GC-MS Techniques for Troubleshooting Off-Flavor, Off-Odor, and Flavor Scalping in Foods, Beverages

Eurofins S-F Analytical Laboratories Inc.
Jamie Willems, Ph.D. & Lily Zehfus, M.S.

August 4, 2022

Presentation Overview

Introduction

Background on Analytical Techniques

Case Studies

Introduction

- Aroma & taste are important components of consumer appeal.
  - Product identity
  - New product formulations
  - Quality assurance
  - Consumer trust

- Off-odors/flavors are off-putting to consumers and may be a sign of serious problems!

- Common causes of off-odors/flavors can include:
  - Packaging Issues
  - Chemical or Microbial Contamination
  - Production Issues

Packaging Issues

- **Packaging incompatibility:** packaging interacts with product
  - Acidic product degrades an improperly coated can aluminum can.
  - Potential safety concern!
  - **Flavor scalping**: loss of vital flavor and odor molecules through absorption into the packaging material
    - Decrease in product quality over time
  - **Leaching**: unwanted molecules from the packaging material migrate into the product
    - Potential safety concern!
- **Product degradation:** degradation of profile components during storage.
  - Molecules sensitive to storage conditions
    - e.g. Light sensitive or heat sensitive
  - Insufficient preservatives or antioxidants

Contamination

Chemical

- Storage issues
  - Leaching from storage receptacle (e.g. holding tank)
  - Leaching from packaging
- Improper Cleaning
  - Insufficient rinsing procedure
  - New cleaning agent
- User error
  - Excess lubricant added to machinery during maintenance contaminates product line

Microbial

- Improper storage
  - Too long at room temperature
- Improper handling
  - Poor hygiene
- Cross-contamination
  - Exposure to raw product (e.g. meat)
- Processing failure
  - Incomplete pasteurization

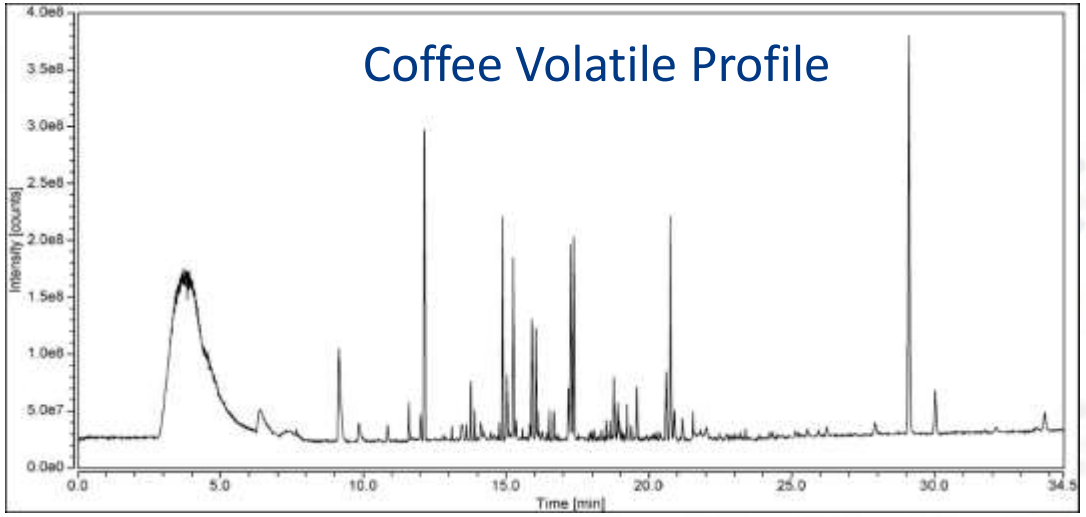
Production Issues

- Equipment failure
  - Oven fails to reach proper temperature leading to spoilage
- Process failure
  - Failure to complete defatting/pasteurization, etc.
- User error
  - Failure to recognize out of calibration equipment leads to improperly measured or processed ingredients
- Ingredient issue
  - Impure ingredient due to incomplete reaction
  - Change in quality of ingredients with new supplier

In many cases, an off-odor or flavor is caused by a combination of system and human errors!

Technical Approach: Aromatic Profiling

- The profile or ‘fingerprint’ of volatile compounds contributes to the overall aroma of a product.
- By identifying profile differences between a good and bad sample, compounds which may contribute to off-odors can be identified.
  - These compounds can then be further investigated and linked to a cause or source of the off odor.



Compound	RT (min)	Odor/Aroma
Methyl-pyrazine	11.28	Nutty, roasted
2,5-dimethyl-pyrazine	12.31	Nutty, roasted
2,6-dimethyl-pyrazine	12.41	Nutty, roasted
4-propyl-pyridine	13.40	Green, fatty
2,3,5-trimethyl-pyrazine	13.74	Nutty, roasted
Acetic acid	14.27	Sour, acidic
1-(acetyloxy)-2-propanone	14.50	Fruity
Furfural	14.56	Bready, baked
Benzaldehyde	15.65	Almond, cherry

