

Too Hot to Handle: Chemical Profiling of Six Global Peppercorn Varieties

Trop chaud pour être manipulé : Profil chimique de six variétés de grains de poivre à travers le monde

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Abstract | Résumé

For centuries, peppercorns have been valued for their aroma, spice, and distinctive flavour profiles across many cultures. While piperine is widely recognized as the primary compound responsible for their characteristic spicy taste, less is known about the broader chemical composition that contributes to their flavour, especially across different varieties of peppercorns. The goal of this study is to identify and compare the volatile compounds present in six types of peppercorns: black pepper (*Piper nigrum*), white pepper (*Piper nigrum*), red Pondicherry pepper (*Piper nigrum*), Indian long pepper (*Piper longum* var. *longum*), pink peppercorn (*Schinus terebinthifolius*), and Szechuan pepper (*Zanthoxylum bungeanum*). Particular attention is given to assessing the distribution of piperine within the *Piper* species. Ground pepper samples were subjected to supercritical carbon dioxide (sc-CO₂) extraction, and the resulting extracts were diluted in ethyl acetate before analysis using gas chromatography-mass spectrometry (GC-MS). The analysis enabled comparisons between *Piper* and non-*Piper* peppercorns regarding their dominant chemical constituents, revealing clear differences between true peppercorns and botanically distinct species. Additionally, the pepper samples were subjected to headspace analysis using solid phase microextraction (SPME) before GC-MS analysis. It was found that while all peppercorn varieties shared many of the same volatile compounds in varying amounts, the false peppercorns contained additional unique compounds not observed in the samples belonging to the *Piper* genus. These findings underscore the potential role of distinct volatile constituents in shaping aromatic differences among peppercorn varieties.

Depuis des siècles, les grains de poivre sont appréciés pour leur arôme, leur piquant et leurs profils de saveur distinctifs dans de nombreuses cultures. Bien que la pipérine soit largement reconnue comme le principal composé responsable de leur goût piquant caractéristique, on en sait peu sur la composition chimique globale qui contribue à leur saveur, en particulier entre les différentes variétés de poivre. L'objectif de cette étude est d'identifier et de comparer les composés volatils présents dans six types de poivres : le poivre noir (*Piper nigrum*), le poivre blanc (*Piper nigrum*), le poivre rouge de Pondichéry (*Piper nigrum*), le poivre long indien (*Piper longum* var. *longum*), le poivre rose (*Schinus terebinthifolius*) et le poivre de Sichuan (*Zanthoxylum bungeanum*). Une attention particulière a été portée à la répartition de la pipérine au sein des espèces du genre *Piper*. Les échantillons de poivre moulu ont été soumis à une extraction au dioxyde de carbone supercritique (CO₂ sc), puis les extraits obtenus ont été dilués dans de l'acétate d'éthyle avant d'être analysés par chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC-MS). L'analyse a permis de comparer les poivres du genre *Piper* et ceux qui n'en font pas partie en fonction de leurs constituants chimiques dominants, révélant des différences nettes entre les « vrais » poivres et les espèces botaniquement distinctes. De plus, les échantillons ont été analysés en phase gazeuse (headspace) à l'aide d'une microextraction en phase solide (SPME) avant l'analyse GC-MS. Il a été observé que, bien que toutes les variétés de poivre partagent de nombreux composés volatils en proportions variables, les « faux » poivres contiennent des composés supplémentaires uniques qui ne sont pas présents dans les échantillons appartenant au genre *Piper*. Ces résultats mettent en évidence le rôle potentiel de composés volatils distincts dans la formation des différences aromatiques entre les variétés de poivre.

