Volatile Profiling of Indonesian Culinary Mushrooms Using SPME Coupled to GC-MS and Chemometrics

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Abstract: *Volatile organic compounds (VOCs) are important in defining aroma and acting as distinctive chemical signatures for food identification. In this context, culinary mushrooms have become increasingly popular in daily diets, particularly in Indonesia, which offers a rich diversity. Investigations on volatile phytochemical profiles of Indonesian culinary mushrooms has been limited. Therefore, this research aimed to thoroughly characterize the VOC profiles of Indonesian culinary mushrooms for the purposes of aroma discrimination and identification. The extraction and analysis of mushroom VOCs were carried out using solid phase microextraction gas chromatography-mass spectrometry (SPME-GC/MS). Principal component analysis (PCA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA) successfully classified 13 Indonesian culinary mushrooms into 5 groups based on similar volatile chemical profiles, independent of genetic backgrounds. The results showed that markers contributing to the characteristic aroma of mushrooms, including 1-octen-3-ol, 3-octanol, octanal, and 3-octanone, were identified in nearly all samples.* A. mesenterica, A. delicata, *and* L. squarrosolus *were distinctly reported, with octanal, acetic acid, and 3-octanol serving as the strongest aroma markers, respectively.*

Keywords: *aroma; chemical marker; discriminant; key-odorant; volatile organic compounds*

INTRODUCTION

Volatile organic compounds (VOCs) are low-molecular-weight compounds that readily evaporate at room temperature and are readily detected in the air [1]. VOCs play an important role in defining the aroma perception and overall sensory perception of food commodities, therefore influencing consumer acceptance and market value. For instance, certain VOCs, such as α-pinene, propylbenzene, and *d*-limonene, have been identified as chemical markers to distinguish durum wheat from common types [2]. Furthermore, VOCs are the primary sources of food aroma and act as key odorants. Zabaleta et al. [3] reported that lactones and ketones in cheese contributed significantly to the overall aroma, imparting floral, apricot, and coconut notes.

Culinary mushrooms are increasingly valued for their nutritional benefits and gastronomic appeal [4] Mushrooms are known to produce a diverse range of VOCs. Many factors, including geographic conditions, developmental stages, and genetic variation [5], influenced the diversity in mushroom VOC profiles [6-8]. Consequently, comprehensive profiling of mushroom volatiles has become an important focus in food chemistry and flavor research, particularly for quality control and authentication. For instance, Malheiro et al. [9] discriminated six wild mushroom species, consisting of *Clitocybe odora*, *Clitocybe fragrans*, *Hebeloma crustuliniforme*, *Lepista nuda*, *Tricholoma fracticum*, and *Tricholoma terreum* based on unique VOCs. This research successfully identified specific chemical markers for each cluster, such as *p*-anisaldehyde for *Clitocybe* species, 3-octanol for *L. nuda*, 3-octanone for *H. crustuliniforme*, linalool for *T. fracticum*, and hexanal for *T. terreum*. Additionally, C-8 aliphatic compounds such as 3-octanol, 1-octanol, 1-octen-3-one, and 3-octanone have been identified as significant odorants in various culinary mushrooms [10-11].

Volatilomics is a metabolomics-based approach used to extensively characterize volatile compounds and their variation under different conditions [5]. Volatilomics provides a comprehensive framework for characterizing volatile metabolites that are highly responsive to processing and matrix effects in different food commodities including mushrooms. The solvent-free nature of SPME minimizes artifact formation and matrix interference, allowing representative capture of volatile compounds. Coupled with GC–MS, this approach enables sensitive separation and reliable identification of complex volatile mixtures [12]. Chemometric analysis was subsequently employed to manage the high-dimensional volatilomics data, facilitating discrimination among treatments and identification of relevant key volatile markers.