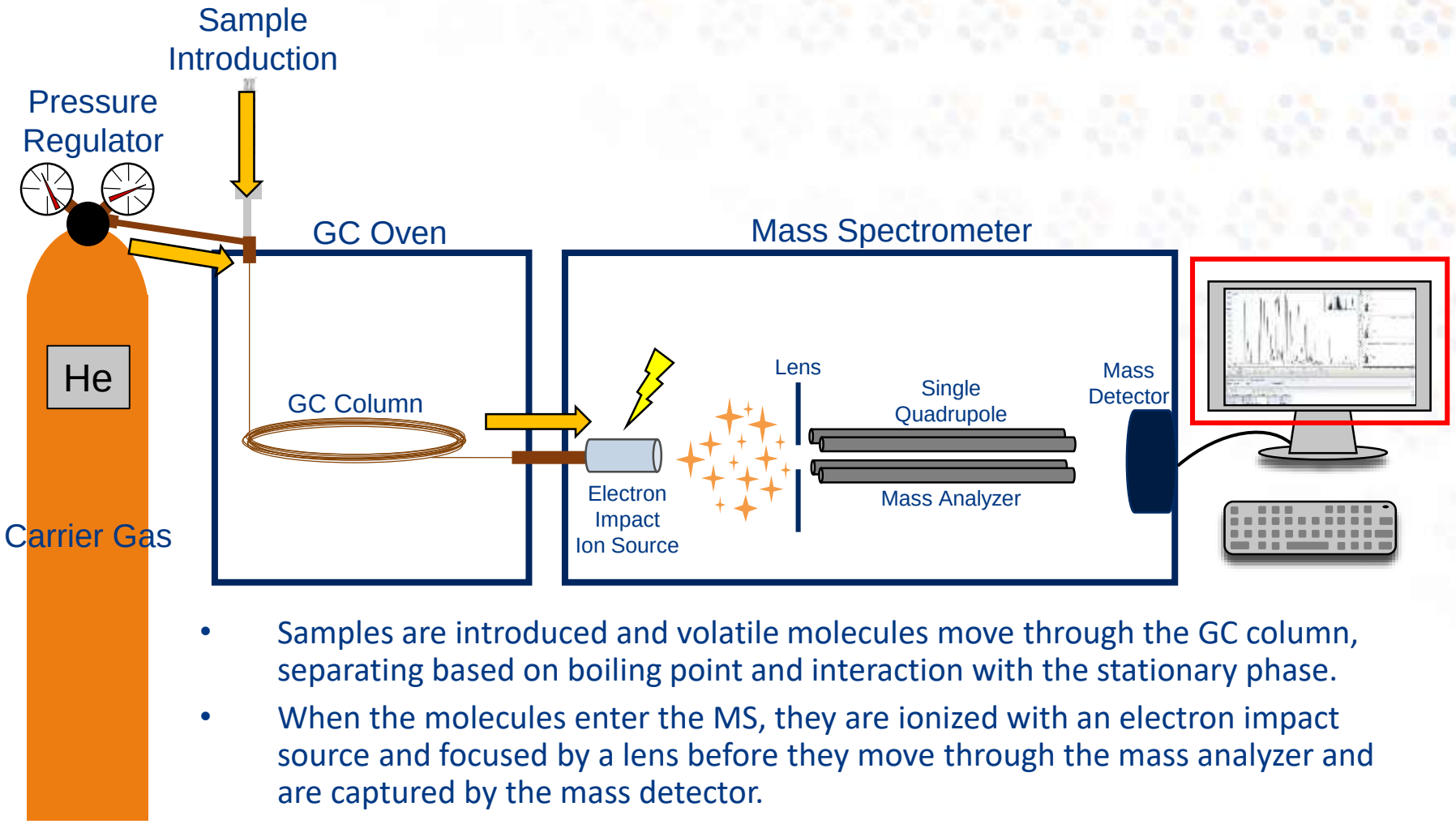
Analytical Technique

eurofins

Gas Chromatography with Mass Spectrometry

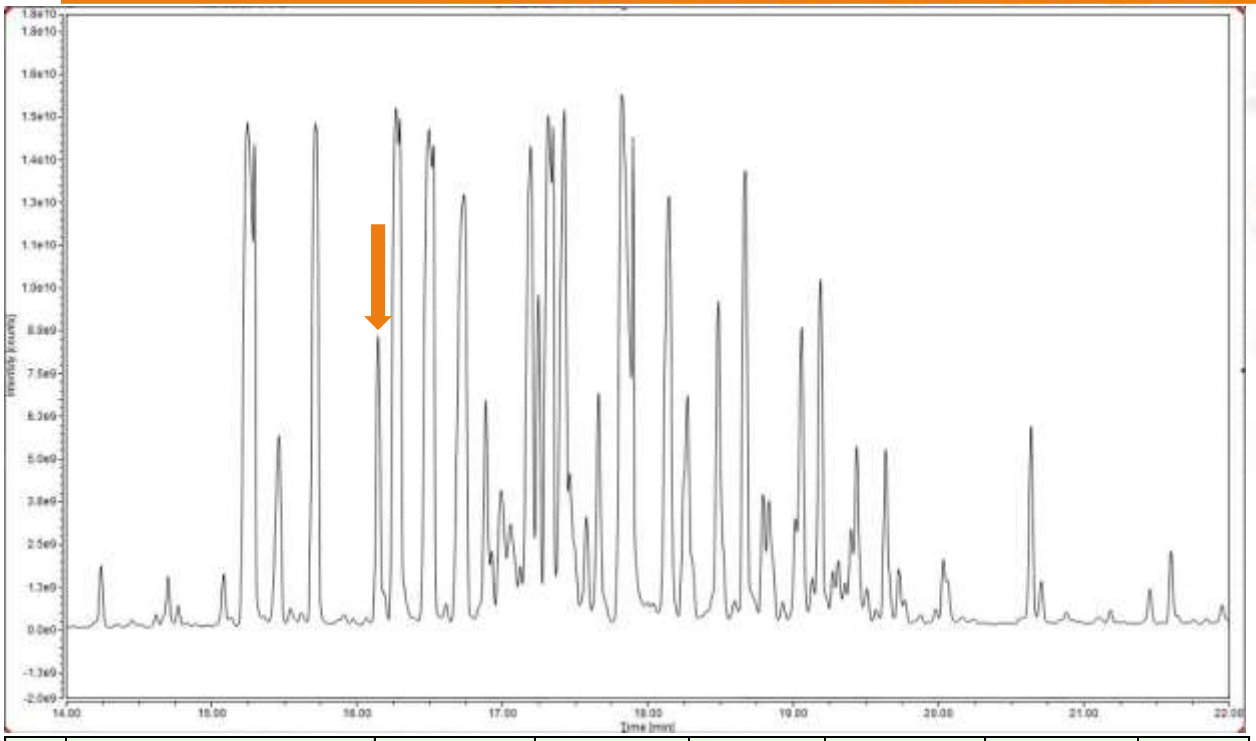
- Gas chromatography coupled with mass spectrometry (GC-MS) is a powerful technique and our flagship approach for the analysis of volatile profiles.
  - Compounds are volatilized and separated in a gas phase based on their affinity to the stationary phase.
- Mass spectrometry is used for compound identification based on characteristic fragmentation patterns.
  - Library of >300,000 compounds

Gas Chromatography with Mass Spectrometry

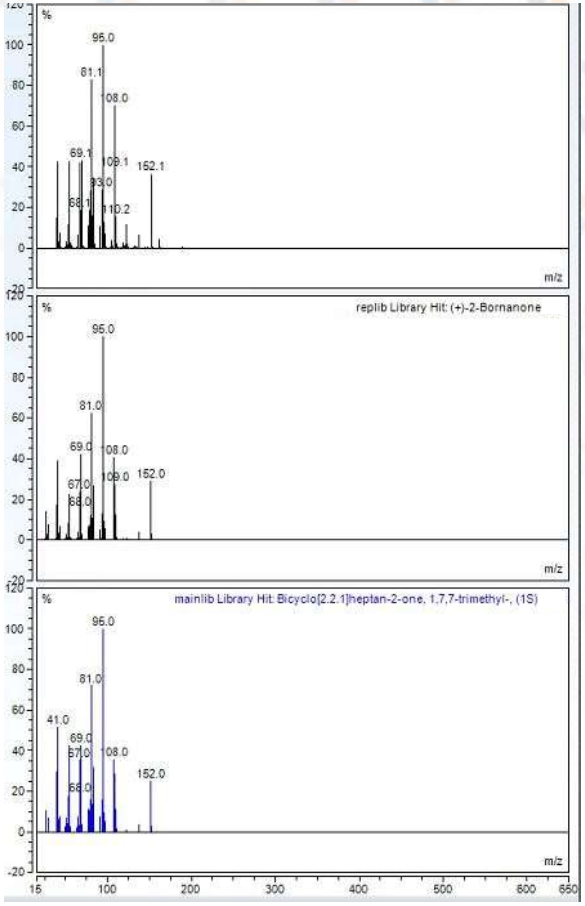


- Samples are introduced and volatile molecules move through the GC column, separating based on boiling point and interaction with the stationary phase.
- When the molecules enter the MS, they are ionized with an electron impact source and focused by a lens before they move through the mass analyzer and are captured by the mass detector.

