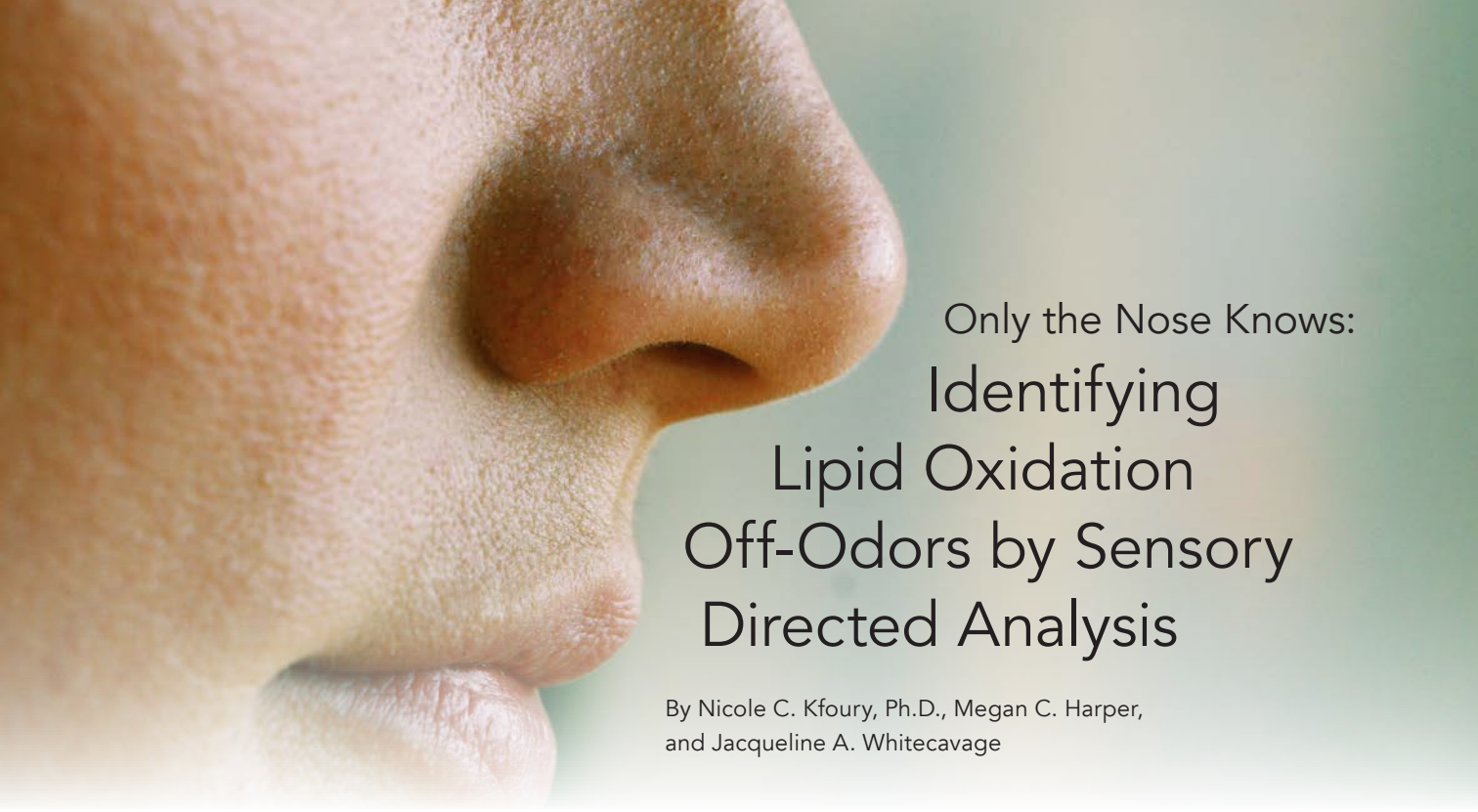

Only the Nose Knows: Identifying Lipid Oxidation Off-Odors by Sensory Directed Analysis

