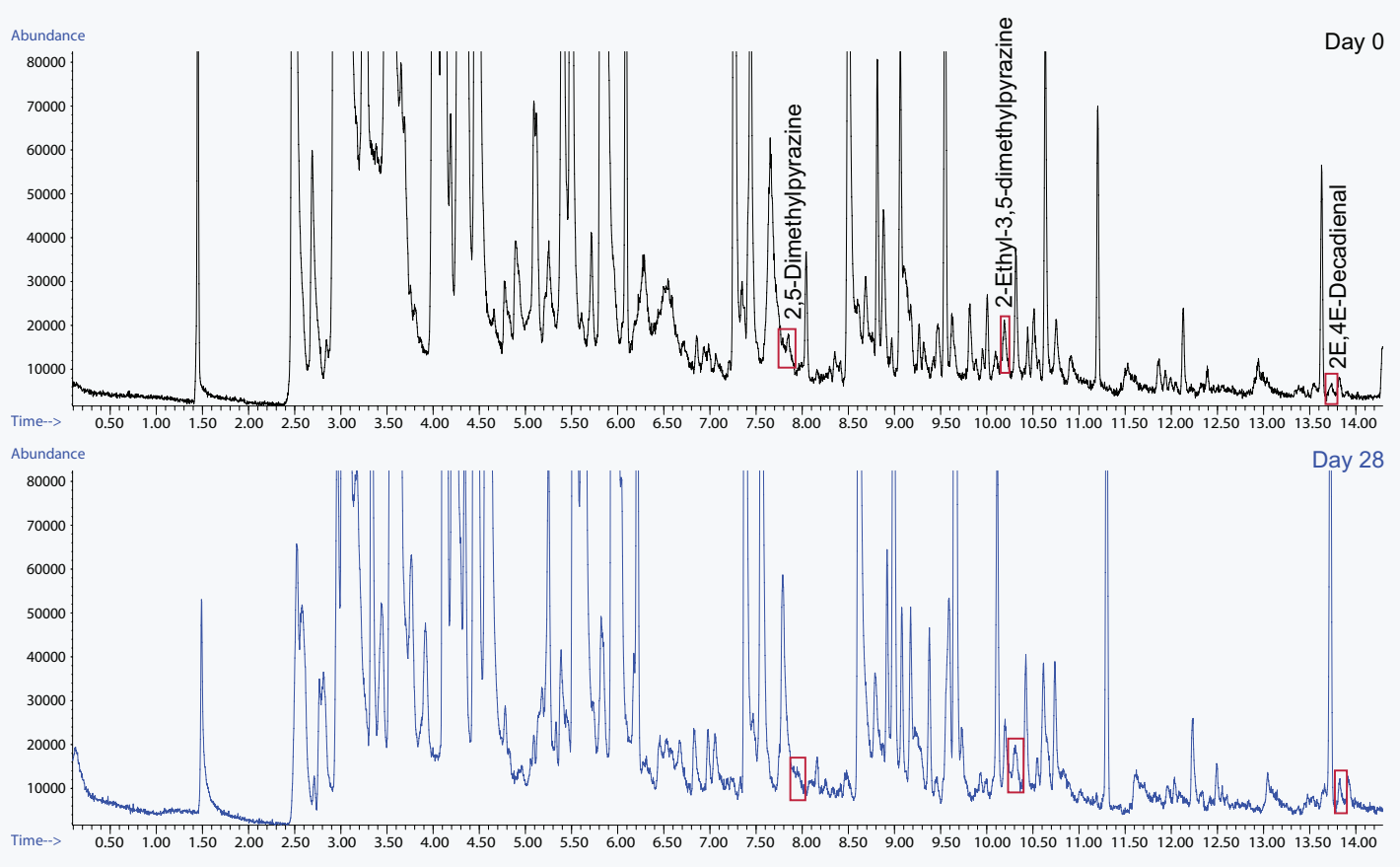

Figure 9: Stacked views of TICs of treated patties on day 0 (black) and day 28 (blue).

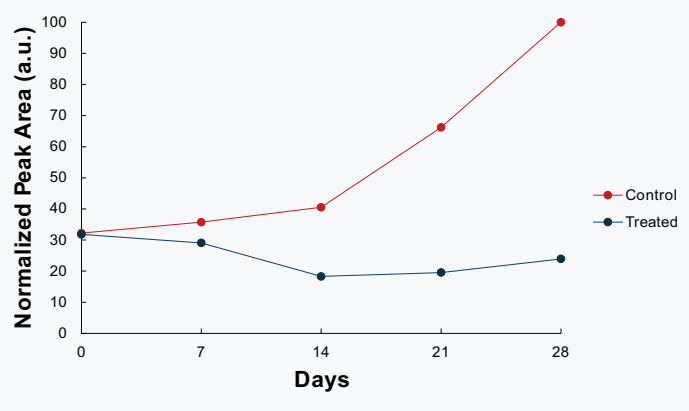

Figure 10: Increase of peak area counts as a function of storage time on the example of 2,5-dimethylpyrazine.

Table 5: Normalized peak area counts for 2,5-dimethylpyrazine (m/z = 108) in the control and treated patties during storage.

Days	Control	Treated
0	32.2	31.8
7	35.7	29.1
14	40.5	18.3
21	66.2	19.5
28	100.0	23.9

Conclusion

This study has demonstrated the ability of an SDA methodology to identify key sensory-active compounds responsible for aging and oxidation in plant-based meat alternative patties. The data shows differences in chromatography and sensory perception between the control and rosemary extract treated patties. DHS extraction on a Tenax® TA tube allows for the efficient and optimal concentration of essential sensory-active compounds from the samples. When DHS is equipped with the ODP 4 and MS detectors, off-odor regions can be correlated to the corresponding compounds. This work proves that the rosemary extract made the plant-based meat alternative patties less susceptible to off-odors to prolong freshness.

References

[1] https://plantbasedwithamy.com/does-plant-based-meat-go-bads.

[2] Zhogolevaa, A., Alas, M., & Rosenvalda, S. (**2023**, June 21). *Characterization of odor-active compounds of various pea preparations by GC-MS, GC-O, and their correlation with sensory attributes*. Future Foods. https://www.sciencedirect.com/science/article/pii/S2666833523000291#cebibl1

[3] Trindler, C., Kopf-Bolanz, K. A., & Denkel, C. (2021a, **December 22**). *Aroma of peas, its constituents and reduction strategies – effects from breeding to processing. Food Chemistry*. https://www.sciencedirect.com/science/article/pii/S0308814621028983?pes=vor

[4] Veenstra JP, Johnson JJ. Rosemary (Salvia rosmarinus): *Health-promoting benefits and food preservative properties*. Int J Nutr. **2021**;6(4):1-10. Epub 2021 Jun 24. PMID: 34651071; PMCID: PMC8513767.

[5] https://www.thegoodscentscompany.com/data/rw1426491.html

[6] https://www.thegoodscentscompany.com/data/rw1464601.html