Indonesia is recognized for its rich diversity of culinary mushrooms; however, research on these resources has largely focused on non-volatile constituents [13], while their volatile profiles remain poorly documented. To date, no comprehensive characterization of VOCs in Indonesian culinary mushrooms using SPME–GC–MS has been reported. Volatile profiling is particularly valuable for biodiverse food materials, as VOCs provide species-specific chemical fingerprints that can be used for discrimination, authentication, and quality characterization. The objectives of this study were, therefore, to characterize and compare the VOC profiles of 13 Indonesian culinary mushrooms and to identify key volatile markers that enable chemical differentiation among mushroom species. The mushrooms' volatilomics study was conducted using SPME–GC–MS, and the resulting datasets were interpreted using multivariate analysis, including principal component analysis (PCA) and orthogonal partial least squares–discriminant analysis (OPLS-DA), to characterize and discriminate mushroom species based on their volatile profiles.

■ EXPERIMENTAL SECTION

Materials

A total of 13 species of culinary mushrooms were obtained from various regions across Indonesia (Table 1). After harvesting, the fresh samples were thoroughly washed and shade-dried for 48 h. The dried specimens were transported to IPB University and stored immediately at −20 °C until extraction. Mushroom identification was conducted by Heri Santoso from the Foundation of *Generasi Biologi* Indonesia, and the voucher specimens were deposited in the same institution

Instrumentation

The volatile compounds analysis was performed using GC-MS (QP2010 Plus, Shimadzu, QP2010 Plus), with a capillary column RTX-5MS (crossbonds, 5% diphenyl, 95% dimethyl polysiloxane equal to DB-5MS, 30 m × 0.25 mm). The extraction process was carried out using SPME containing DVB/CAR/PDMS 50/30m with a 1 cm length and a 24-gauge needle (SPME, Sigma-Aldrich, Germany).

Procedure

Volatiles extraction using SPME

Frozen mushroom samples were defrosted, sliced into small pieces. A 5-g portion was placed into a 20 mL headspace vial and securely sealed with a PTFE-silicon septum. The fiber was preconditioned in the GC-MS port injector at 250 °C for 2 min to eliminate contaminants, and the chromatogram was recorded. Before each extraction, the fiber was also exposed to the empty vial

Table 1. List of 13 culinary mushrooms obtained across Indonesian region

Mushrooms	Sample Code	Common Name/Family
Pleurotus ostreatus	PLO	Oyster mushroom/ *Pleurotaceae*
Auricularia mesenterica	AUM	Tripe mushroom/ *Auriculariaceae*
Lentinula edodes	LED	Shitake mushroom/ *Marasmiaceae*
Pleurotus cystidiosus	PLC	Maple oyster mushroom/ *Pleurotaceae*
Auricularia auricula-judae	AUJ	Wood ear mushroom/ *Auriculariaceae*
Auricularia cornea	AUC	Cloud ear mushroom/ *Auriculariaceae*
Auricularia delicata	AUD	Jelly mushroom/ *Auriculariaceae*
Auricularia polytricha	AUP	Jelly ear mushroom/ *Auriculariaceae*
Lentinus squarrosulus	LES	Lot mushroom/ *Polyporaceae*
Tremella fuciformis	TRF	Snow mushroom/ *Tremellaceae*
Agaricus bisporus	AGB	Button mushroom/ *Agaricaceae*
Favolus tenuiculus	FAT	Tropical white polypore mushroom/*Polyporaceae*
Schizophyllum commune	SCC	Split gill mushroom/*Schizophyllaceae*

and injected into the GC-MS port injector for 2 min as the blank. For the extraction process, the sample in the vial was heated at 50 °C, and the SPME fiber was inserted into the vial and exposed for 30 min. After the exposure, the SPME fiber was immediately injected into the GC-MS port injector for 2 min [14].

GC-MS analysis

GC-MS analysis was conducted using the previously described method [1]. Initially, the column oven temperature was set at 40 °C for 1.2 and 3 min, increased at rates of 4, 5, and 10 °C/min to 120, 135, and 180 °C, respectively. The temperature was increased to 230 °C at 10 °C/min and held for 8 min. Helium was adopted as the mobile phase at flow rate 1 mL/min. For the MS condition, electron ionization mode was used, with detector and interface temperatures set to 230 and 250 °C, respectively.