Keywords: peppercorns; *Piper nigrum*; piperine; GC-MS; SPME; supercritical CO₂ extraction; volatile compounds; terpenes; pepper aroma; flavor chemistry

Introduction

Peppercorns, the dried fruits of various *Piperaceae* species, play a central role in global cuisines and have reported medicinal properties. Their use in traditional medicine is due to their anti-inflammatory and antibacterial effects (1). The pungency of *Piper*

nigrum is primarily attributed to piperine, an alkaloid that activates the Transient Receptor Potential Vanilloid 1 (TRPV1) receptor, contributing to the characteristic sensation of heat. While piperine has been widely studied and is considered the principal bioactive component in *P. nigrum* varieties, peppercorns from other species, such as *Schinus terebinthifolius* (pink peppercorns)

and *Zanthoxylum bungeanum* (Szechuan pepper), are not considered “true peppercorns” and display distinct sensory properties that suggest the presence of additional and/or alternative compounds contributing to their spiciness. All peppercorns, whether true or false, possess a similar smell. The olfactory sense is commonly said to contribute to about 75-95% of the flavours that are tasted (2), a fact that motivated an investigation into the similarity of false peppercorns to true peppercorns.

Previous studies largely focused on obtaining essential oils using traditional extraction methods such as steam distillation, hydrodistillation, and organic solvent extraction to identify volatile compounds (3). When analyzing the chemical composition of *P. nigrum* extracted essential oils, the compounds found were 3-carene, limonene, β -caryophyllene, and α - and β -pinene (4). For *Piper longum* var. *longum* (Indian long pepper), β -caryophyllene, pentadecane, and β -bisabolene were the main chemical constituents identified (5). For the *S. terebinthifolius* sample, the main compounds identified were 3-carene, limonene, α -phellandrene, and α -pinene (6). Lastly, for the *Z. bungeanum*, the major compounds detected were β -pinene, 1,8-terpinene, cis-piperitol acetate, oleic acid, palmitic acid, and 4-terpineol (7).

To expand the study of these peppercorns, this study employed both supercritical carbon dioxide (sc-CO₂) extraction and solid-phase microextraction (SPME) to elucidate their chemical profile. The former will extract non-volatiles from the ground peppercorns, while SPME will capture volatile components in the headspace above a ground sample. When using SPME as a method of extraction, the same variety of molecules, which are largely terpenes, are expected to be observed (8-10).

Methods

Whole peppercorns were purchased from various commercial sources. 10 g of each peppercorn was ground up in an electric grinder and placed into extraction thimbles and covered with cotton wool. The extraction thimble was placed in the sample chamber of the SFT-250 supercritical CO₂ System (Supercritical Fluid Technologies Inc.). The CO₂ was pressurized to 300 bar at a temperature of 40°C. 1 μ L of the extract was diluted in 2 mL of ethyl acetate. 1 μ L of this solution was injected into the gas chromatography-mass spectrometry (GC-MS, Agilent 6890 GC with MSD) having a 30 m Agilent 19091J-433 HP-5 column, with an internal diameter of 250 μ m, a stationary phase thickness of 0.25 μ m, and helium carrier gas having a flow rate of 30 cm/s. The oven temperature was programmed from 150°C to 320°C at 20°C/min, with a hold time of 5 minutes. The front inlet temperature was set at 250°C. Compound identification was by mass spectral comparisons to the NIST database and retention times of standards, although a limitation of the study was the absence of Kováts retention indices (11).

Solid phase microextraction (SPME) was implemented to sample the headspace of the ground peppercorns. A small amount of each

peppercorn, 100 mg, was ground and placed into a sealed vial. The vial was heated on a hot plate at 40°C for 5 minutes, allowing for volatile components to enter the gas phase and be absorbed by the coated fibre of the SPME syringe. The syringe was then injected into the GC-MS, running the previously described program.

Results and Discussion

GC-MS analysis of peppercorn extracts obtained via sc-CO₂ extraction revealed that piperine was the major component in samples belonging to the *P. nigrum* species (white pepper is shown in Figure 1A, others in Figure 2). This was expected of the black, white, and red Pondicherry Peppercorns based on the literature outlined previously. However, not all of these species were *P. nigrum* peppercorns, and thus, the other peppercorns had different chemical compositions. While *P. longum* is from the same genus as *P. nigrum*, it contains piperine, isobutyl-2,4-decadienamide (which gives a spicy, herbal odour, complementing piperine) and simple hydrocarbons. There was an unidentified peak at 9 min. This unidentified compound was also apparent in *Z. bungeanum*, the extract of which also contained palmitic (C16:0) and oleic (C18:1) fatty acids (Figure 2E).