By Nicole C. Kfoury, Ph.D., Megan C. Harper, and Jacqueline A. Whitecavage

Introduction

Lipids are vital to human nutrition, providing energy for biological processes, maintaining brain function, and facilitating the absorption of fat-soluble vitamins [1,2]. Lipid oxidation in food is therefore associated with loss of nutritional value, but as lipids break down, degradation products that are sensory-active even at very low concentrations are also formed, resulting in noticeable rancidity off-odors and reduced customer acceptance[2,3]. Sensory Directed Analysis (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 in parallel enables determination of sensory-active regions of the chromatogram and simultaneous mass spectral identification of the associated compounds. As a result, SDA is the ideal technique for solving sensory-related challenges in food products.

In this study, direct thermal extraction (DTE) and dynamic headspace (DHS) were used as automated, solventless means of extracting and concentrating analytes from different sample types. DTE is performed directly in the Thermal Desorption Unit (TDU 2) to determine volatile and semi-volatile organic compounds (VOCs and SVOCs) at trace levels. DHS involves purging the headspace above a solid or liquid sample with inert gas, extracting and then concentrating volatiles on a sorbent-filled trap, resulting in improved recovery and extremely low limits of detection compared with equilibrium headspace. TD Multidesorption Mode can be used to stack analytes from multiple extractions for increased mass on column in areas of interest where no peak signal is initially seen.

Experimental

Instrumentation

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

Standard Preparation

Standards of hexanal, octanal, nonanal, 2E-octenal, 2E-nonenal, 2E,4E-heptadienal, 2E,4E-nonadienal, 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. To confirm the identity of the lipid oxidation off-odor compounds, standards were analyzed using the same instrument conditions.

Sample Preparation

Fresh and aged canola oil, wheat crackers, and cheddar crackers were analyzed to determine the lipid oxidation off-odors. The canola oil was aged at 40 °C for several weeks. Wheat and cheddar crackers were aged at ambient temperature for one year. The 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 micro-vial, transferred to an empty glass TDU tube, capped with a transport adapter, and placed in a sealed position in a tray on the MPS robotic autosampler for DTE analysis. A 2.0 g sample of each cracker was weighed into individual 20 mL screw-capped vials and placed in the sample tray on the MPS robotic for DHS analysis.

Sample Introduction

Oil samples were extracted by DTE at 90 °C for 15 minutes with a 50 mL/min helium flow. Analytes were trapped in the CIS 4 inlet using a glass bead-filled liner at -120 °C. After the extraction was completed, the trapped analytes were transferred to the GC column in split mode (5:1) by rapidly heating the CIS 4 inlet to 280 °C.

The cracker samples were incubated in the DHS module at 40 °C for 2 minutes and then extracted for 20 minutes at 50 mL/min helium flow for a total purge volume of 1000 mL. Analytes were trapped at 25 °C on a Tenax®TA packed tube. For analysis, the tubes were desorbed in the TDU 2 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. Following tube desorption, the trapped analytes were transferred to the GC column in split mode (5:1) by rapidly heating the inlet to 280 °C.

The GC oven temperature started at 35 °C (2 min), ramping 15 °C/min to 280 °C (2 min). The carrier gas was kept at a constant flow of 1 mL/min and the column used was a 30 m HP-5MS UI, di = 0.25 mm, df = 0.25 µm. The MSD was operated in full scan mode (40-350 amu).

Olfactometry

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

Results and Discussion

Each sample was initially subjected to direct sensory evaluation to determine the odors of interest associated with lipid oxidation, as shown in Table 1:

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 fresh samples exhibited the odors expected from each respective food product, 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, a Thermal Extractor (TE 2) was used to release and individually assess the total odor extracted from each oil and and each DHS sorbent trap. The released total extracted odors were assessed by direct olfactory detection a the TE 2 outlet.