Identification of volatiles

Volatiles were identified by matching with the mass spectra library (NIST MS 14.0 standard version) as a reference and comparing linear retention index (LRI) with the value in the NIST chemistry book (webbook.nist.gov). A series of homologous *n*-alkane solutions (C8-40, Polyscience, Niles, IL, USA; 5 mg/L) was used to calculate the LRI of the sample under the same analysis conditions. In this context, LRI was counted using Eq. (1) in GC-MS [15].

$$\text{LRI (compound)} = \left(\frac{t_R\,\text{compound} - t_R(n)}{t_R(N) - t_R(n)} \right) \times 100\% \qquad (1)$$

LRI is the LRI of the compound, t_R is the time retention, n and N are the numbers of carbon atoms in eluting alkanes before and after producing the product, respectively.

Data analysis

PCA and OPLS-DA were used to classify the GC-MS data collected in an unsupervised and supervised manner using SIMCA version 18 (Sartorius Stedim Biotech GmbH, Göttingen, Germany). The validity of the models was assessed using permutation plots, model accuracy (R^2X), and predictive accuracy (Q^2Y). Additionally, variable influence on projection (VIP) value and Y-related coefficients were used as the assessment tools to select marker compounds for each mushroom class [16].

■ RESULTS AND DISCUSSION

Volatile Profiles of Indonesian Culinary Mushrooms

Approximately 164 volatiles (Table S1) were identified across mushroom samples and categorized into hydrocarbons, alcohols, terpenoids, ketones, aldehydes, acids, and others. Hydrocarbons were the most abundant group, followed by terpenoids and alcohols, using headspace SPME-GC-MS. This result was consistent with previous research, which reported the classes as the dominant volatiles in mushrooms [7,9,11]. Representative chromatograms for each sample are provided in Fig. S1. The data showed that volatile

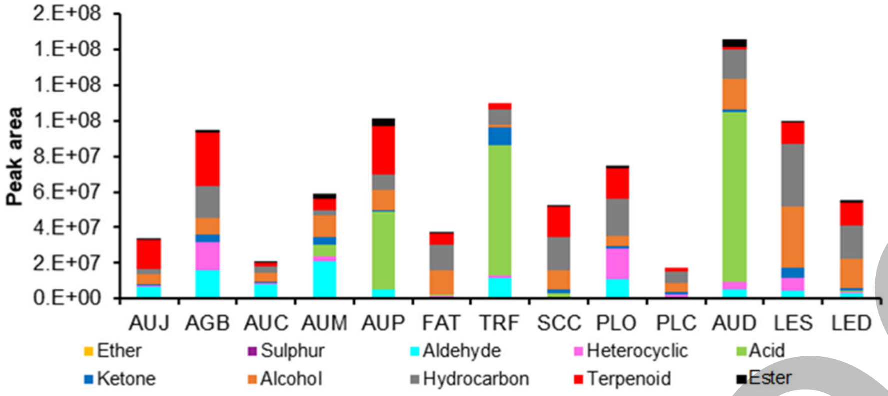

Fig 1. Relative distribution of a group of volatile compounds from 13 culinary mushrooms detected using SPME GC-MS analysis (for abbreviation see Table 1)

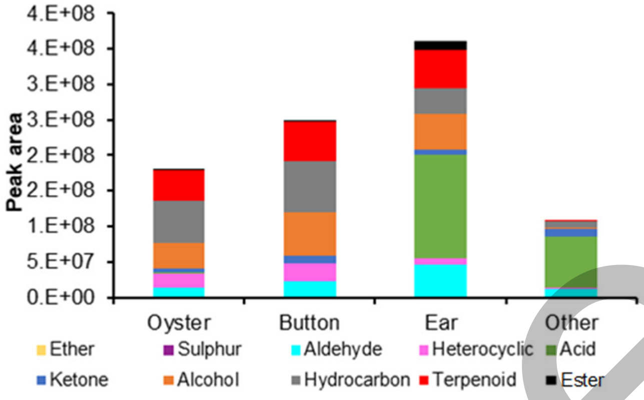

Fig 2. Profiles of the relative volatile compounds proportion in different mushroom groups