Gas Chromatography with Mass Spectrometry



Mass Spectrum for Peak at 16.18 min



No.	Name	CAS Number	Mol. Weight	SI	Retention Time min	Area counts*min	Rel.Area %
34	Geranyl isovalerate	109-20-6	238	700	14.874	59486439.8	0.10
35	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	1783-84-2	306	767	14.954	21643464.4	0.04
36	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5	15537-55-0	154	844	15.135	78151582.8	0.13
37	Cyclohexanone, 5-methyl-2-(1-meth	491-07-6	154	876	15.229	1442065034.4	2.41
38	Cyclohexanone, 5-methyl-2-(1-meth	491-07-6	154	892	15.387	193198954.1	0.32
39	2,4-Cycloheptadien-1-one, 2,6,6-trim	503-93-5	150	726	15.538	1165428620.9	1.95
40	Cyclohexanone, 5-methyl-2-(1-meth	491-07-6	154	883	15.725	1028330997.8	1.72
41	Cyclohexanone, 5-methyl-2-(1-meth	491-07-6	154	851	15.799	542304025.5	0.91
42	Copaene	3856-25-5	204	894	15.880	238977025.8	0.40
43	Longifolene	475-20-7	204	854	16.034	47118297.5	0.08
44	1,2,4-Methenoazulene, decahydro-1	1137-12-8	204	841	16.071	57765842.6	0.10
45	(+)-2-Bornanone	464-49-3	152	900	16.175	775344200.3	1.29

Figure 1. GC-MS chromatogram and compound identifications for essential oil.

eurofins

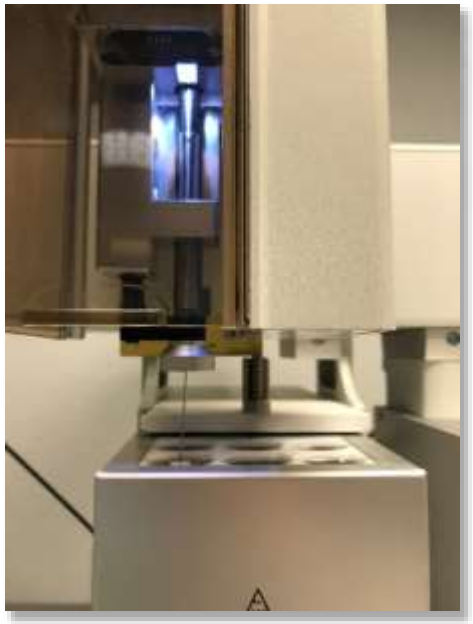
Technique: Solid Phase Micro-Extraction (SPME)

- SPME is a technique for the selective extraction of volatile components from a sample.
- SPME employs the use of fibers coated with a thin layer of polymer material.
- This material acts as an extracting phase allowing for selective extraction and concentration of volatile compounds.
- Fiber coatings can be selected based on compound classes of interest.

Technique: Solid Phase Micro-Extraction (SPME)

SPME Tool

- Samples are incubated in headspace vials to release the volatile compounds.
- The SPME fiber is exposed to the generated headspace gases and allowed to equilibrate.
- Any adsorbed compounds are then thermally desorbed into the GC inlet and analyzed by GC-MS.



Technique: Headspace-GC-MS

- For some highly aromatic products the concentration effect from SPME may not be desired.
- In these cases, an aliquot of the un-concentrated headspace gas is used.

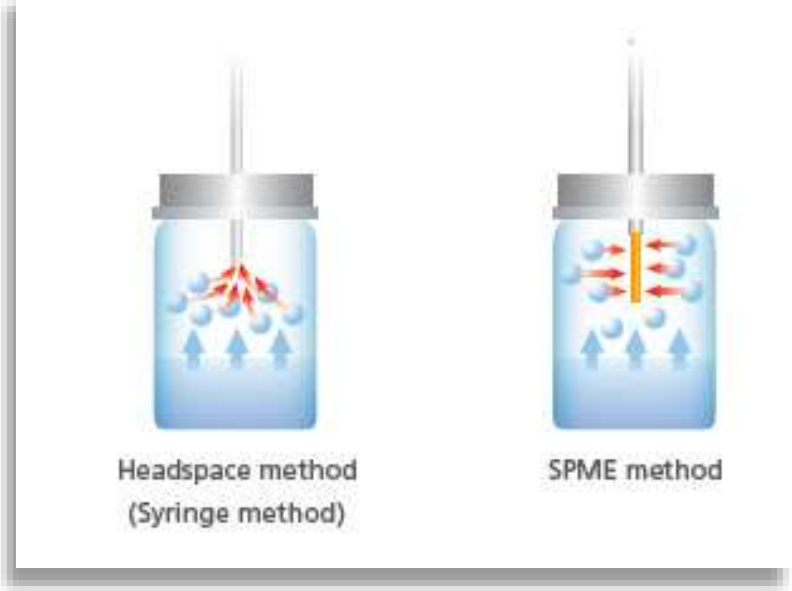


Image Credit. Shimadzu

Case Studies

eurofins

Case Study 1: Fruity Drink Contamination Investigation

After a consumer discovered an oddly colored fruity drink with an unusual aroma, they were concerned about the possibility of contamination.