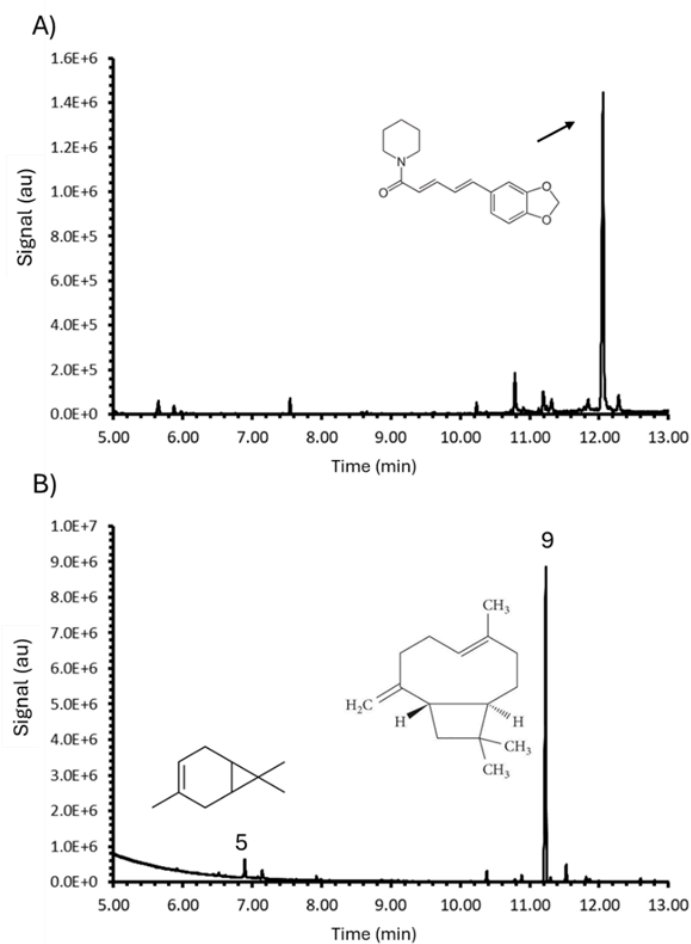


Figure 1. GC-MS results for the analysis of White Peppercorn (*P. nigrum*). A) sc-CO₂ extraction results. B) Solid phase microextraction results. The chromatograms show piperidine (12.1 min), 3-carene (6.9 min) and β -caryophyllene (11.2 min) as major compounds.

