CHEM 133.02 LAB2-AC2
Experiment #3: *Extraction and Identification of the Major Components of Essential Oils by Gas Chromatography – Mass Spectrometry (GC-MS)*
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Abstract

In this experiment, the major components of an unknown sample of ginger essential oil (EO) were analyzed and quantified via gas chromatography-mass spectrometry (GC-MS). Despite finding compounds consistent with known ginger essential oil components, GC-MS results showed multiple unexpected compounds present in the ginger essential oil sample, Possible reasons for the aforementioned anomalies in the sample, chemical degradation and contamination from storage conditions and treatment of sample, were unable to be verified due to the storage conditions and treatment of the sample being unknown. To address this, it was recommended for future research to be informed of the storage and treatment histories of essential oil samples to allow for the GC-MS analysis to be more comprehensive.

Introduction

Essential oils are flammable and volatile products extracted from plant parts by various methods including steam distillation and dry distillation (Salahaddin, 2021; Ali *et al*., 2015). They are valuable products due to their versatility and have many industrial applications in fields such as medicines, food, cosmetics and agriculture (Ali *et al*., 2015; Salahaddin, 2021; Bolouri *et al*., 2022). Many of the applications of essential oils involve close contact with the human body including penetration into skin surfaces, inhalation and oral consumption (Ali *et al*., 2015). This makes identification and quantitative analysis of their components essential to ensure their purity and safety before use, as oils containing significant amounts of toxic and poisonous compounds can lead to toxic and lethal reactions from customers who use them (Salahaddin, 2021).

Gas chromatography is a common method used for quantitative analysis of the components of essential oils (Salahaddin, 2021). When coupled with mass spectroscopy, gas chromatography-mass spectrometry (GC-MS) can be used to accurately identify and quantify the multiple components of essential oil efficiently (Salahaddin, 2021). GC-MS is almost exclusively used for the quantitative analysis of volatile products (Salahaddin, 2021), making it suitable for quantitative analysis of essential oils which are volatile mixtures.

In this experiment, the major components of an unknown sample of ginger essential oil (EO) were analyzed and quantified via gas chromatography-mass spectrometry (GC-MS). The

relative compositions of each component and the likely identities corresponding to each peak or GC fraction were identified in relation to the oil's overall purity and quality.

Method

Sample Preparation

The unknown ginger oil sample was first diluted 1000-fold with ethyl acetate solvent. Initial dilution used 20 μL of ginger essential oil with 980 μL of ethyl acetate as solvent to achieve a 50 fold dilution. A second dilution was prepared, 20 μL of the 50-fold diluted solution was dissolved in 980 μL of ethyl acetate. The 1000-fold diluted solution was used as the sample analyzed by the GC-MS instrument (Shimadzu GCMS-QP2010 Ultra).

GC-MS Initialization

Helium pressure gas canisters connected were opened and followed with GC-MS along with the program. A 2 hour auto-start was performed to initialize the vacuum pump and the ion source temperature. After initialization, leak check and auto-tuning were performed. The GC-MS instrument was set at the following parameters before elution: washing volume: 8uL, column oven temperature: 50.0 °C, injection temperature, 250.00 °C, splitless injection mode, sampling time: 1.00 min., linear velocity option for flow control mode, pressure: 52.7 kPa, total flow rate: 13.19 mL min^{-1}, column flow rate: 0.99 mL min^{-1}, linear rate: 36.1 cm s^{-1}, purge flow rate: 3.0 mL min^{-1}, and split ratio: 10.0.

GCMS Sample Run

The GC-MS method was opened and downloaded followed by loading of ginger oil samples and subsequent elution for 36 minutes. Further autotuning for the GC-MS run was performed by increased solvent rinsing from 1 to 2. The run employed a specific oven temperature program which set column temperature at 50.0 °C for 2 minutes, followed by ramps to 80, 120, and 200 °C at rates of 2, 5, and 30 °C/min, respectively, and held at 280 °C for 5 minutes. The resulting chromatogram was further cleaned with deletion of initial integration and manual selection of peaks.