Case Study 1: Fruity Drink Contamination Investigation

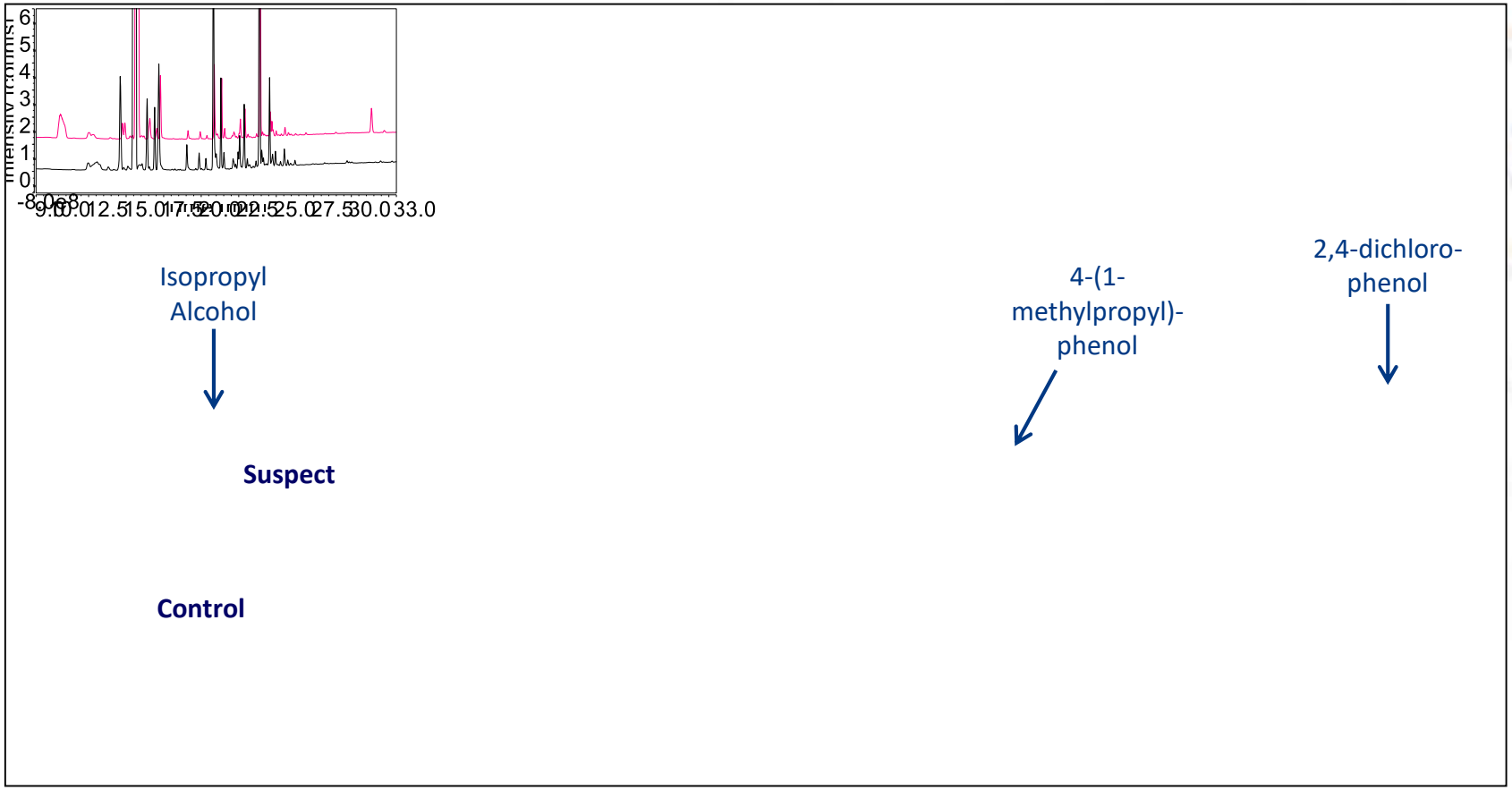


Figure 2. GC-MS profile of a complaint sample (top/pink) and the control sample (bottom/black).

Case Study 1: Fruity Drink Contamination Investigation

- The off odor sample was found to contain isopropyl alcohol, 4-(1-methylpropyl)-phenol and 2,4-dichlorophenol.
- These solvents are associated with cleaning products and disinfecting agents and **strongly** supports that the sample was contaminated.

Additional Analyses:

- Based on GC-MS results, additional analysis was performed to gain more information about the contaminating substance.
- Since the suspect had a clear difference in color from the control, the samples were analyzed using an internally developed and validated LC-UV method to screen for the major FD&C approved dyes: Blue 1, Blue 2, Yellow 5, Yellow 6, Red 3, Red 40, and Green 3.