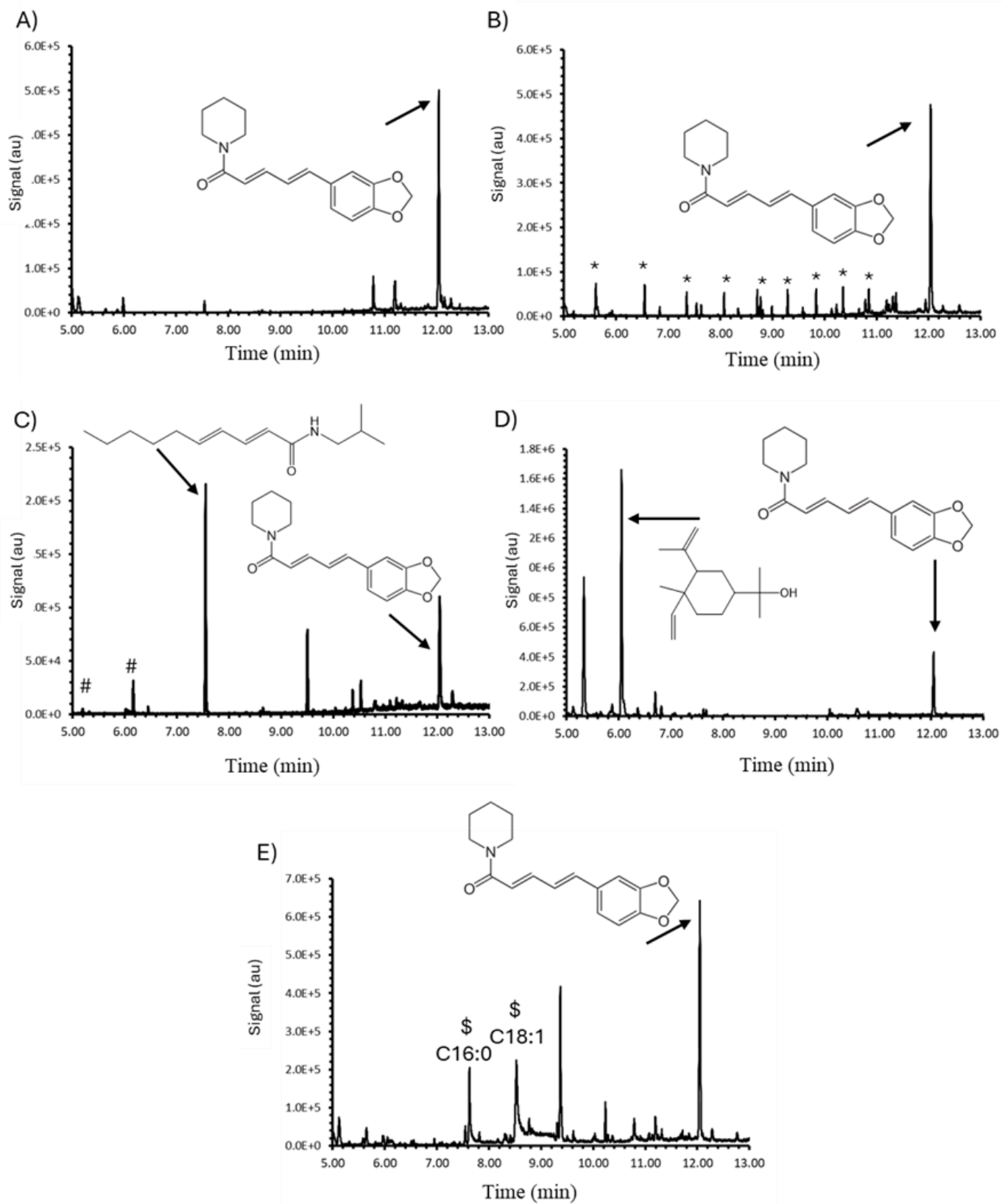


Figure 2. GC-MS results for the analysis of sc-CO₂ extracts of A) Black Peppercorn (*P. nigrum*), B) Red Pondicherry Pepper (*P. nigrum*), C) Indian Long Pepper (*P. longum*), D) Pink Peppercorn (*S. terebinthifolius*), and E) Szechuan Pepper (*Z. bungeanum*). * represents column bleed. # represents hydrocarbon and \$ represents fatty acid.