Identification of Structures Associated with GC-MS Peaks

The PDF output file printed from the GC-MS instrument which summarized chromatographic information on ginger oil was interpreted and analyzed via a computer program developed in Python 3.13 (Sanguyo *et al.*, 2025). Information on the major peaks, retention times, percent peak areas, MS base peaks, and all MS molecule hits with the highest similarity indices were automatically extracted from the output file. Likely candidates for each peak were manually narrowed down to a few possible structures with common names that are known to be found in ginger oil and other essential oils.

Results

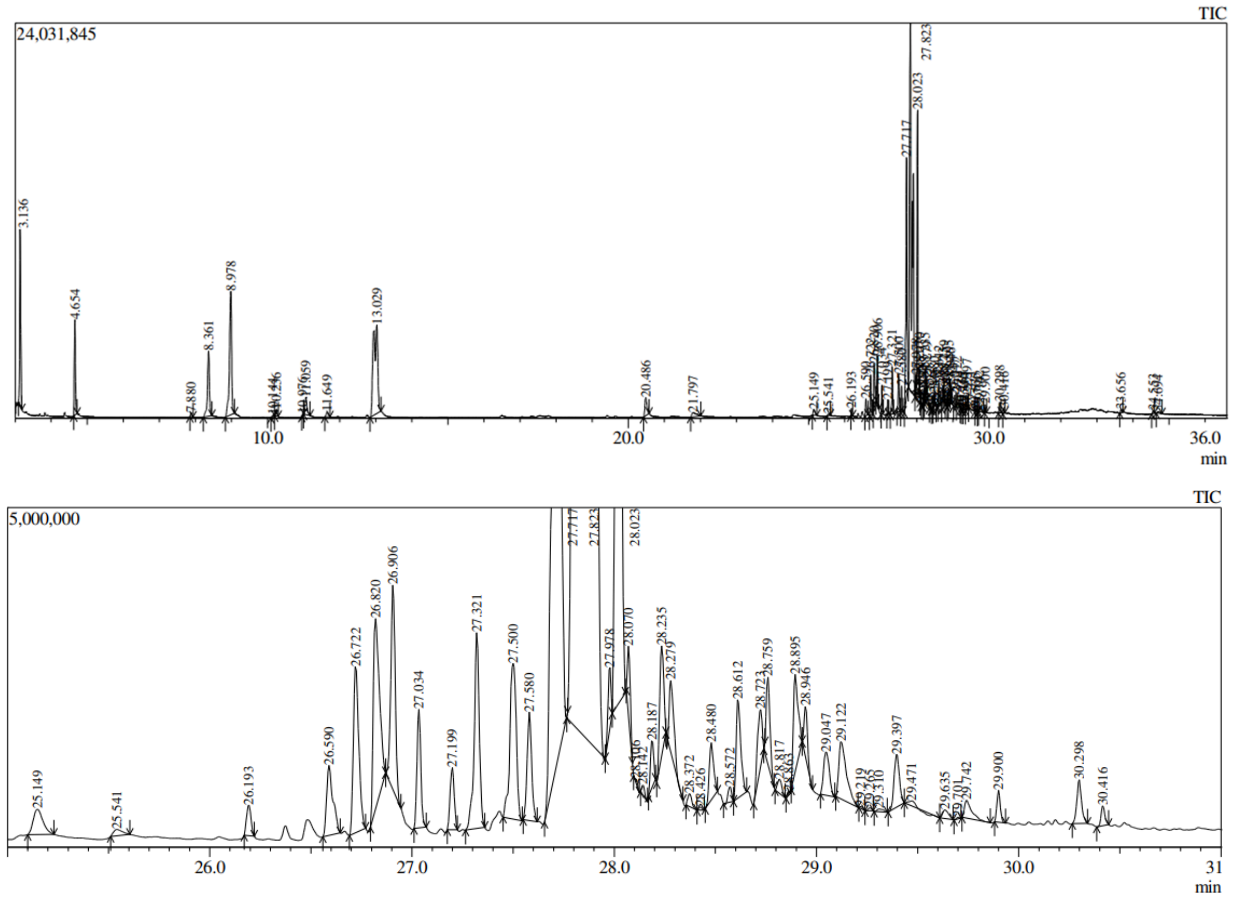

Figure 1. GC Chromatogram of Ginger Oil from (a) 0 to 36.0 Minutes and (b) 25.0 to 31.0 Minutes, with Retention Times Indicated Above Each Peak

