LabWorks APPNOTE

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Identification of Off-Odor Compounds Associated with Lipid Oxidation in Food Products Using Sensory Directed Analysis

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Keywords

Lipid oxidation, Off-odors, Canola Oil, Crackers, Sensory Directed Analysis, Direct Thermal Extraction, Dynamic Headspace, Olfactory Detection Port

Abstract

Edible oils and food products containing edible oils are prone to off-odors due to lipid oxidation. However, most studies focus on the increase of aldehydes and other compounds in oxidized samples without relating the results to the sensory attributes of the sample. In this study, a sensory directed analysis (SDA) method was employed to identify key sensory-active compounds responsible for these off-odors. Off-odors were explored in cooking oil and crackers. In each case, an aged sample was compared to a fresh, positive control sample. The method utilized a variety of sample preparation techniques appropriate to each sample type and gas chromatographic separation paired with simultaneous olfactory and mass spectral detection. The ability to smell individual sensory-active compounds and determine their identity is crucial to developing processes to avoid off-odors and creating the highest quality food products.

Introduction

Lipid oxidation is an important reaction in food chemistry and is associated with a loss of quality and nutritional value. Lipids are vital to human nutrition, providing energy for biological processes, maintaining brain function, and facilitating the absorption of fat-soluble vitamins [1,2]. As these lipids break down, secondary degradation products are formed, including alkanes, alcohols, esters, aldehydes, and ketones. Some of these volatiles are sensory-active, even at very low concentrations, resulting in off-odor formation and reduced acceptability in food products [2,3].

SDA is a process that utilizes gas chromatography in combination with 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. As a result, SDA can be used to solve sensory-related challenges by determining the compounds responsible for producing off-odors in food products.

In this study, direct thermal extraction (DTE) and dynamic headspace (DHS) were used as automated, solventless means of extracting analytes from different sample types. DTE requires very little sample, which is extracted directly in the thermal desorption unit, and can be used for trace analysis of volatile and semi-volatile organic compounds (VOCs and SVOCs) in solid or liquid samples. DHS is a technique that purges the headspace above a solid or liquid, extracting volatiles and concentrating them onto a sorbent-filled trap. Because it is a non-equilibrium technique, more volatiles are driven into the headspace, resulting in improved recovery and extremely low limits of detection. For both techniques, the TD Multidesorption Mode, which can be selected in the GERSTEL Maestro software, can be used to stack multiple injections onto the inlet for increased analyte mass on column in areas of interest where no peak signal is initially seen.

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Experimental

Instrumentation

GERSTEL MPS LabWorks Platform with Dynamic Headspace (DHS) and Olfactory Detection Port (ODP 4) on Agilent 8890/5977C GC-MSD, GERSTEL Thermal Extractor (TE 2).

Analysis Conditions LabWorks Platform

DHS	
Trap	Tenax® TA
Incubation	40 °C (2 min)
Sampling	Sample 40 °C
	Trap 25 °C
	Volume 1000 mL (50 mL/min)
TDU 2	
Pneumatics mode	Splitless
Temperature	40 °C; 720 °C/min; 280 °C (3 min) (DHS)
	40 °C; 720 °C/min; 90 °C (15 min) (DTE)
CIS 4	
Liner	Glass bead packed
Pneumatic mode	Solvent vent (50 mL/min), split 5:1
Temperature	-120 °C; 12 °C/s; 280 °C (3 min)

Analysis Conditions Agilent 8890 GC

Pneumatics	He; Pi = 13.066 psi
	Constant flow = 1 mL/min
Column	30 m HP-5MS UI
	d_i = 0.25 mm d_f = 0.25 µm
Oven	35 °C (2 min); 15 °C/min; 280 °C (2 min)

Analysis Conditions Agilent 5977C MSD

Mode	Full scan, 40-350 amu

Standard Preparation

Standards of hexanal, octanal, nonanal, 2E-octenal, 2E-nonenal, 2E,4E-heptadienal, 2E,4E-nonnadienal, and 2E,4E-decadienal were prepared in methanol. One microliter of the 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 lipid oxidation.