profiles varied considerably among mushroom species, both in the number of compounds and in their relative abundances. PLC and TRF exhibited the highest and lowest numbers of volatiles (84 and 51, respectively). The volatile profile of PLC and AUC was characterized by low signal intensity. In contrast, AUD and TRF exhibited a more focused profile with high intensity for fewer compounds (Fig. 1). This suggested that the aroma of AUC and TRF is dominated by a small number of strong odorants, while the aroma of AUC and PLC results from a more complex mixture of less impactful compounds.

Based on morphological characteristics, mushrooms used in this study can be classified into four distinct groups: the oyster (PLO, PLC, FAT, SCC), button (AGB, LES, LED), ear (AUJ, AUC, AUM, AUP, AUD), and other (TRF). Fig. 2 reports the volatile profiles of each morphological group, showing distinct volatiles among mushroom types. The distribution of the classes was highly type-specific. Ear-type mushrooms were predominantly characterized by high intensity of acids, aldehydes, and terpenoids. Oyster- and button-types primarily contained hydrocarbons, alcohols, terpenoids, and heterocyclics. TRF, which does not belong to the three groups, showed a high acid content but the lowest intensity among the other compound groups.

Hydrocarbons

Hydrocarbons are the product of lipid degradation through autooxidation [17]. In this research, hydrocarbons were the most frequently detected group, with 62 compounds putatively identified. The distribution of volatile hydrocarbon groups among the 13 Indonesian culinary mushrooms (Fig. 3) showed both similarities and differences depending on taxonomic affiliation. Although LES and LED are taxonomically related, their distributions of hydrocarbon groups differed significantly, indicating species-specific regulation of volatile biosynthesis. A similar pattern was observed for PLO and PLC, which also exhibited distinct relative distributions of hydrocarbon groups despite belonging to the same genus. In contrast, species within the Auricularia genus (AUJ, AUD, and AUM) displayed highly comparable relative peak areas across the major hydrocarbon classes. This inconsistency suggests that the discriminatory power of hydrocarbon grouping is limited for closely related Auricularia species.

The majority of hydrocarbons were aliphatic alkanes and alkenes, with the highest total abundance reported in LES. Moreover, 1-undecane and 1-tridecane

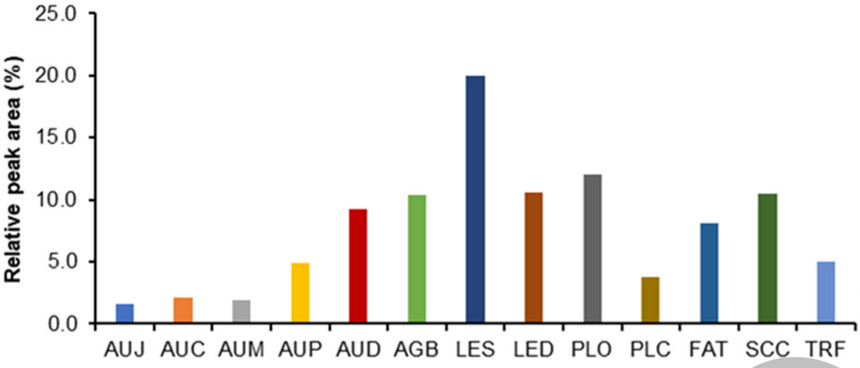

Fig 3. Relative distribution of hydrocarbon group in each mushroom sample based on relative peak area as detected by SPME GC-MS analysis (for abbreviation see Table 1)

were detected as the most abundant individual hydrocarbons (Table S1). 1-Undecane was detected at the highest intensity in AUP, accounting for 70% of the total hydrocarbon area. Meanwhile, 1-tridecane was the most intense in the LES, accounting for 40% of the total hydrocarbon area. The detection of the compounds was consistent with Mohamed and Farghaly [18], who reported 1-undecane and 1-tridecane in mushroom samples, including *P. ostreatus*. Hydrocarbons primarily have an alkane-like odor and a high threshold value, indicating that the contribution to the overall aroma is minimal [8]. This result was consistent with Qin et al. [19], who found that hydrocarbons were not the key aroma-active compounds in *L. edodes*. Furthermore, other hydrocarbons were presented at relative abundance below 10% of the total area in each mushroom.