Table 1. Identified Components of Ginger Oil Based on the Top 17 Peaks in the GC Chromatogram in Figure 1

Entry No.	Retention Time (min.)	Possible Major Compound(s)	% Area or % Composition	MS Base Peak (m/z)
1	27.82	β-curcumene, zingiberene	26.7	119
2	13.03	linalyl formate	12.8	93
3	27.72	α-curcumene, geranyl-p-cymene	9.00	132
4	8.978	camphene	8.91	93
5	28.02	β-sesquiphellandrene, β-bisabolene, β-cis-farnesene	7.90	69

Table 1. Identified Components of Ginger Oil Based on the Top 17 Peaks in the GC Chromatogram in Figure 1 (cont.)

Entry No.	Retention Time (min.)	Possible Major Compound(s)	% Area or % Composition	MS Base Peak (m/z)
6	8.361	D-α-pinene	4.06	93
7	26.82	geranyl acetate, nerol acetate, geraniol butyrate, geranyl isobutyrate	1.69	69
8	27.50	β-trans-farnesene	1.43	69
9	27.32	germacrene B	1.40	93
10	20.49	L-borneol	1.31	95
11	26.72	ylangene	1.29	119
12	26.91	β-humulene	1.28	93
13	11.06	β-myrcene	1.16	93
15	28.90	β-eudesmol	0.68	59
16	28.61	α-bisabolol	0.65	69
17	21.80	α-terpineol	0.64	59

Table 2. Comparison of the Reported % Composition of 14 Major Components with Literature Data on Other Variants of Fresh Ginger Oil

Major Component	% in Unknown Ginger Oil	% in Ecuadorian Ginger Oil[a]	% in Indian Ginger Oil[b]	% in Nedumangad Ginger Oil[c]
bisabolene	-	5.8	11.2	5.8
bisabolol	0.65	-	-	0.30
cadinene	-	3.5	3.5	2.2
camphene	8.91	7.8	15.9	4.0
citral (geranial and neral)	-	19.6	33.5	8.9
α-curcumene	< 9.00	5.6	22.1	5.6
β-curcumene	< 26.7	-	-	-
β-eudesmol	0.68	0.2	8.19	2.0

Table 2. Comparison of the Reported % Composition of 14 Major Components with Literature Data on Other Variants of Fresh Ginger Oil (cont.)

Major Component	% in Unknown Ginger Oil	% in Ecuadorian Ginger Oil[a]	% in Indian Ginger Oil[b]	% in Nedumangad Ginger Oil[c]
α-farnesene	-	6.8	7.6	-
β-cis-farnesene and β-trans-farnesene	< 9.33	0.2	-	0.1
Esters of geranic acid (geranyl acetate, geranyl isobutyrate)	< 10.69	0.2	5.8	0.1
α-terpineol	0.64	0.5	8.8	1.3
ylangene	1.29	-	-	-
zingiberene	< 26.7	17.4	11.7	28.6

[a] Data from Hoferl *et al.* (2015)

[b] Data from Munda *et al.* (2018)

[c] Data from Sasirahan & Menon (2010)

Discussion

The GC-MS chromatogram for ginger oil, showing 61 peaks in total, is shown in Figure 1. As shown in Table 1, more than 17 major oil components, corresponding with the top 17 peaks in terms of peak areas, were detected and assigned their likely identities. The relative concentration of each oil component was estimated as its corresponding percent peak area. Some of these components found in the top 6 peaks included several sesquiterpenes such as β-curcumene (< 26.7%), zingiberene (< 26.7%), β-sesquiphellandrene (< 7.90%), β-bisabolene (< 7.90%), and β-cis-farnesene (< 7.90%). Other components such as linalyl formate (12.8%), α-curcumene (< 9.00%), geranyl-p-cymene (< 9.00%), camphene (8.91%), and D-α-pinene (4.06%) were monoterpenes and their derivatives (AtQ#4). The structures of some components are shown in Figure 2. Based on peaks 7 to 17, many other sesquiterpenes, monoterpenes, and their derivatives with concentrations above 0.64% were detected in the oil sample. All other peaks indicated the presence of organic compounds in trace concentrations, but their likely identities were not able to be determined.