Sample Preparation

Fresh and aged canola oil, wheat crackers, and cheddar crackers were analyzed to determine lipid oxidation off-odors. The canola oil was aged at 40 °C for several weeks. The wheat and cheddar crackers were aged at ambient temperature for one year. A very aged cheddar cracker sample was aged at 40 °C for one year.

A 50 mg sample of canola oil was weighed into a slitted microvial and placed into an empty glass thermal desorption tube. The tube was capped with a transport adapter with o-ring seal and placed in a 40-position tray on the MPS robotic autosampler for DTE analysis. A 2.0 g sample of each cracker was weighed into 20 mL screw-capped vials for DHS extraction.

Sample Introduction

The oils were extracted at 90 °C for 15 minutes with a 50 mL/min helium flow and cold trapped in the CIS 4 inlet using a glass bead-filled liner at -120 °C. When extraction was complete, samples were transferred to the column in split mode (5:1) by rapidly heating the inlet to 280 °C.

The crackers 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 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 (5:1) by rapidly heating the inlet to 280 °C.

Olfactometry

GC-O analysis was performed with the column effluent split 2:1 between the ODP and MS detectors, 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.

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Results and Discussion

Each sample was subjected to a sensory analysis to determine the odors of interest associated with lipid oxidation, as shown in Table 1. The fresh samples exhibited the odors expected from each of the respective food products, with only very slight oxidation odors. The aged samples had very characteristic lipid oxidation off-odors, including green, painty, oily, fatty, waxy, and rancid. To confirm that DTE and DHS successfully extracted the odors of interest from each sample, the Thermal Extractor (TE 2) was used to smell the total odor released from the oils and sorbent tubes.

Figures 1-2 show the stacked view of aged (top) and fresh (bottom) canola oil and wheat crackers, respectively. Figure 3 shows the stacked view of very aged (top), aged (middle), and fresh (bottom) cheddar crackers. The chromatograms in red are overlaid with the olfactory regions in yellow. Odor regions that represent key sensory characteristics determined in the samples are marked in blue. There is significantly greater peak signal and more odor regions in the very aged and aged samples compared to the fresh samples. The key sensory characteristics detected in each sample, the identified compound, and the peak area normalized to the fresh sample are shown in Tables 2-4.

Table 1: Sensory characteristics of fresh, aged, and very aged samples.

Sample	Fresh	Aged	Very Aged
Canola oil	canola oil, slight green/aldehydic	oxidized, painty, green, aldehydic	n/a
Wheat crackers	cracker, slight green/oily	stale, oily, fatty, oxidized	n/a
Cheddar crackers	cracker, cheddar, slight waxy	stale, oily, fatty, waxy	rancid, fatty, waxy, chemical

The aged canola oil was described as oxidized, painty, green, and aldehydic. Oxidized and painty odors were detected at the ODP and were identified as 1-penten-3-one, 2E,4E-heptadienal, and 2E,4E-decadienal. 2E,4E-heptadienal was detected by the MS in both samples but could only be smelled at the ODP in the aged sample, this is likely due to its concentration being below the odor threshold in the fresh sample. On the other hand, 2E,4E-decadienal has a very low odor threshold and could be detected at the ODP in both but was below the instrument detection limit in the fresh sample.