Case Study 1: Fruity Drink Contamination Investigation

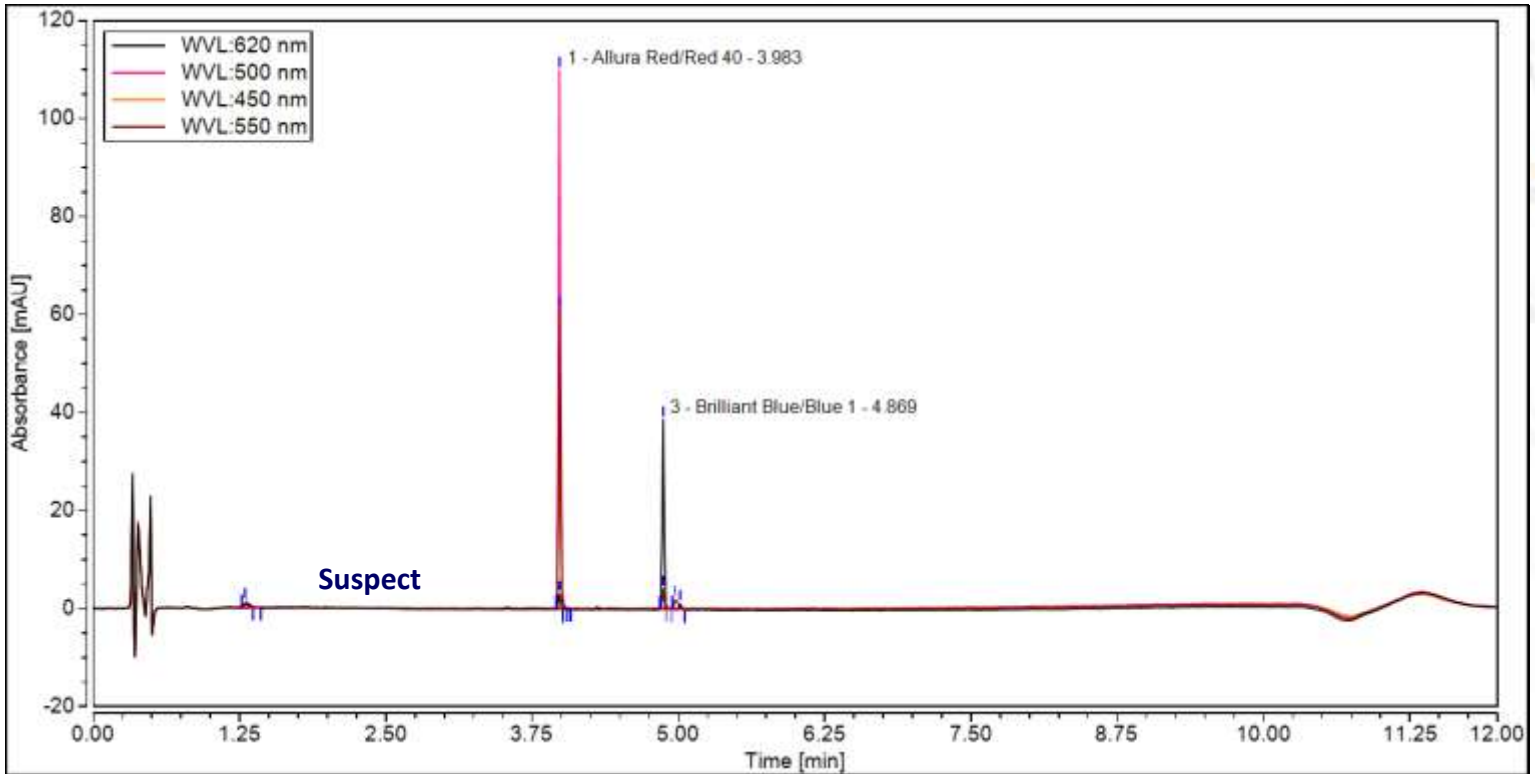


Figure 4. LC-UV chromatograms for suspect sample.

- The suspect sample contained the expected dye (Red 40) in addition to an ingredient from the contaminant: Blue 1.

Contamination!

Case Study 2: Dairy Product Off-Gassing and Off Odor Investigation

Manufacturers of a newly developed fermented dairy product noticed that some samples had a foamy surface with an unpleasant off odor.

Case Study 2: Dairy Product Off-Gassing and Off Odor Investigation

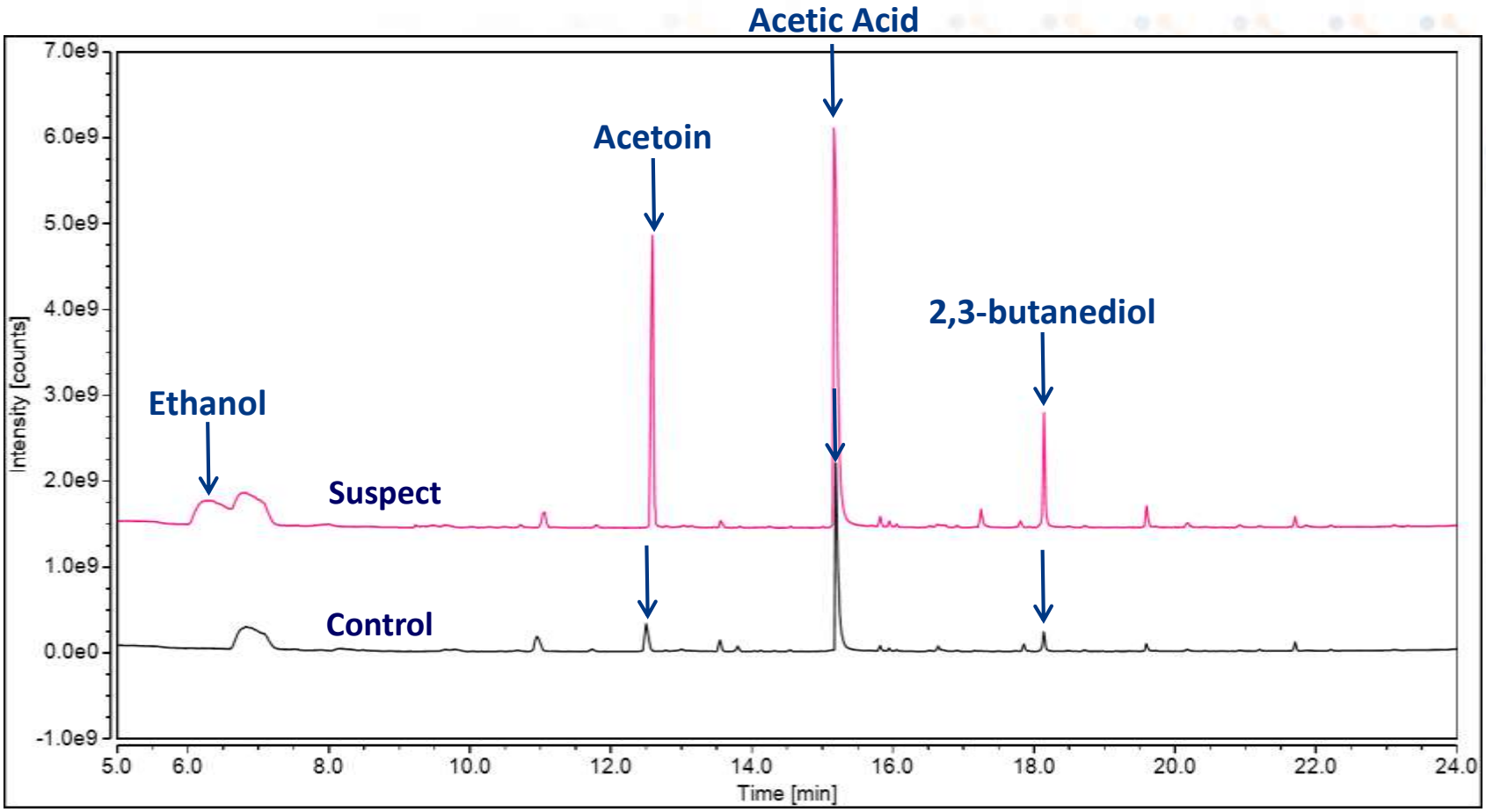
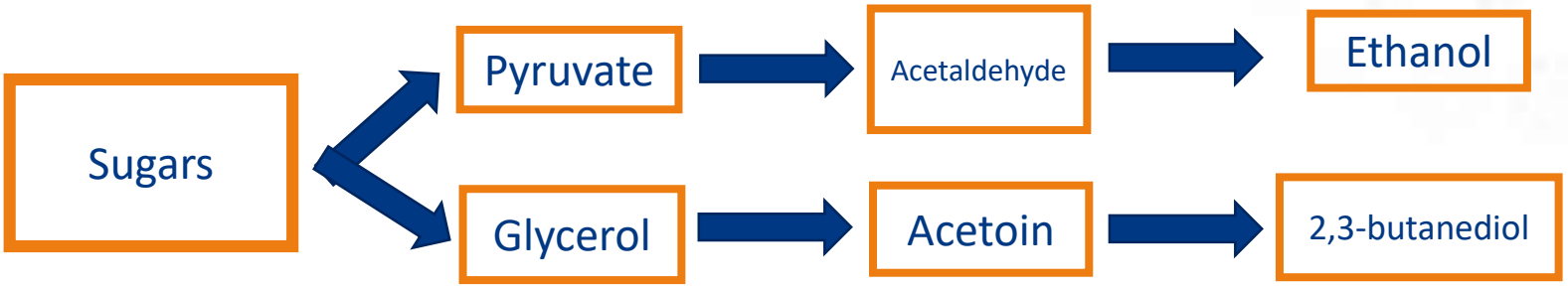


Figure 5. GC-MS chromatograms of the suspect (top/pink) and control (bottom/black) dairy products.