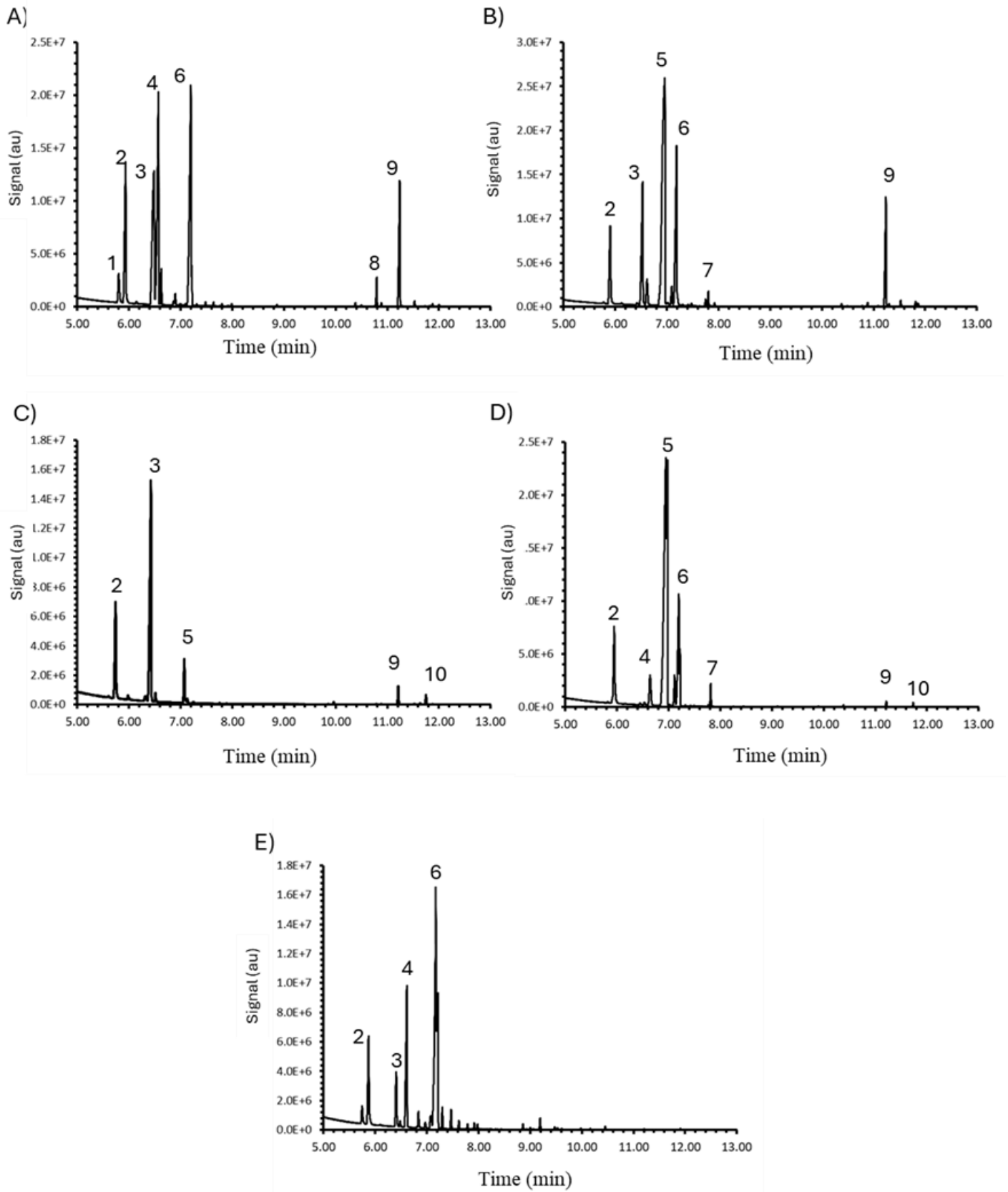


Figure 3. GC-MS results for the analysis of SPME extracts of A) Black Peppercorn (*P. nigrum*), B) Red Pondicherry Pepper (*P. nigrum*), C) Indian Long Pepper (*P. longum*), D) Pink Peppercorn (*S. terebinthifolius*), and E) Szechuan Pepper (*Z. bungeanum*). Compound assignments are in Table 1.

Table 1. Major compounds detected by SPME across the six peppercorn varieties. Numbers refer to the peak labels in Figure 3.

Peppercorn	Black Pepper (<i>P. nigrum</i>)	White Pepper (<i>P. nigrum</i>)	Red Pondicherry Pepper (<i>P. nigrum</i>)	Indian Long Pepper (<i>P. longum</i>)	Pink Peppercorn (<i>S. terebinthifolius</i>)	Szechuan Pepper (<i>Z. bungeanum</i>)
Identified Compound						
α -phellandrene	1	-	-	-	-	-
α -pinene	2	-	2	2	2	2
β -pinene	3	-	3	3	-	3
myrcene	4	-	-	-	4	4
3-carene	-	5	5	-	5	-
limonene	6	-	6	6	6	6
1-methyl-4-(1-methyl)ethylidene	-	-	-	-	7	-
Germacrene D	8	-	-	-	-	-
β -caryophyllene	9	9	9	9	9	-
pentadecane	-	-	-	10	10	-