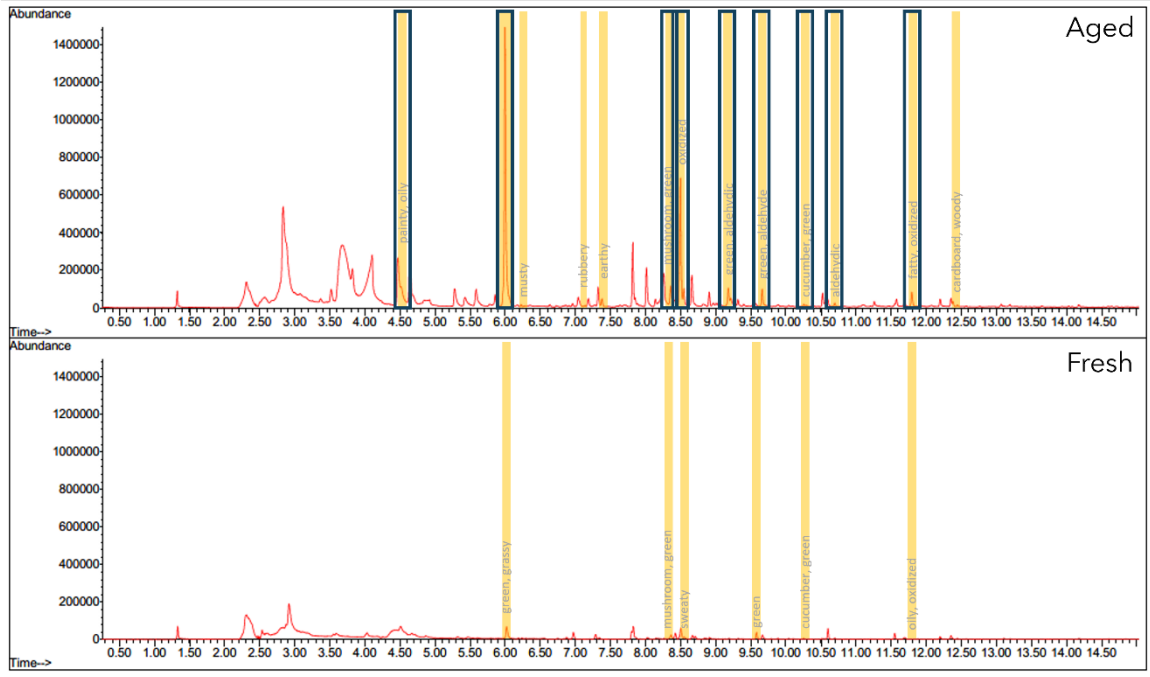

Figure 1: Stacked view of total ion chromatograms of aged (top) and fresh (bottom) canola oil.

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Table 2: Key sensory characteristics, identified compounds, and relative peak areas for fresh and aged canola oil.

RT	Odor Characeristic		Compound	Peak Area	
	Fresh	Aged		Fresh	Aged
4.58		painty, oily	1-Penten-3-one	n.d.	1.0
6.01	green, grassy	green, grassy	Hexanal	1.0	19.2
8.26	mushroom, green	mushroom, green	1-Octen-3-ol	n.d.	1.0
8.48		oxidized	2E,4E-Heptadienal	1.0	10.8
9.18		green, aldehydic	2E-Octenal	n.d.	1.0
9.65	green	green, aldehydic	Nonanal	1.0	4.8
10.26	cucumber, green	cucumber, green	n.d.		
10.70		aldehydic	Decanal	n.d.	1.0
11.81	oily, oxidized	fatty, oxidized	2E,4E-Decadienal	n.d.	1.0

Note: n.d. = not detected

The fresh canola oil had a slight green and aldehydic aroma, whereas the same odor was much more prominent in the aged sample. There were several green and aldehydic odors detected at the ODP. Hexanal and nonanal were described as green and aldehydic in both samples but were present at much higher levels in the aged sample. 2E-octenal and decanal were also described as green and aldehydic but were only found in the aged samples.

Many of the compounds that were identified in the wheat crackers were also found in the canola oils. These include hexanal, 1-octen-3-ol, 2E,4E-heptadienal, and 2E-octenal. Some additional compounds were identified, including acetic acid, heptanal, and octanal. The former two were identified in both the fresh and aged samples at the MS but were only smelled at the ODP in the aged samples, again suggesting that they are present at a concentration below their odor threshold in the fresh samples and thus not contributing to any off-odor smell in the sample.

