LabWorks APPNOTE

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# Identification of Key Sensory-Active Flavor Compounds in Plant-Based Tuna Fish using Sensory Directed Analysis

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## Keywords

Sensory Directed Analysis (SDA), Plant-Based Protein, Dynamic Headspace (DHS), Selectable $^{1}$D/$^{2}$D-Gas Chromatography-Olfactometry/Mass Spectrometry ($^{1}$D/$^{2}$D-GC-O/MS), Sensory-Active Compounds, Flavor, Olfactory Detection Port.

## Abstract

Plant-based proteins are a rapidly growing market with plant-based seafood replacement also seeing significant growth in recent years. The key to the overall food experience is the aroma perceived while consuming the product. The challenge with plant-based protein sources is that they often produce aromas not traditionally associated with the product of interest. In this study, a sensory directed analysis method was employed to identify and compare key sensory-active flavor compounds in plant-based tuna with those in real tuna fish. This method utilized dynamic headspace extraction with gas chromatographic separation and simultaneous olfactory and mass spectral detection. Combining olfactory detection of individual sensory-active compounds with GC-MS determination of their identity is crucial to understanding the most desirable aromas and to best replicating them in food products.

## Introduction

The plant-based food market is projected to reach nearly $200 billion by 2030. As health awareness grows, consumers are turning towards natural, plant-based products over animal-based products. Concerns about red-meat consumption, antibiotics in livestock, and climate change are leading factors for the booming plant-based meat industry. In a similar fashion, fears of overfishing, heavy-metal consumption, and microplastics are fueling the demand for plant-based seafood replacement. Plant-based tuna fish is one of the first such products on the market. As a result, manufacturers face the challenge of replicating the taste and texture of animal products in plant-based foods.

Sensory directed analysis (SDA) is a process that utilizes gas chromatography in combination with the human nose and mass spectrometry to identify sensory-active flavor compounds. The use of olfactory and MS detection enables simultaneous determination of sensory-active regions of the chromatogram and mass spectral identification of the associated flavor compounds. As a result, SDA can be used to solve sensory-related challenges by determining the compounds responsible for producing desirable flavors in food products.

In this study, dynamic headspace (DHS) was used as an automated, solventless means of extracting analytes. Selectable $^{1}$D/$^{2}$D-Gas Chromatography-Olfactometry/Mass Spectrometry ($^{1}$D/$^{2}$D-GC-O/MS) or "heart-cutting" GC was used to resolve components in the complex matrix. The system is configured with two low thermal mass (LTM) GC columns with dissimilar column phases and a valveless, software-controlled column switching device to easily implement a 2D GC separation. The combination of techniques in an SDA approach enabled the identification of key flavor compounds in real and plant-based tuna fish products.

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## Experimental

*Instrumentation*

GERSTEL LabWorks Platform with Dynamic Headspace (DHS), Selectable $^{1}D/^{2}D$ System and Olfactory Detection Port (ODP 4) on Agilent 8890/5977B GC/MSD with LTM option, GERSTEL Thermal Extractor (TE 2)

*Analysis Conditions*

| | |
|---|---|
| LabWorks Platform | |
| DHS | |
| Trap | Tenax® TA |
| Incubation | 30 °C (5 min) |
| Sampling | Sample 30 °C |
| | Trap 25 °C |
| | Volume 200 mL (20 mL/min) |
| TDU | |
| Pneumatics mode | Solvent venting |
| Temperature | 40 °C (1 min); 720 °C/min to 280 °C (3 min) |
| CIS | |
| Liner | Glass bead packed |
| Pneumatics mode | Solvent vent (50 mL/min), split 10:1 |
| Temperature | -120 °C; 12 °C/sec to 280 °C (3 min) |
| Agilent 8890 GC | |
| Pneumatics | He, $p_i$ = 335.17 kPa |
| | Constant pressure ($^{1}D$) |
| | Ramped pressure with backflush ($^{2}D$) |
| Oven | 250 °C, isothermal |
| LTM-Column 1 | 30 m DB-5MS UI (Agilent) |
| | $d_i$=0.25 mm, $d_f$=0.25 µm |
| Temperature | 40 °C (1 min); 10 °C/min to 280 °C (3 min) |
| LTM-Column 2 | 30 m DB-WAX (Agilent) |
| | $d_i$=0.25 mm, $d_f$=0.25 µm |
| Temperatures | 40 °C (3.1 min); 5 °C/min to 240 °C (real tuna) |
| | 40 °C (4.5 min); 10 °C/min to 240 °C (plant-based tuna 1) |
| | 40 °C (4.15 min); 10 °C/min to 240 °C (plant-based tuna 2) |
| Agilent 5977B MSD | |
| Mode | Full scan, 40 – 350 amu |

*Sample Preparation*

Two plant-based products and one real canned tuna fish product were purchased from a local store. A 2.5 g sample of each was taken and these were placed into separate 20 mL screw-capped vials for DHS extraction.