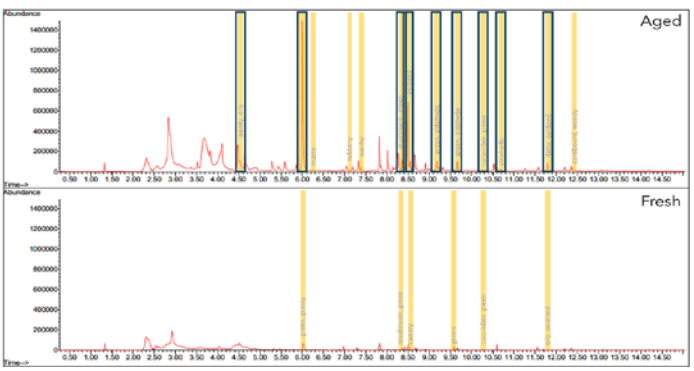

Figure 1: Stacked view of total ion chromatograms of aged (top) and fresh (bottom) canola oil.

Figures 1-2 show the stacked view of aged (top) and fresh (bottom) canola oil and wheat crackers. 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 are significantly greater peak signals 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 compounds, and the peak areas normalized to the fresh samples are shown in Tables 2-3.

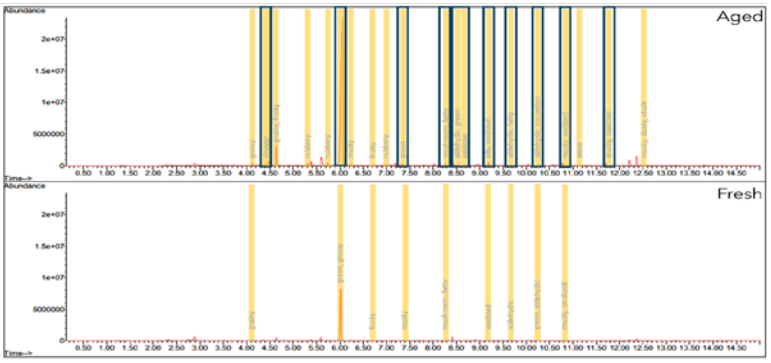

Figure 2: Stacked view of total ion chromatograms of aged (top) and fresh (bottom) wheat crackers.

The aged canola oil was described as oxidized, painty, green, and aldehydic. Oxidized and painty odors detected at the ODP 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, likely because the concentration in the fresh sample was below the odor threshold. 2E,4E-decadienal, on the other hand, has a very low odor threshold and could be detected at the ODP in both samples, but was below the instrument limit of detection in the fresh sample.
The fresh canola oil had only a slight green and aldehydic aroma; in the aged sample this was much more pronounced. Several green and aldehydic odors were detected at the ODP. Hexanal and nonanal were assessed 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 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, as can be seen in Figure 2.The former two were identified in both the fresh and aged samples at the MS but were only detected 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, as can be seen in Table 3.
In three regions, green and oxidized odors were detected at the ODP but not by the MS. The compounds in the two regions around 10.83 and 11.82 minutes were identified by increasing mass on column through the TD Multidesorption Mode, as can be seen 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

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

RT	Odor Characteristic		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
10.2	cucumber, green	cucumber, green	n.d.		
10.7		aldehydic	Decanal	n.d.	1.0
11.8	oily, oxidized	fatty, oxidized	2E,4E-Decadienal	n.d.	1.0

Note: n.d. = not detected

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

and odor descriptor as the unidentified compound in the canola oil, which could be identified in the very aged cheddar crackers as 2E-nonenal. 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 were drastically increased in the very aged samples, potentially due to exposure to elevated temperatures for a prolonged time. These fatty acids cause 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 crackers but are no longer present in the very aged sample. It is likely that these compounds are breaking down and forming methyl ketones at the elevated temperature, to which the very aged sample was exposed[1]. The methyl ketones also contribute to the fatty and rancid odors in the very aged sample. For more detailed information, please see the associated GERSTEL AppNote[5].

Conclusion

Sensory Directed Analysis (SDA) can help identify key sensory-active off-odor compounds formed by lipid oxidation. The data shows distinct differences in chromatography and sensory perception between fresh, aged, and very aged samples[5]. Notably, the presence or absence of a compound in the MS chromatogram does not prove or disprove its sensory impact on the sample. Many compounds were detected by the MS but produced no detectable odor at the ODP. In contrast, several compounds were smelled at the ODP, but no peak signal was seen. Invaluable information is missed in an MS-only approach. The SDA approach can readily be used for a wide variety of applications to identify sensory-active compounds. SDA is a critical tool when creating high-quality food products and working to maintain brand loyalty and customer satisfaction.