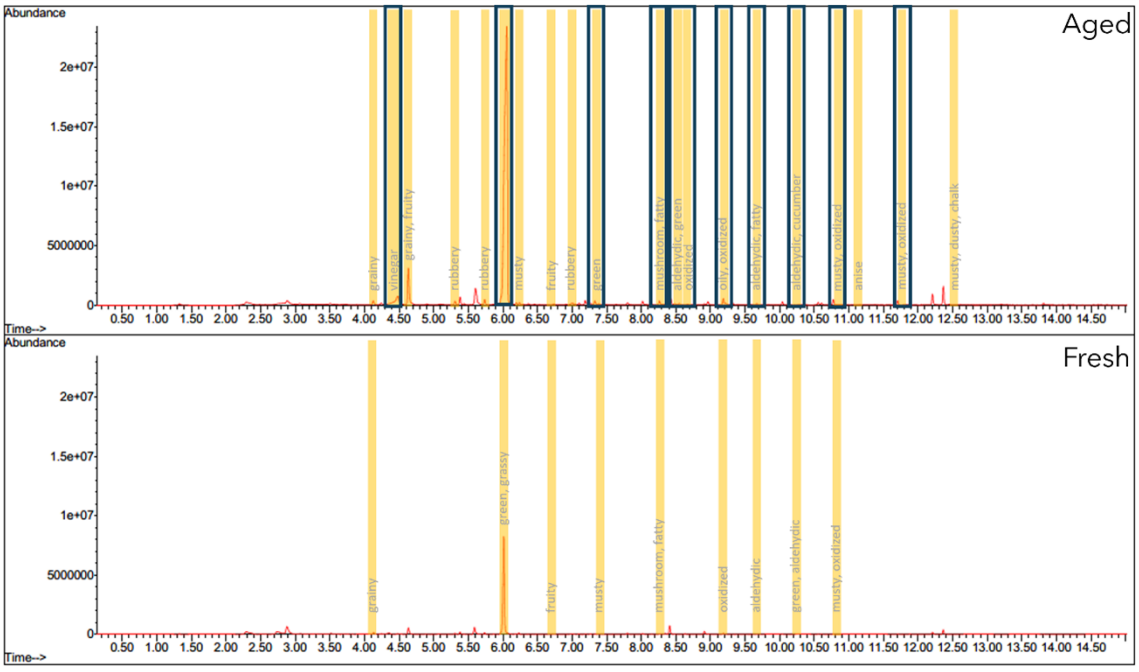

Figure 2: Stacked view of total ion chromatograms of aged (top) and fresh (bottom) wheat crackers.

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Table 3: Key sensory characteristics, identified compounds, and relative peak areas for fresh and aged wheat crackers.

RT	Odor Characteristic		Compound	Peak Area	
	Fresh	Aged		Fresh	Aged
4.45		vinegar	Acetic acid	1.0	6.6
6.04	green, grassy	green, grassy	Hexanal	1.0	5.5
7.35		green	Heptanal	1.0	7.6
8.26	mushroom, fatty	mushroom, fatty	1-Octen-3-ol	1.0	8.3
8.58		aldehydic, green	Octanal	n.d.	1.0
8.69		oxidized	2E,4E-Heptadienal	n.d.	1.0
9.22	oxidized	oily, oxidized	2E-Octenal	1.0	4.7
9.68	aldehydic	aldehydic, fatty	Nonanal	n.d.	1.0
10.28	green, aldehydic	aldehydic, cucumber	n.d.		
10.83	musty, oxidized	musty, oxidized	n.d.		
11.82		musty, oxidized	n.d.		

Note: n.d. = not detected

There were three regions where green and oxidized odors were detected at the ODP, but no peak signal was seen at the MS. The latter two regions at 10.83 and 11.82 minutes were identified by increasing mass on column, using the TD Multidesorption Mode in the Maestro software, as shown in Figure 4. 2E,4E-decadienal was previously identified in the canola oil, but 2E,4E-nonadienal was newly identified in the wheat crackers. The first region, at 10.28 minutes, has the same retention time and odor descriptor as the unidentified compound in the canola oil. However, it could be identified in the very aged cheddar crackers as 2E-nonenal.