Terpenoids

Terpenoids represent one of the largest and most diverse groups of volatiles found in mushrooms [9,11], which is primarily comprised of monoterpenes and sesquiterpenes. The biosynthesis of terpenoids is conducted through the mevalonate pathway, which produces the universal five-carbon precursors, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). In this research, terpenoids were the second most frequently detected group of compounds, with 30 individual compounds putatively identified (Fig. 4). Terpenoid groups among the thirteen Indonesian culinary mushrooms showed a high variability in relative contribution across species, indicating that terpenoid composition is not uniformly conserved among taxa. Only LED and LES, which have the same genus, showed comparable terpenoid group distributions, with similar relative peak areas across samples.

Among the identified terpenoids, *d*-limonene and β-sabinene were the most abundant (Table S1).

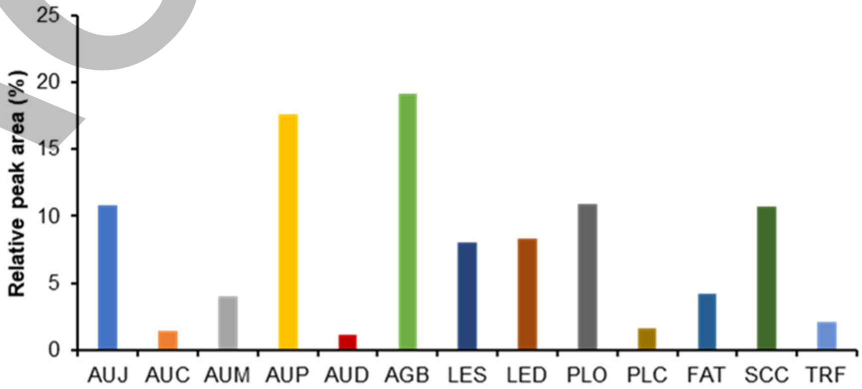

Fig 4. Relative distribution of terpenoid group in each mushroom sample detected by SPME GC-MS analysis (for abbreviation see Table 1)

d-Limonene was highly specific, representing 56% of the total terpenoid area in AUP, while β-sabinene reached its highest intensity in AUD, representing 60% of the total terpenoid area. β-sabinene was also detected consistently across nearly all mushroom samples. Fu et al. [20] showed that *d*-limonene was detected in *Auricularia* mushrooms from Shanghai, with citrus and mint notes. However, *d*-limonene was not attributed to the mushroom's aroma. Beyond aromatic profile, the compound exhibited many biological activities, including antioxidant and neuroprotective effects [21]. Similarly, β-Sabinene reported turpentine and wood odor but did not significantly contribute to the overall aroma. Judžentienė et al. [22] showed that β-sabinene could inhibit the growth of several gram-positive bacteria, namely *B. cereus* and *S. aureus,* as well as certain gram-negative bacteria and yeasts.

Alcohols

Alcohols were the third-most-frequently detected volatile group, with 24 individual compounds identified. However, the overall intensity was lower across the samples. This low intensity was due to the oxidation of alcohols into other compounds, such as aldehydes or acids, during the shade-drying process. This result was similar to Sun et al. [23], where alcohol concentration in *F. velutipes* was reduced significantly during the natural drying process. Similar to the previous group of compounds, the distribution of alcohol groups among the thirteen Indonesian culinary mushrooms does not consistently follow taxonomic boundaries (Fig. 5). The observed patterns do not show clear discrimination between closely related taxa, indicating that alcohol group abundance alone is insufficient as a standalone descriptor for mushroom species differentiation. Only two species of ear mushrooms, AUP and AUM, showed almost comparable alcohol distribution.