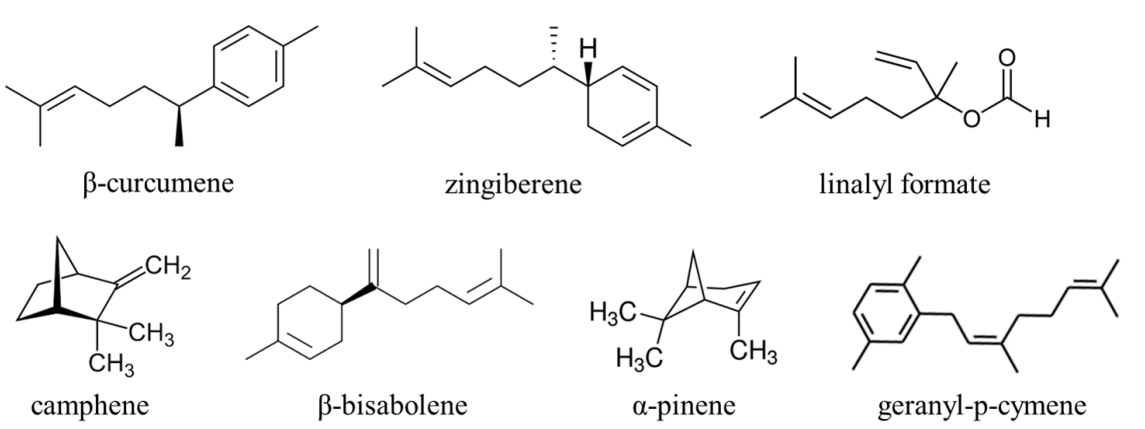

Figure 2. Structures of Major Components Found in Ginger Oil

The above structures for sesquiterpenes, monoterpenes, and derivatives were determined as likely candidates for the oil components as their base peaks shown in Table 1 were found to be generally consistent with the predictions of various electron ionization (EI) mechanisms. Some of the chief EI fragmentation mechanisms resulting in cations corresponding to their base peaks include the formation of allylic intermediates for sesquiterpenes such as β-curcumene and zingiberene, McLafferty rearrangement for carbonyl-containing terpenes such as linalyl formate and geranyl acetate, and sigma cleavage near highly-substituted carbons (Dunnivant, 2017; McMurry, 2016) (AtQ#5). Depending on the functional groups and arrangement of atoms in these components, some of these mechanisms may be more dominant in producing the base peak, although more complex mechanisms may be involved for bicyclic terpenes such as camphene and α-pinene. Figure 3 shows an example on the formation of an allylic intermediate as a possible candidate for the m/z = 119 base peak used to identify the most abundant components being β-curcumene and zingiberene.

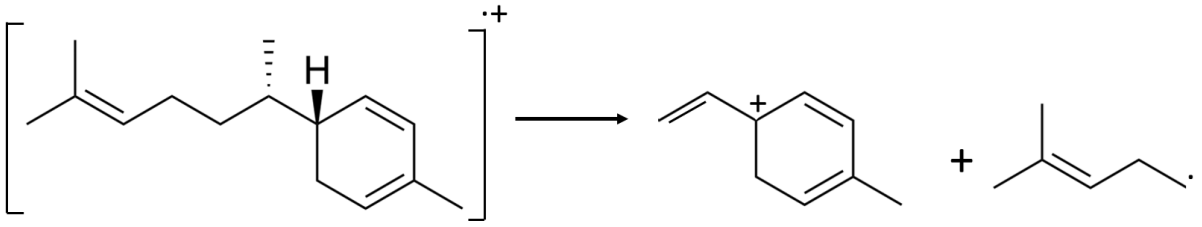

Figure 3. Proposed Pathway for the m/z = 119 Base Peak in Identification of Zingiberene via MS