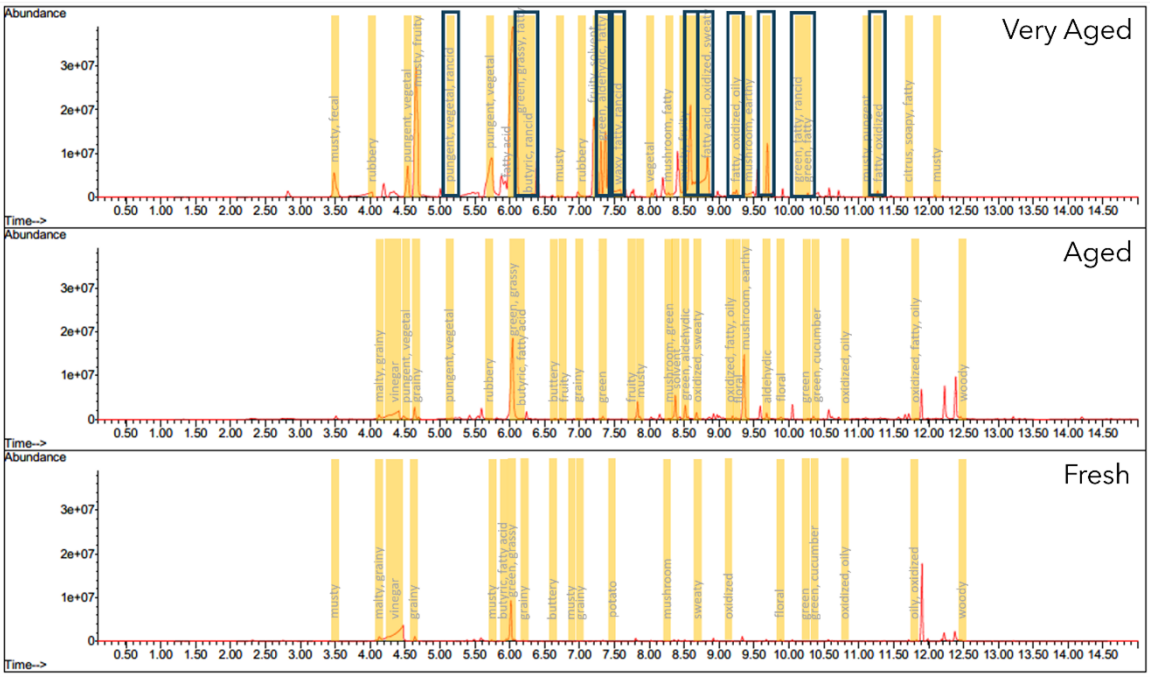

Figure 3: Stacked view of total ion chromatograms of very aged (top), aged (middle), and fresh (bottom) cheddar crackers.

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In addition to the aldehydes detected in the oils and wheat crackers, fatty acids and methyl ketones were found in the cheddar crackers. The fatty acids are found at relatively low levels in the fresh and aged samples, likely due to their natural presence in cheddar [4]. However, the fatty acid levels drastically increased in the very aged samples, potentially due to exposure to elevated temperatures for a prolonged time. As a result, these fatty acids contribute to the waxy, fatty, and rancid aromas in the very aged crackers. Interestingly, 2E,4E-nonadienal and 2E-4E-decadienal levels increase in the aged compared to the fresh cracker but are no longer present in the very aged sample. It is likely that these compounds are breaking down into the methyl ketones that are present due to the increased temperature the very aged sample was exposed to [1]. The methyl ketones also contribute to the fatty and rancid odors in the very aged sample.

Table 3: Key sensory characteristics, identified compounds, and relative peak areas for fresh, aged, and very aged cheddar crackers.