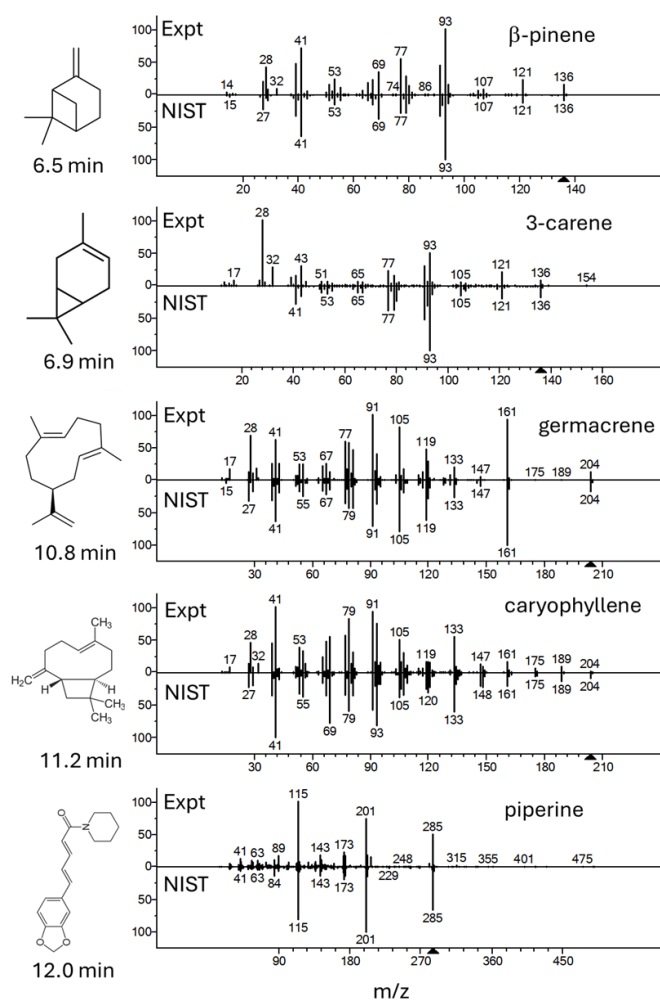


Figure 4. Comparison of selected observed and NIST mass spectra. Included compounds are β -pinene, 3-carene, germacrene, caryophyllene, and piperine. "Expt" represents observed mass spectra.

Across all peppercorn samples, the SPME results revealed a largely overlapping profile dominated by monoterpenes and sesquiterpenes, including α -pinene, β -pinene, β -caryophyllene, and limonene (see Figure 1B for White Pepper and Figure 3 for the other peppers studied, and Figure 4 for representative mass spectral identifications). Table 1 summarizes the results. It should be noted that most of the monoterpenes exhibit very similar mass spectra, and thus identification is based on both mass spectra and retention time. These compounds are commonly associated with woody and citrusy aromas and are consistent with previously reported volatile profiles of peppercorn essential oils. Despite these shared components, differences in relative abundance and the presence of additional minor compounds were observed among the false peppercorns. *S. terebinthifolius* and *Z. bungeanum* displayed unique volatile constituents not detected in the *Piper* species, which may contribute to subtle differences in perceived aroma despite their overall similarity. For instance, *Z. bungeanum* is dominated by the monoterpenes, contributing to its pungent and citrus aroma. These findings support the hypothesis that while true or false peppercorns share a common aromatic framework of terpenes, the species-specific volatile compounds may underlie their distinct sensory characteristics.

Conclusion

This study compared the volatile chemical composition of six peppercorn varieties using sc-CO₂ extraction and SPME coupled with GC-MS analysis. As predicted, piperine was identified as the main compound in the *P. nigrum* samples, confirming its presence and relative dominance with true peppercorns. In contrast, *P. longum*, *S. terebinthifolius*, and *Z. bungeanum* exhibited non-piperine dominant chemical profiles, highlighting the compositional diversity among peppercorn species and the difference between true and false peppercorns. SPME analysis revealed that both true and false peppercorns share a similar

volatile framework composed of terpenes, specifically monoterpenes and sesquiterpenes, explaining their comparable aromas. However, the false peppercorns contained additional unique volatile compounds not observed in the *Piper* species, suggesting that species-specific terpenes contribute to subtle aromatic differences. Future studies could investigate interactions between piperine identified by GC-MS and the endocannabinoid system, particularly its reported inhibition of fatty acid amide hydrolase. Such work may help elucidate biochemical mechanisms underlying the anti-inflammatory properties traditionally attributed to peppercorns.

Acknowledgements

Thank you to the JLH Mass Spectrometry Core Facility of the University of Ottawa for providing instrument access and consumables related to this project. A special thank you to Dr. Paul Mayer, who has granted me this wonderful opportunity, and to Dr. Sharon Barden for helping me along the way.

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