*Standards Preparation*

Standards of trimethylamine, 1-penten-3-ol, allyl methyl sulfide, diallyl sulfide, 2-ethylfuran, and 3-methylbutanal were prepared in methanol. One microliter of standard was spiked onto the glass frit of a 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.

*Olfactometry*

GC-O analysis was performed on a Selectable $^{1}D/^{2}D$-GC-MS system equipped with an Olfactory Detection Port 4 (ODP 4) as shown in Figure 1. The column effluent was split 2:1 between the ODP 4 and MS, respectively. The ODP transfer line was heated to 280 °C. The mixing chamber was heated at 150 °C and purged with humidified nitrogen to prevent olfactory fatigue and nasal dehydration.


**Figure 1:** Selectable $^{1}D/^{2}D$-GC-O/MS system.

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## Results and Discussion

Each sample was subjected to a sensory analysis to identify key odors of interest as shown in Table 1. While the real tuna sample smelled fishy and meaty, the two plant-based tunas had distinct vegetal aromas. To confirm that the DHS successfully extracted the odors of interest the Thermal Extractor (TE 2) was used to smell the total odor released from the sorbent tube.

**Table 1:** Sensory characteristics of real and plant-based tuna samples.

| Sample | Sensory Characteristic |
|---|---|
| Real Tuna | fishy, brothy, meaty |
| Plant-Based Tuna 1 | beany, vegetal |
| Plant-Based Tuna 2 | grainy, vegetal, seaweed |

Figure 2 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 a total odor profile of the extract. The DHS extractions were found to be representative of the odors detected in each of the tuna samples.

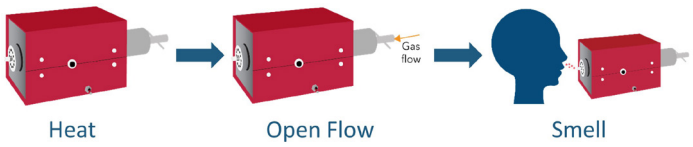


**Figure 2:** TE setup for smelling the total odor of the DHS extracts.

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Figure 3 shows the stacked view of real tuna (top), plant-based tuna 1 (middle), and plant-based tuna 2 (bottom). The chromatograms, in red, are overlaid with the olfactory regions in yellow. Odor regions that are representative of key sensory characteristics determined in the samples are marked in blue. The odor regions of interest are different in the three tuna samples, confirming the use of plant-based ingredients produced odors that are not always like those in the real product.