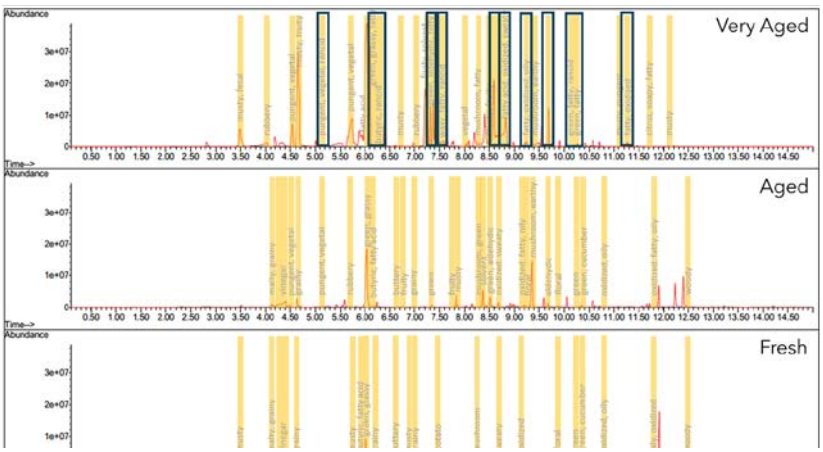

Figure 3: Stacked view of total ion chromatograms of very aged (top), aged (middle), and fresh (bottom) cheddar crackers.

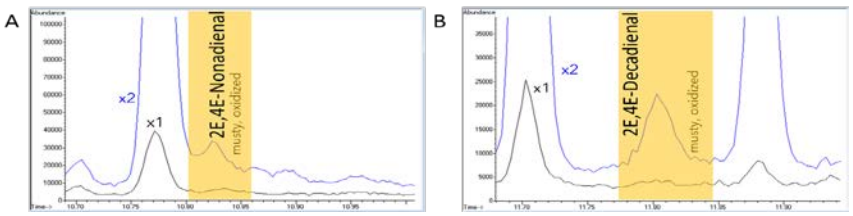

Figure 4: TD Multi-Desorption mode for identification of 2E,4E-nonadienal (A) and 2E,4E-Decadienal (B).

References:

[1] S. Grebenteuch, C. Kanzler, S. Klaussnitzer, L. W. Kroh, and S. Rohn. The formation of methyl ketones during lipid oxidation at elevated temperatures. Molecules 26 (2021).

[2] S. Böttcher, U. Steinhäuser, S. Drusch. Off-flavor masking of secondary lipid oxidation products by pea dextrin. Food Chemistry 169 (2015) pp. 492-498.

[3] P. Goméz-Cortés, G. L. Sacks, J. T. Brenna. Quantitative analysis of volatiles in edible oils following accelerated oxidation using broad spectrum isotope standards. Food Chemistry 174 (2015) pp. 310-318.

[4] Hayaloglu A. A., Karabulut, I. Characterization and comparison of free fatty acid profiles of eleven varieties of Turkish cheeses. Int J Food Prop 16 (2013) pp. 1407-1416.

[5] Nicole C. Kfoury, Megan C. Harper, and Jackie A. Whitecavage. GERSTEL AppNote: Identification of Off-Odor Compounds Associated with Lipid Oxidation in Food Products Using Sensory Directed Analysis.

SDA Workshops in the US and Germany

GERSTEL's 3-day SDA Workshop is designed for analytical chemists and sensory scientists. The workshop integrates sensory and instrumental analysis for investigating sensory-active compounds to solve critical challenges in a wide variety of products and sample types. It includes lectures, hands-on demonstrations, and interactive discussions with our Analytical Services Group. Interested? Please contact: workshop@gerstel.de