Among the alcohols, 2-ethylhexanol and 1-octen-3-ol were the most abundant. In two species of ear mushrooms, AAJ and AUM (Table S1), in which 2-ethylhexanol was detected at the highest intensity, representing 70% of the total alcohol area. Moreover, 1-octen-3-ol constituted 53% of the total alcohol area in PLC and was detected in all samples. 2-ethylhexanol was the only branched C-8 compound and had a green and rose aroma. This compound was detected in several popular mushrooms, such as button and king oyster [24]. Both 1-octen-3-ol, an aliphatic C-8 compound, and the characteristic odor. The compound existed in two enantiomeric forms with a distinct aroma profile. *R*-(-)-1-octen-3-ol has a unique fruity mushroom-like characteristic, while *S*-(-)-1-octen-3-ol is described as a moldy and grassy note. Beyond aroma's role, 1-octen-3-ol exhibited antimicrobial activities against five common food-related bacteria and inhibited the fungal growth [25]. Another key mushroom odorant, 3-octanol, was detected in only 4 of 13 samples. This compound was characterized by a nutty, mushroom-like odor [10]. Additionally, the concentrations of key odorants varied with growth stage. Feng et al. [6] reported that immature mushrooms typically produced higher levels of key odorant compounds than mature mushrooms.

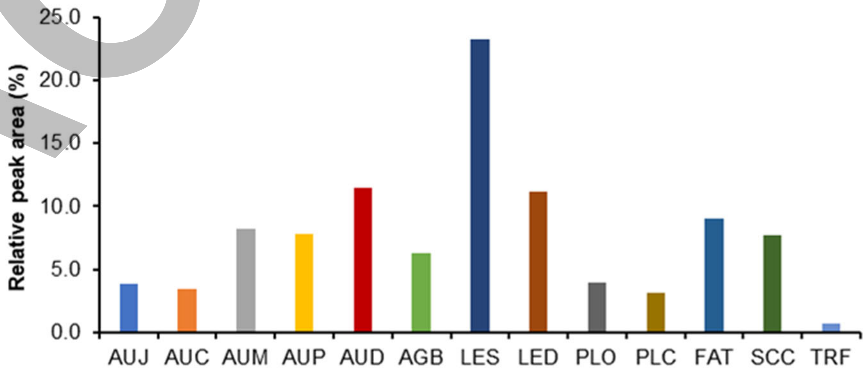

Fig 5. Relative distribution of alcohol group in each mushroom sample detected by SPME GC-MS analysis (for abbreviation see Table 1)

Aldehydes

Aldehydes are another common volatile produced by the oxidation of unsaturated fatty acids in mushrooms. A total of 12 aldehydes were putatively identified in this research. In Fig. 6, it is shown that aldehyde group abundance does not consistently separate all mushrooms, particularly among closely related species. AUM showed the highest abundance among all samples, whereas in SCC it was almost negligible. PLO and PLC show very different aldehyde-group relative abundances, despite belonging to the same genus. The relative contribution of aldehydes is not conserved between the two species, indicating species-specific differences in the aldehyde-related metabolic pathway. This suggests that aldehyde group profiling is sensitive enough to capture interspecies variability within the Pleurotus genus, although it should be interpreted in combination with other volatile groups for comprehensive characterization.

Nonanal, benzaldehyde, and octanal were identified as the most dominant aldehyde types (Table S1). Nonanal, detected at the highest intensity in AAJ and AUC, accounted for 56% of the total aldehyde area. Benzaldehyde was detected in 90% of the total alcohol area in AGB. Octanal was identified at the highest intensity in PLC, accounting for 52% of the total alcohol area. Aldehyde has a low aroma threshold and can significantly influence the odor characteristic of mushrooms at low temperatures [26]. Nosaka and Miyazawa [27] identified nonanal as the main volatile responsible for the mushroom-like and fatty odors in *B. leucomelas*. Another important compound, benzaldehyde, produces a distinct aroma similar to bitter almond and burnt sugar. Octanal, an eight-carbon aliphatic compound, is a key odorant in mushrooms, contributing a citrus and green aroma. Nonanal, benzaldehyde, and octanal have been detected in many types of mushroom species, such as *B. edulis* and *C. comatus* [10]. Nonanal and octanal were reported to inhibit the growth of *A. flavus* in crops [28], while benzaldehyde exhibited a potent insecticidal activity and antimicrobial activity [29].