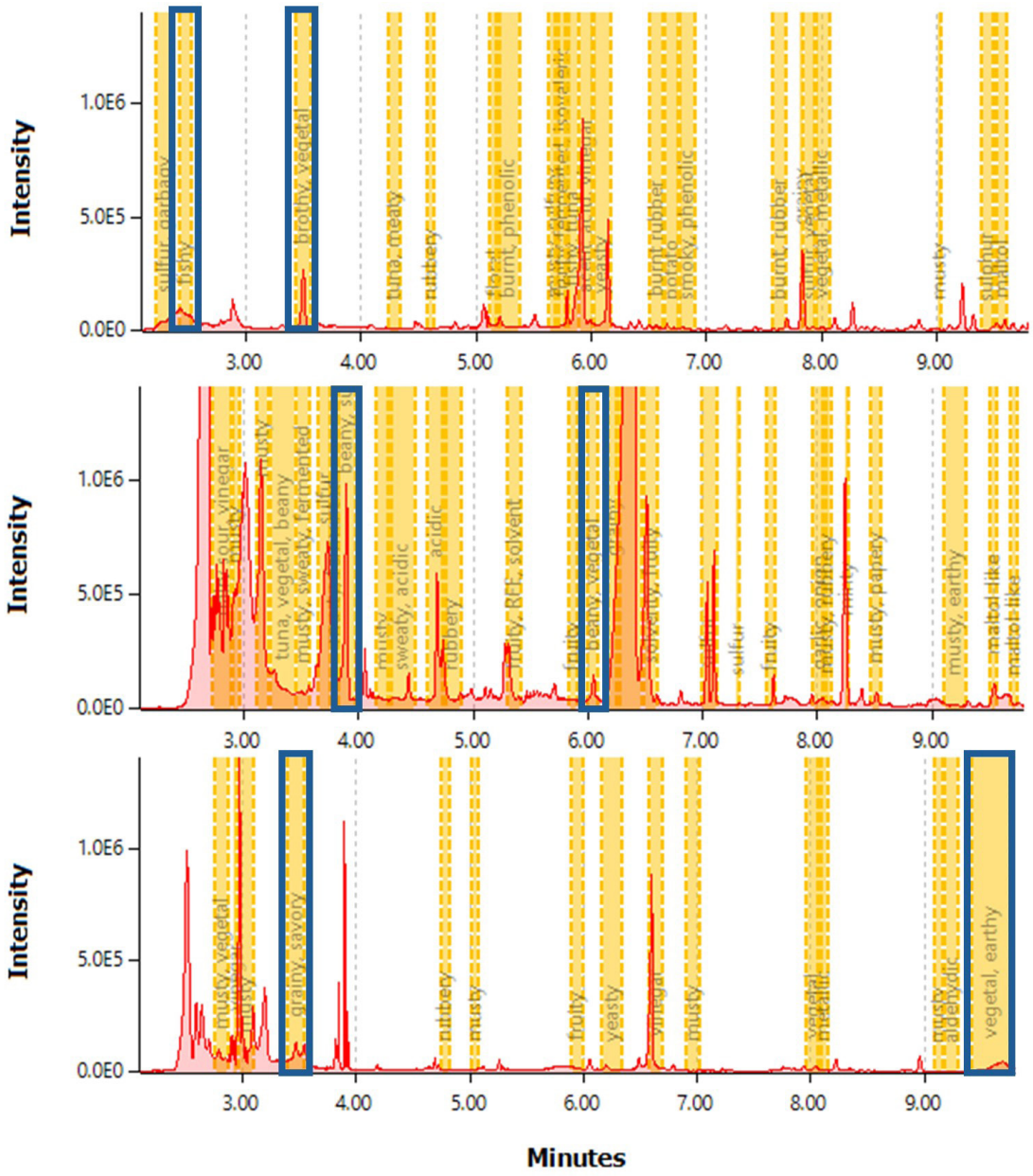


**Figure 3:** Stacked view of real tuna (top), plant-based tuna 1 (middle), and plant-based tuna 2 (bottom) GC-O/MS data with odors regions of interest marked in blue.

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For the real tuna samples, there are two odor regions of interest in the sample as shown in the top chromatogram of Figure 3. Figure 4 shows the peak at 3.5 minutes, which was described as brothy and vegetal and identified as 1-penten-3-ol. A standard of 1-penten-3-ol was analyzed to confirm that the retention time, mass spectrum, and odor matched that of the sample. Figure 5A shows the region of interest at 2.5 minutes, which had a fishy odor, but coelution prevented compound identification. Therefore, the region was heart-cut to the second column for additional separation. Figure 5B shows the $^{2}$D chromatogram where the peak with the fishy odor was separated and identified as trimethylamine. A standard of trimethylamine was analyzed by both $^{1}$D and $^{2}$D GC-O/MS to confirm that the retention time, mass spectrum, and odor matched that of the sample.