Case Study 2: Dairy Product Off-Gassing and Off Odor Investigation

- Four compounds were identified in elevated amounts in the suspect versus control: ethanol, acetoin, acetic acid, and 2,3-butanediol.
- The elevation of these compounds, in particular ethanol and the foam/gas buildup (CO_2), indicate over-fermenting of the product via multiple pathways.



Production Issue!

Case Study 3: Vanilla Smoothie Off Odor Investigation

A freshly manufactured batch of vanilla smoothie was reported to have an uncharacteristic smell, described as solvent-like in some cases.

Case Study 3: Vanilla Smoothie Off Odor Investigation

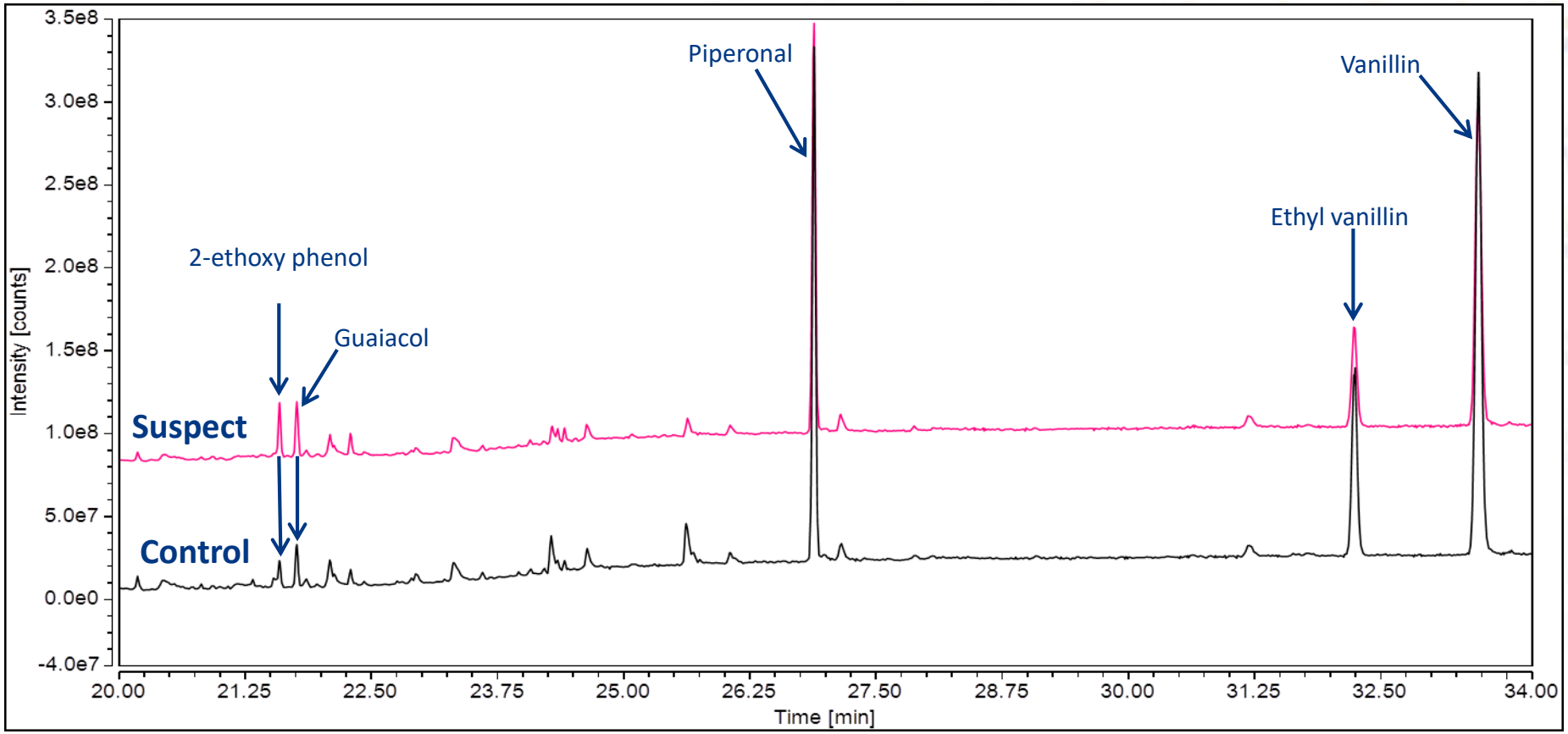


Figure 6. SPME-GC-MS profile of the complaint vanilla beverage (top/pink) and the control beverage (bottom/black).

Case Study 3: Vanilla Smoothie Off Odor Investigation

- The suspect sample was found to have less of the main flavor and odor components of vanilla:
  - Vanillin
  - Ethyl vanillin
  - Piperonal
- The suspect also had higher intensities of two compounds that are industrial precursors of ethyl vanillin and vanillin:
  - 2-ethoxyphenol
  - Guaiacol
- The vanilla used in the suspect smoothie was degraded or improperly produced causing the off odor/flavor.

Ingredient Issue!

Case Study 4: Table Sugar Contamination Investigation

A customer complained of discovering a powerful floral odor in their package of table sugar upon opening.