Ketones

Fig. 7 illustrates the relative contributions of ketone groups among the 13 Indonesian culinary mushrooms, expressed as percentage peak area. There was noticeable variation in ketone contribution across species. Similar to the previous discussion on other groups of compounds, these variations are not uniformly aligned with taxonomic relationships, as some closely related species exhibit contrasting ketone contributions, while others show partially overlapping profiles. The formation of ketones is attributed to multiple biochemical pathways, such as the oxidation of polyunsaturated fatty acids, the Maillard reaction, the degradation of amino acids, and microbial oxidation [30] This indicates that ketone formation is strongly species-dependent and influenced by metabolic regulation beyond genus or family classification, suggesting differences in ketone-related metabolic pathways such as lipid oxidation and secondary metabolism. Ketone was found at the highest intensity in TRF, LES, AUM, and AGB. While AUC, AUP, and FAT had the lowest intensity.

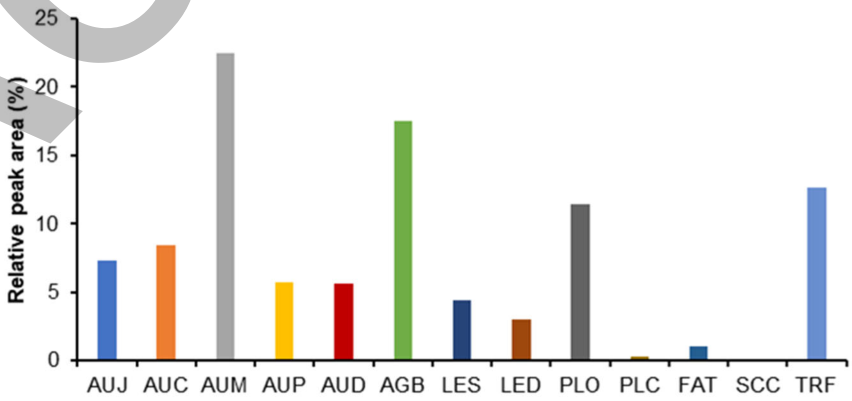

Fig 6. Relative distribution of aldehyde group in each mushroom sample detected by SPME GC-MS analysis (for abbreviation see Table 1)

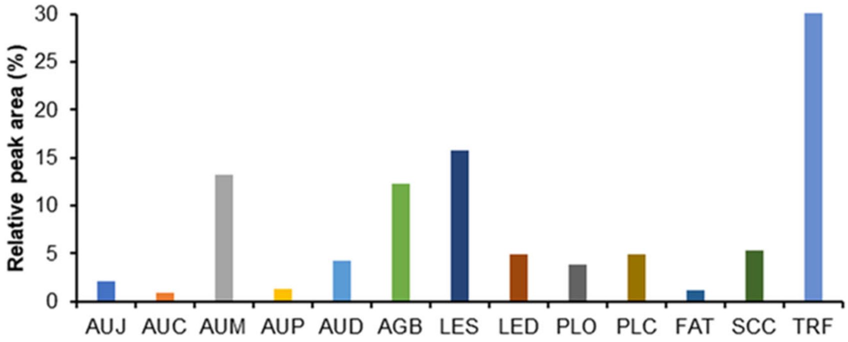

Fig 7. Relative distribution of ketone group in each mushroom sample detected by SPME GC-MS analysis (for abbreviation see Table 1)

Considering relative intensity, Table S1 shows that 3-octanone, 2-undecanone, and 2-nonanone were the most dominant ketones. 3-Octanone was detected at the highest intensity in AUD, LES and LED, and PLC. Furthermore, 2-undecanone was detected at the highest intensity in TRF, accounting for 90% of the total ketone area. 2-Nonanone was found at the highest intensity in AGB. 3-Octanone was a key odorant in mushrooms and possessed a low detection threshold with a buttery, mold-like odor [10]. 2-Undecanone contributed to a fresh and green aroma to mushrooms, similar to *T. fuciformis* [8] This compound was reported to exhibit antifungal activity against *Botrytis cinerea* in tomato both *in vitro* and *in vivo* assays [31]. Moreover, 2-nonanone was characterized by a fragrant and sweet aroma, contributing to the sweet scent in *V. volvacea* [32].