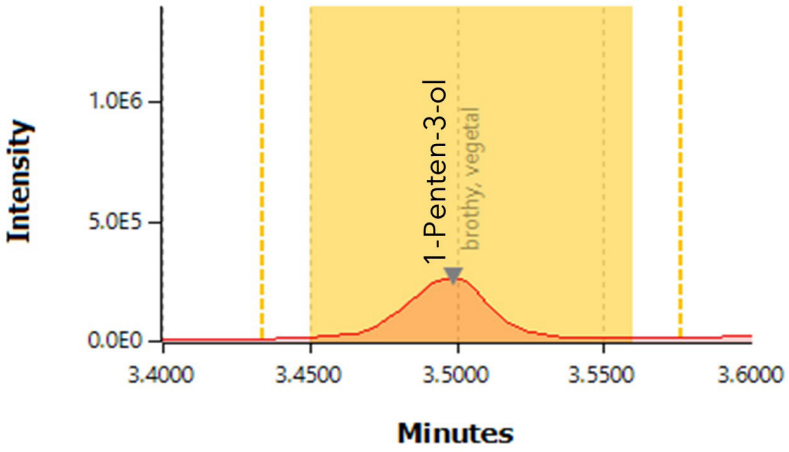


**Figure 4:** Chromatogram of odor region of interest at 3.5 minutes, identified as 1-penten-3-ol, in real tuna.

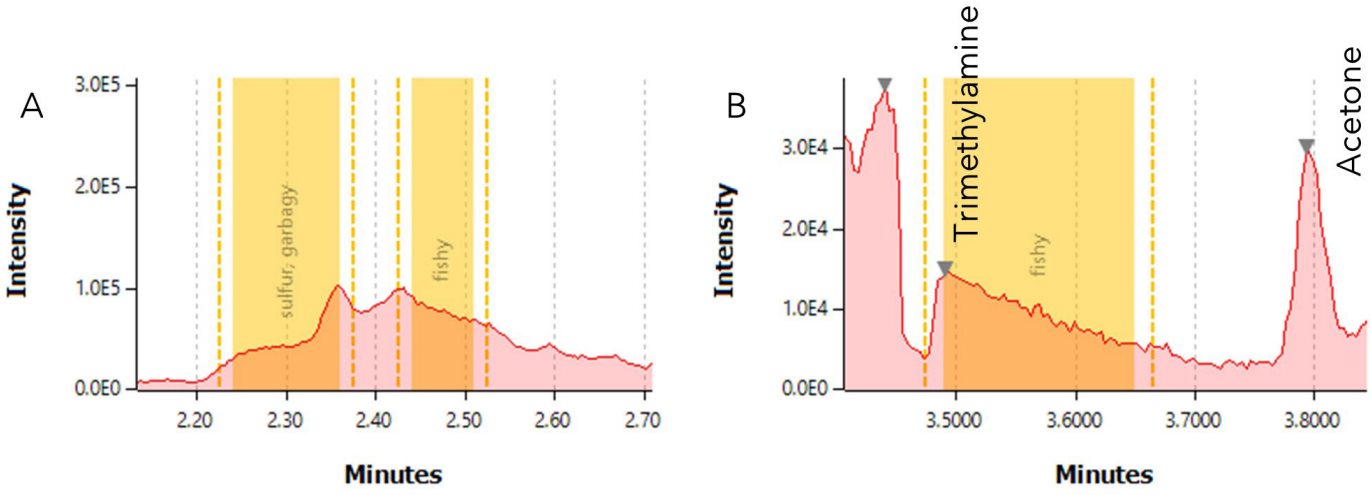


**Figure 5:** $^{1}$D (A) and $^{2}$D (B) chromatograms of the odor region of interest described as fishy and identified as trimethylamine in real tuna.

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For plant-based tuna 1, there are two odor regions of interest in the sample as shown in the middle chromatogram of Figure 3. Figure 6 shows the peak at 6.0 minutes, which was described as beany and vegetal and identified as diallyl sulfide. A standard of diallyl sulfide was analyzed to confirm that the retention time, mass spectrum, and odor matched that of the sample. The region of interest at 3.9 minutes had an odor described as beany and sulfur-like. Figure 7A shows the peak at 3.9 mins, where AMDIS was used to deconvolute two compounds, allyl methyl sulfide and 2-ethylfuran. A heart-cut was employed to discern which peak was responsible for the odor detected. However, when the region of interest was cut to the second column, the two compounds still coeluted, as shown in Figure 7B. Standards of both compounds were purchased and analyzed separately to confirm which was responsible for the odor. The beany, sulfur odor could be attributed to allyl methyl sulfide whereas 2-ethylfuran had no detectable odor.