Table 2 shows a comparison of the composition of the ginger oil in this study with three other reported variants of ginger oil analyzed by gas chromatography-based methods. It can be seen that the oil's overall composition significantly differed from the compositions of Ecuadorian, Indian, and Nedumangad oils as reported by other literature studies (Hoferl *et al*. 2015; Munda *et al*., 2018; Sasirahan & Menon, 2010). Some compounds such as camphene, α-curcumene, and zingiberene were found at relatively similar concentrations with at least one of the reported oils. However, other compounds including several esters of geranic acid, β-cis-farnesene, and β-trans-farnesene were found to be at much higher concentrations compared to literature results. Furthermore, compounds such as bisabolene, cadinine, citral, and α-farnesene that are commonly found in ginger oils were not detected. Finally, some compounds such as ylangene and β-curcumene that were detected in the oil being studied were not previously reported in the literature. It is possible that these differences originated from different conditions employed in the cultivation of the source ginger variant from which the oil was extracted from. According to Munda *et al.* (2018), differences in altitude, location, and other conditions affecting the growth of ginger plants can directly influence the composition of various components in ginger oil.

It is important to note that there are several notable limitations to the GC-MS method employed in this study. First, the method was not sufficiently selective as it was unable to assign unique compounds to several detected peaks, especially peaks with high concentrations (AtQ#5). As shown in Table 1, more than 1 compound, of which are most often constitutional isomers of each other, may be identified from peaks 1, 3, and 5. One explanation is that some compounds with similar structures might have been eluted from the GC column with similar retention times, resulting in peak overlap. Peak 1, for instance, might have originated from the simultaneous elution of β-curcumene and zingiberene, of which have similar overall structure and hydrophobicity (Figure 2). Secondly, the method was not sufficiently sensitive in detecting and identifying all components corresponding to the 61 peaks, as all peaks with areas of less than 0.64% were unable to be identified or narrowed to a few known candidates. Although the choice of method enabled the simultaneous determination of major components from the first 17 peaks within a short time frame of 36 minutes, it was unable to assign likely identities for other minor components present at low or trace concentrations (AtQ#5). It is possible that these compounds

may be identified by performing further analysis on the individual GC-MS fractions, but since these compounds were not yet reported from literature, MS was not sufficient for assigning likely identities to these peaks.

Besides method limitations, there are other limitations that may be attributed to the sample preparation of the ginger oil before analysis. For instance, identification via MS showed that the two peaks in Figure 1, corresponding to retention times of 3.136 and 4.654 minutes, were likely to be propanoic acid and acetic acid, respectively. Lower molecular-weight acids, outside of pentadecanoic acid, are generally not found in ginger essential oils (Munda *et al.*, 2018). It is likely that both acids formed due to the hydrolysis of various ester components during sample preparation and/or storage or due to the intentional addition of acids into the oil before analysis. Another example is the absence of citral, which includes the components geranial and neral that are commonly found in other variants of ginger oils at detectable concentrations (Hoferl *et al*. 2015; Munda *et al*., 2018; Sasirahan & Menon, 2010). One contributing factor is that citral might have degraded into p-cymene and subsequently condensed with other components during oil extraction and/or sample preparation, particularly in steps involving the application of heat (Williamson & Masters, 2011). Finally, possible errors in the measured concentrations of each component may be attributed to errors in the dispensing of volumes via micropipettes during the dilution of the ginger oil.

Answers to Guide Questions

1. Discuss the principles behind steam distillation.

Steam distillation is a form of distillation involving two immiscible liquids, such as ginger oil and water (Atkins *et al.*, 2018). Boiling occurs at lower temperatures than the normal boiling points of either liquid, since it proceeds when the sum of the partial pressures of the immiscible liquids equals the external pressure (Williamson & Masters, 2011). As the components are boiled, the purified distillate is collected as two separate layers just like the undistilled solution. Some of the applications of steam distillation include the isolation of oils and resins, purification of heat-sensitive organic compounds, and elimination of solvents. Some of its advantages over other distillation methods include the requirement of low boiling point and selectivity for liquids with great differences in volatility.