Case Study 4: Table Sugar Contamination Investigation

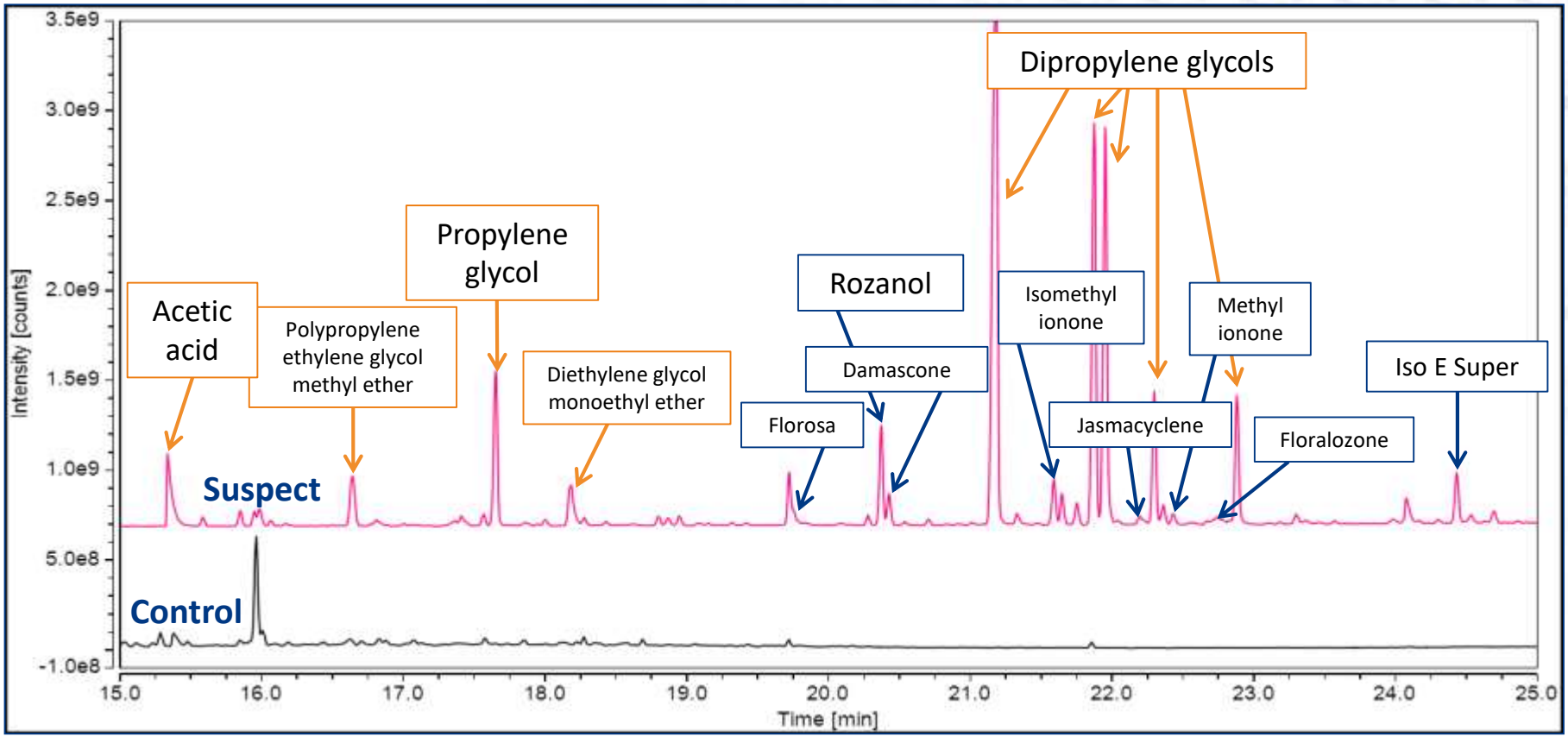


Figure 7. SPME-GC-MS profile of the control and suspect sugar samples showing solvents (orange) and fragrance-related (blue) compounds.

Case Study 4: Table Sugar Contamination Investigation

- The suspect sugar sample was found to contain an abundance of compounds not found in the control including:
  - **Solvents**: acetic acid, polypropylene glycol, polypropylene ethylene glycol methyl ether, diethylene glycol monoethyl ether, and a variety of dipropylene glycols.
  - **Fragrance agents**: florosa, rozanol, damascone, isomethyl ionone, jasmacyclene, methyl ionone, floralozone, and Iso E Super.
- Many of these compounds are common ingredients in a variety of scented products including cosmetics, soaps, detergents, and cleaning products.

Contamination!

Case Study 5: Coconut Flour Off Odor Investigation

An unflavored coconut flour powder was reported to smell ‘funny’ compared to a normal sample. The client submitted fresh control sample along with packaged suspect sample. Due to concern from the client, the packaging material was analyzed as a sample.

Case Study 5: Coconut Flour Off Odor Investigation

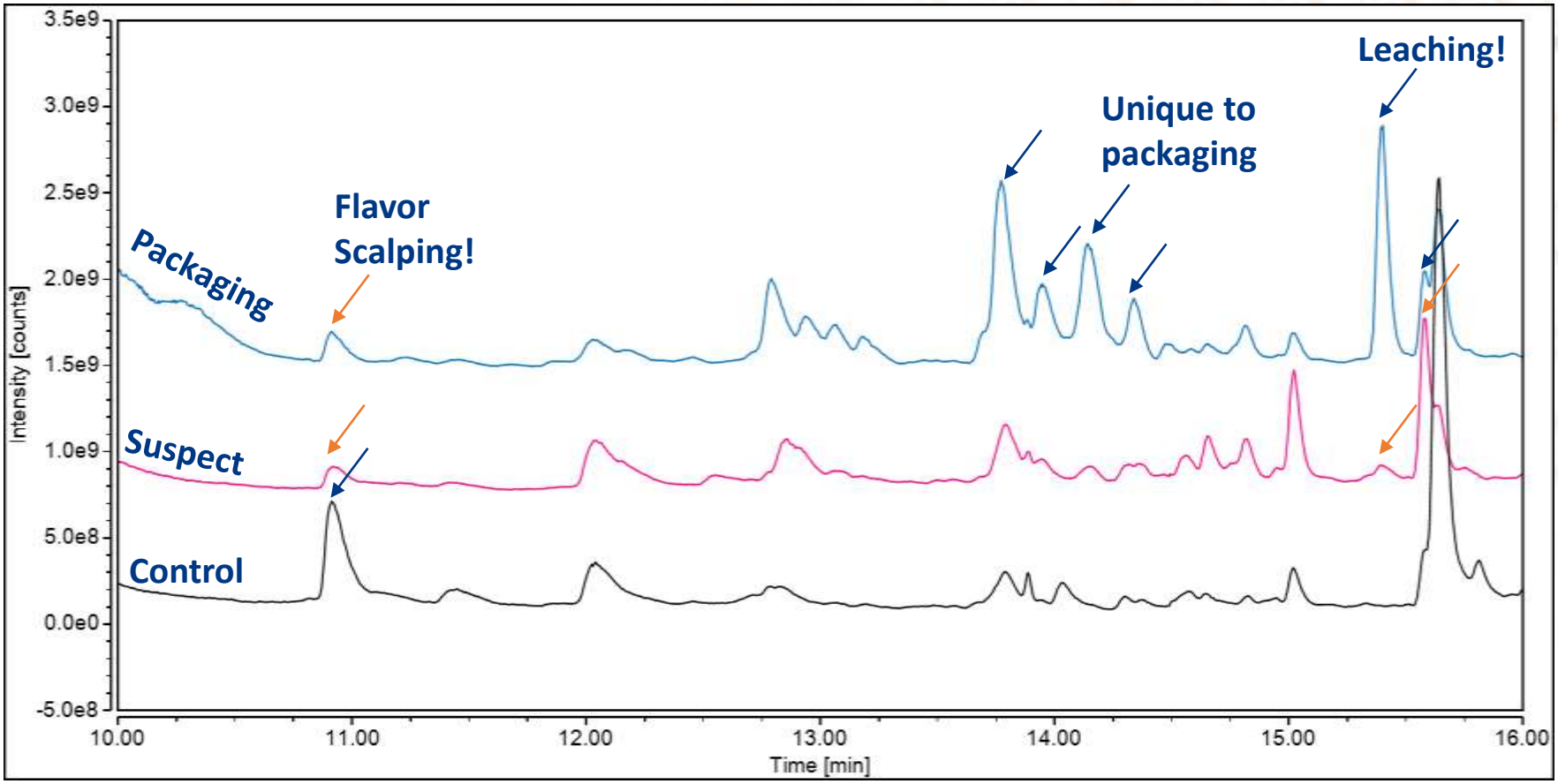


Figure 8. SPME-GC-MS chromatograms for coconut flours and packaging material.