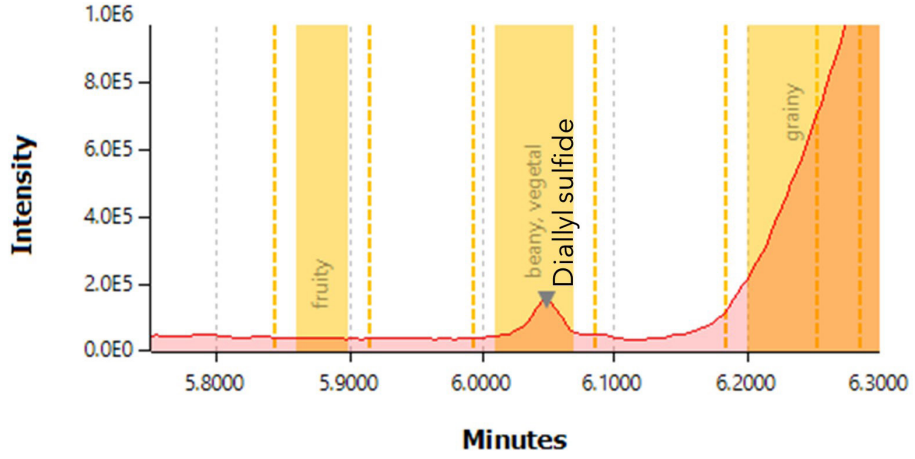


**Figure 6:** Chromatogram of odor region of interest at 6.0 minutes, identified as diallyl sulfide, in plant-based tuna 1.

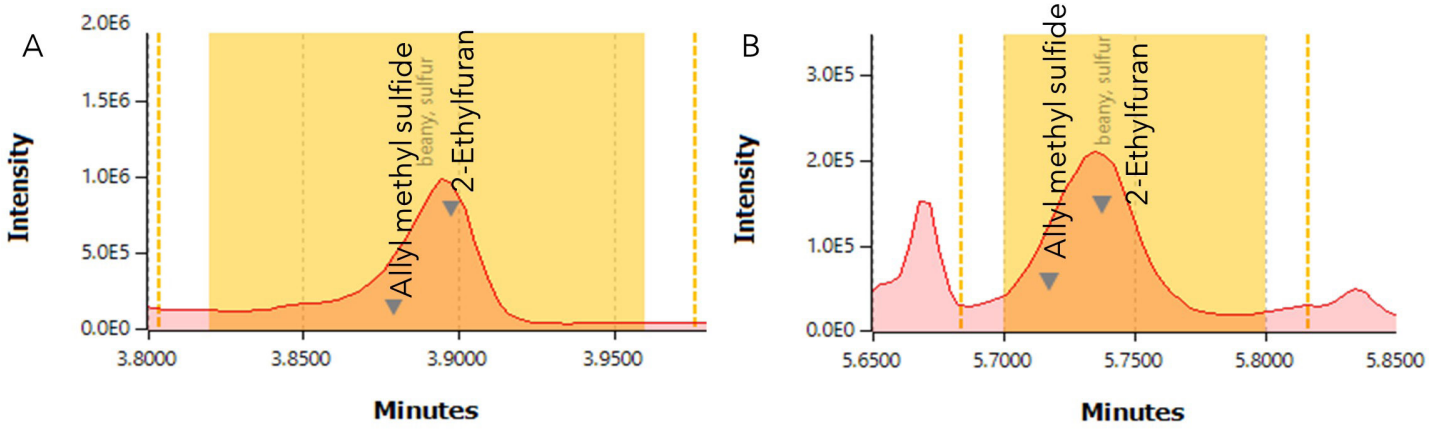


**Figure 7:** $^{1}D$ (A) and $^{2}D$ (B) chromatograms of the odor region of interest described as beany and sulfur identified as allyl methyl sulfide in plant-based tuna 1.

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For plant-based tuna 2, there are two odor regions of interest in the sample as shown in the bottom chromatogram in Figure 3. Figure 8 shows the peak at 9.7 minutes, which was described as vegetal and earthy and identified as 3-ethyl-2,5-dimethylpyrazine. To confirm the identification, a standard would need to be purchased and analyzed to match retention time, mass spectrum and odor to the sample. The odor region of interest at 3.35 minutes is described as grainy and savory. Figure 9A shows the peak at 3.35 mins, where AMDIS was used to deconvolute three compounds, 3-methylbutanal, 2-methylbutanal, and thiophene. Figure 9B shows the heart-cut chromatogram where thiophene is separated from the other two compounds and has no detectable odor. However, 3- and 2-methylbutanal are still coeluting at the odor region of interest. A standard of 3-methylbutanal was analyzed and retention time, mass spectrum, and odor matched that of the sample. However, to confirm if 2-methylbutanal was also contributing to the odor, a standard would be needed to determine if retention time, mass spectrum, and odor matched the sample.