2. Discuss the EI process. Why is 70 eV used as the standard ionization energy in EI?

One of the processes involved in the analysis of organic molecules in mass spectrometry (MS) is electron ionization (EI). In EI, electron bombardment at 70 eV or 6700 kJ/mol transfers enough energy to molecules to convert them into reactive radical cations. (McMurry, 2015)

(Figures 4 and 5). At lower energies, soft ionization occurs as the radical cation doesn't cleave and only peaks corresponding to molecular ions are detected. But at higher energies, including 70 eV, hard ionization occurs and the radical cation cleaves into a fragment cation and a fragment radical via a variety of mechanisms. Due to extensive fragmentation, the resulting cations produce an MS spectrum of detected peaks per molecule involved (Van Bramer, 2025). The lower the energy applied, the more reproducible the fragmentation patterns become For these reasons, 70 eV is often designated as the standard ionization energy for hard-ionization MS.

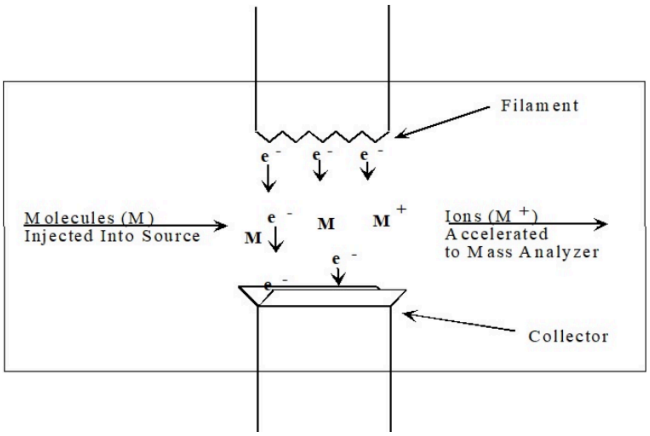

Figure 4. Schematic Representation of Electron Ionization in MS (Van Bramer, 2025)

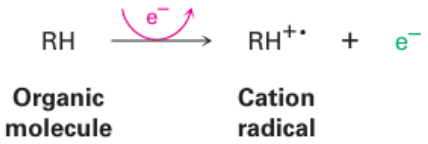

Figure 5. Cation Radical Formation (McMurry, 2016)

4. How do you assure that the identification is reliable? What are the measures of reliability?

For the GC-MS identification to be reliable, it has to be both selective and sensitive. Selective means that it is able to distinguish two or more different analytes with different structures. Sensitive means that it is able to quantify these analytes even at low concentrations.

Conclusion

The researchers determined that the ginger essential oil sample analyzed is likely not of high quality and purity due to the absence of various compounds usually found in ginger oil and the presence of compounds that were absent in literature data for GC-MS analysis for other ginger essential oil samples. The lack of compounds found in other literature for ginger oil such as bisabolene and cardinene and the presence of others not found in literature such as ylangene and β-curcumene were theorized to be attributed to contamination of the sample and chemical degradation due to factors of heat and exposure to harsh chemicals. Another reason for the disparity in composition could be the difference in species of ginger plant the oil was extracted from. However, these hypothesized reasons for the disparity in chemical composition cannot be confirmed as the plant source, storage condition and treatment of the essential oil sample is unknown. Therefore, the results from this experiment emphasize the importance of knowledge of the source of essential oil source and storage history in addition to GC-MS analysis to ensure a comprehensive and accurate understanding of analysis that not only accounts for the essential oil components at the time of the analysis but also the possible reasons for any anomalies in results based on the manufacturing details of the sample.

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Appendix

Data for GC-MS Peaks, Peak Area, and Molecule Hits:
https://docs.google.com/spreadsheets/d/1YjP7W_gWM-KT7i-bDUYECyVSWZfK1RqOnNloWhotsWo

PDF File for Ginger Oil Results (contains GC spectra matched to each peak)
https://drive.google.com/file/d/1W86EXGbjSH_RBq3M0YNaD9twa2PXHj9X/view

Link to Python Program Used for Extracting Information from PDF GC-MS Output File
https://github.com/NotAMadTheorist/GC-MS-of-Ginger-Oil-via-PDF-Scraping/tree/main