Case Study 5: Coconut Flour Off Odor Investigation

- Analysis showed a complex cause of the off odor involving both:
  - **Flavor scalping**: loss of important volatiles as they move from the sample into the packaging.
  - **Leaching**: migration of volatiles into the sample from the packaging.

Packaging Issue!

Case Study 6: Volatile Profiling

Broth vs. Cured Meat

- In addition to off-odor analysis, GC-MS can be used for general flavor profiling which allows for comparative analysis between samples.
- Also, can be used to determine the impact of processing, ingredients, storage, etc. on the flavor profile of a product.

Case Study 6: Volatile Profiling

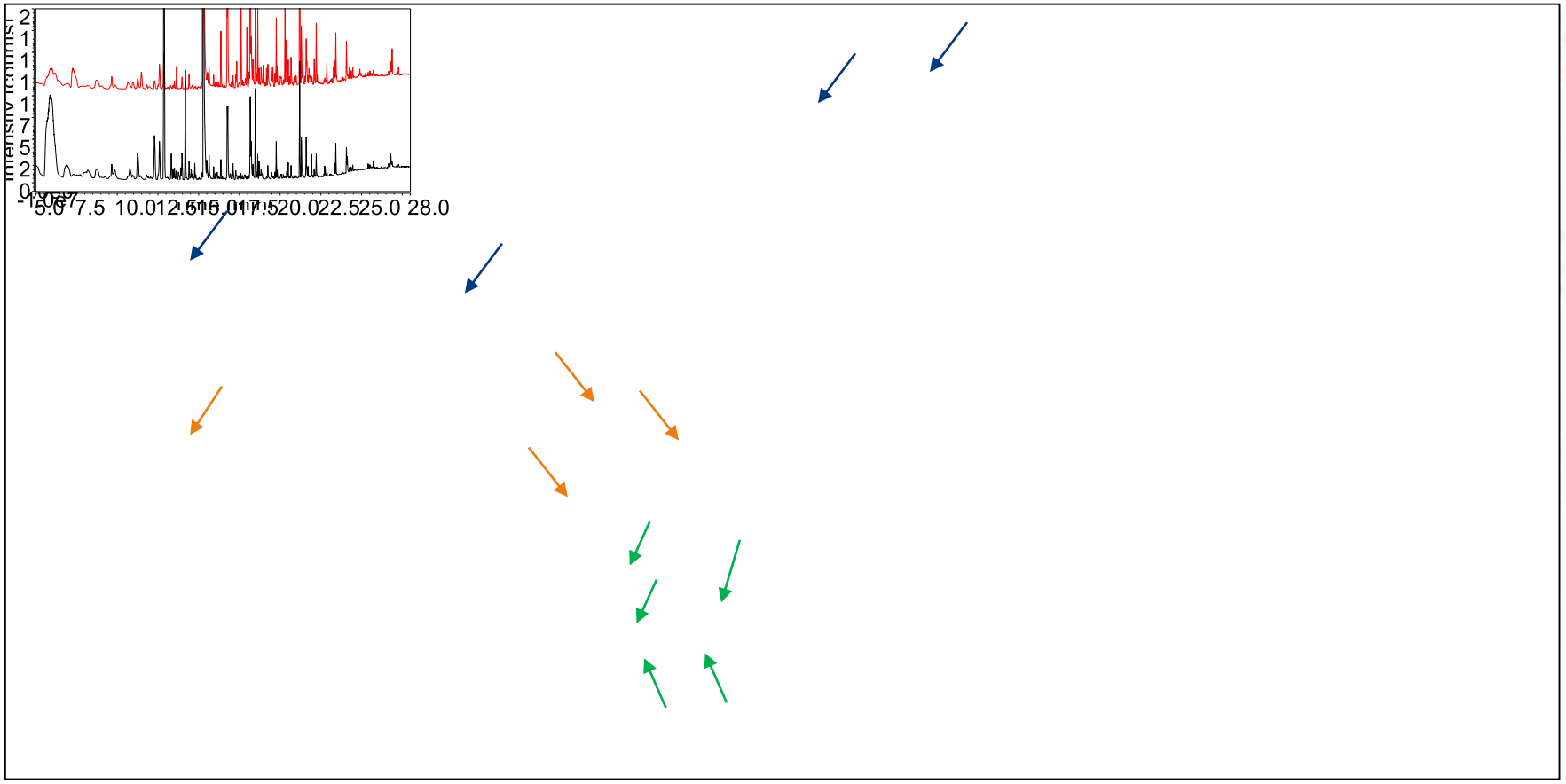


Figure 9. GC-MS profile of cured meat (bottom) and broth (top).

Case Study 6: Volatile Profiling

- The two samples shared a number of major volatiles (acetic acid, butanone and 1-hydroxy-2-propanone).
- The cured meat also contained compounds which are associated with sweet, buttery aromas (e.g., 2,3-pentanedione) and roasted odors (pyrazines).
- The broth had much higher intensities of fatty (e.g. 2-heptenal, 2-octenal) and acidic compounds (e.g. acetic acid).

Additional Techniques

- GC-MS/MS for increased sensitivity/selectivity.
  - Low level quantitation
- Complementary approaches for off-odor analysis:
  - ICP-OES: metals
  - ICP-MS: heavy metals
  - Microbial analysis
  - Sensory panel
  - Acidity/alkalinity
  - Peroxide
  - Non-volatiles (LC-MS)

Summary & Conclusions

- SPME-GC-MS is a powerful technique for off-odor analysis and volatile profiling.
- By comparison of the profile of a control and suspect sample, compounds that could be responsible for the off-odor can be identified.
- This can allow for identification and correction of the underlying issue.

Questions?

Jamie Willems, Ph.D. & Lily Zehfus, M.S.
Eurofins SF Analytical
Contact Us At:
262-754-5300
ESFA_Project@FT.Eurofinsus.com

Follow us online!

Eurofins North America Food and Feed

Eurofins US Food Group

@eurofins