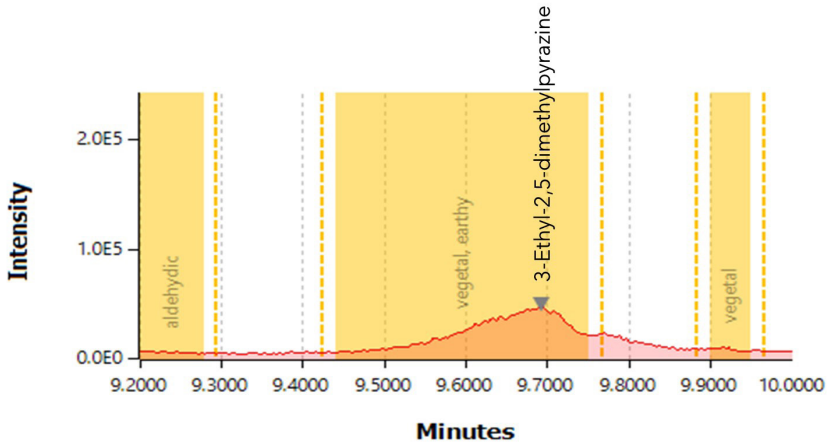


**Figure 8:** Chromatogram of odor region of interest at 9.7 minutes, identified as a 3-ethyl-2,5-dimethylpyrazine, in plant-based tuna 2.

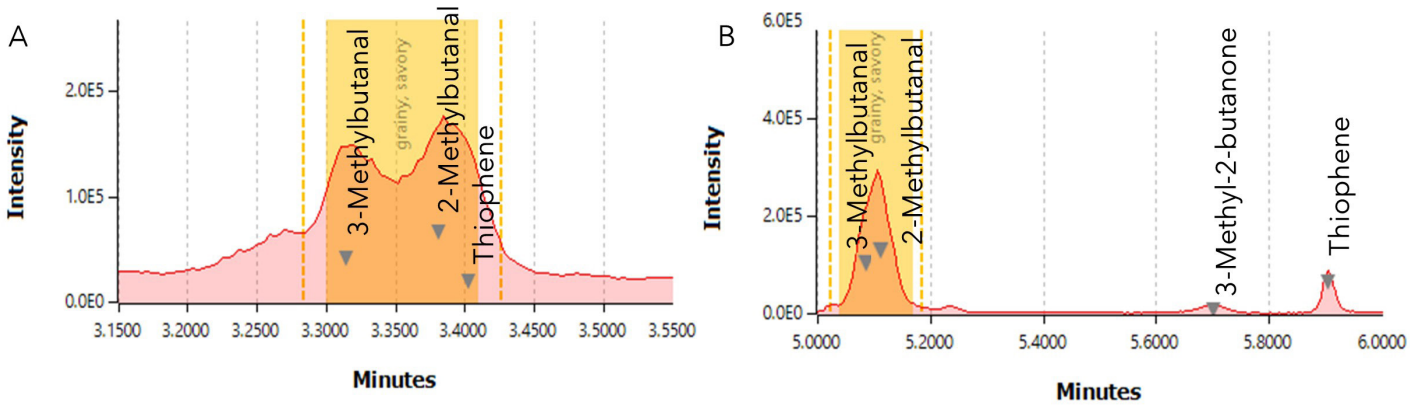


**Figure 9:** [1]D (A) and [2]D (B) chromatograms of the odor region of interest described as grainy and savory in plant-based tuna 2.

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## Conclusion

This study has demonstrated the ability of an SDA methodology to identify key sensory-active compounds in tuna fish samples. The use of DHS provided representative sample extracts for analysis and $^{1}D/^{2}D$-GC-O/MS resolved areas where coelution occurred. This approach could be readily used for identifying sensory-active compounds in a variety of sample types, enabling manufacturers of plant-based replacement products to better replicate the real product flavor, and thus increasing the likelihood of consumer acceptance and appreciation.