RT	Odor Characeristic			Compound	Peak Area		
	Fresh	Aged	Very Aged		Fresh	Aged	Very Aged
5.26		pungent, vegetal	pungent, vegetal, rancid	3E-Penten-2-one	n.d.	1.0	2.0
6.01	green, grassy	green, grassy	green, grassy, fatty	Hexanal	1.0	3.1	10.8
6.09	butyric, fatty acid	butyric, fatty acid	butyric, rancid	Butyric acid	1.0	3.0	28.3
7.35		green	green, aldehydic, fatty	Heptanal	1.0	2.8	78.2
7.53			waxy, fatty, rancid	Pentanoic acid	1.0	2.0	233.5
8.56		green, aldehydic	green, aldehydic, fatty	Octanal	1.0	3.8	255.6
8.68	sweaty	oxidized, sweaty	fatty acid, oxidized, sweaty	Hexanoic acid	1.0	1.9	380.6
9.20	oxidized	oxidized, fatty, oily	fatty, oxidized, oily	2E-Octenal	1.0	4.1	7.9
9.69		aldehydic	green, aldehydic, fatty	Nonanal	1.0	3.2	31.0
10.06	green	green	green, fatty, rancid	3E-Nonen-2-one	n.d.	n.d.	1.0
10.26	green, cucumber	green, cucumber	green, fatty	2E-Nonenal	n.d.	n.d.	1.0
10.85	oxidized, oily	oxidized, oily		2E,4E-Nonadienal	1.0	6.4	-
11.26			fatty, oxidized	2E-Decenal	n.d.	n.d.	1.0
11.84	oily, oxidized	oxidized, fatty, oily		2E,4-Decadienal	1.0	2.9	-
8.68	sweaty	oxidized, sweaty	fatty acid, oxidized, sweaty	Hexanoic acid	1.0	1.9	380.6
9.20	oxidized	oxidized, fatty, oily	fatty, oxidized, oily	2E-Octenal	1.0	4.1	7.9
9.69		aldehydic	green, aldehydic, fatty	Nonanal	1.0	3.2	31.0
10.06	green	green	green, fatty, rancid	3E-Nonen-2-one	n.d.	n.d.	1.0
10.26	green, cucumber	green, cucumber	green, fatty	2E-Nonenal	n.d.	n.d.	1.0
10.85	oxidized, oily	oxidized, oily		2E,4E-Nonadienal	1.0	6.4	-
11.26			fatty, oxidized	2E-Decenal	n.d.	n.d.	1.0
11.84	oily, oxidized	oxidized, fatty, oily		2E,4-Decadienal	1.0	2.9	-

Note: n.d. = not detected

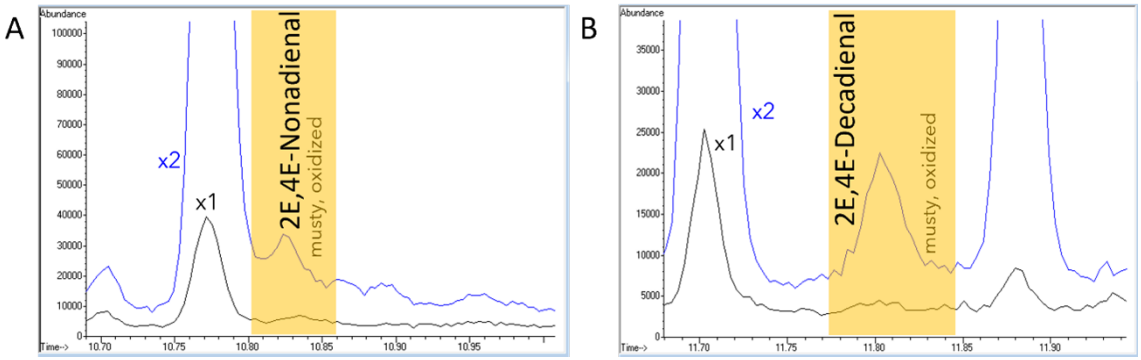

Figure 4: TD multidesorption mode for identification of 2E,4E-Nonadianal (A) and 2E,4E-Decadienal (B).

Conclusion

This study has demonstrated the ability of an SDA methodology to identify key sensory-active compounds responsible for off-odors formed by lipid oxidation. The data shows distinct differences in chromatography and sensory perception between fresh, aged, and very aged samples. Notably, the presence or absence of a compound does not necessarily provide insight into its sensory impact on the sample. Many compounds were detected by the MS but produced no odor at the ODP. In contrast, several compounds were smelled at the ODP, but no peak signal was seen. The invaluable information would be missed in an MS-only approach. The SDA approach could be readily used for a wide variety of applications to identify sensory-active compounds and, as a result, create high-quality food products and enhance consumer satisfaction.

References

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