Acids

The contribution of acid-type volatiles varied across species, indicating differences in organic acid formation and related metabolic processes, as shown in Fig. 8. However, as with other volatile groups, the variation in acid contribution does not consistently follow taxonomic relationships. Some species within the same genus exhibited contrasting acid contributions, such as in ear mushrooms, suggesting that acid formation is influenced by species-specific metabolism rather than taxonomy alone. The absence of acid compounds in several mushroom samples suggests species-specific metabolic characteristics, such as lower production of volatile organic acids. It is also possible that acid-type volatiles were either present at levels below the detection limit of the SPME–GC–MS method or were not favorably released under the SPME extraction condition (Fig. 8). As shown in Table S1, acetic acid was detected as the most abundant at 90% of the total area in 3 out of 13 mushrooms (AUP, AUD, and TRF). This result was consistent with Fu et al. [8], where acetic acid was detected at high abundance in *T. fuciformis* and a high abundance of acetic acid in various *A. auricula.* Acetic acid is a byproduct of carbohydrate metabolism and

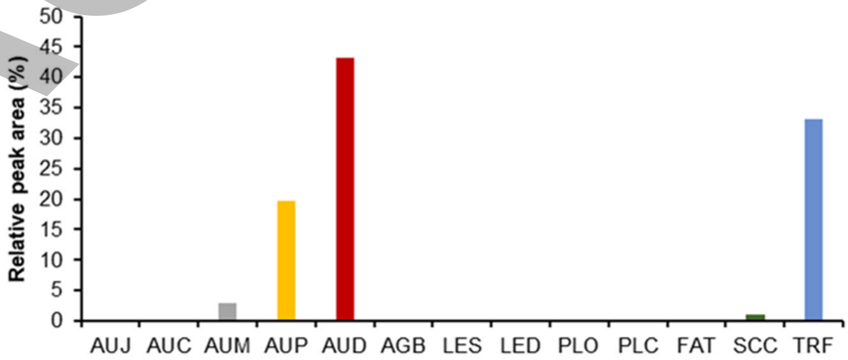

Fig 8. Relative distribution of acid group in each mushroom sample detected by SPME GC-MS analysis (for abbreviation see Table 1)

contributes to the acidic, pungent aroma of mushrooms [20]. Due to a high threshold value, acetic acid has a minimal impact on the overall aroma of mushrooms. Pangprasit et al. [33] showed that acetic acid exhibited strong antibacterial activity against certain pathogenic bacteria.

Heterocyclic compounds were detected in mushroom samples. According to Misharina et al. [7] heterocyclic compounds form during the drying process, leading to the Maillard reaction. In this research, mushrooms were subjected to a natural drying process, resulting in the detection of heterocyclics at lower intensities. Additionally, other volatiles, such as ethers, esters, and sulfur, were detected at low intensity and did not contribute to the aroma of mushrooms. Moreover, the variation in volatiles in these mushrooms is due to many factors, including genetics, variety, different growth stages, environmental conditions, and processing methods [10]. A study by Zhuang et al. [34] showed that *Boletus* varieties produced distinct volatile profiles. It was reported that *B. edulis* is characterized by 2,6-dimethylpyrazine, while *Boletus aereu* is characterized by 2-octenal. Therefore, this indicates that the volatile profile can be utilized for mushroom discrimination.

Collectively, the volatile profiles in Fig. 3–8 reveal that individual compound classes (hydrocarbons, terpenoids, alcohols, aldehydes, ketones, and acids) are insufficient to distinguish between the studied mushroom species. This variability highlights the complex, species-specific metabolism of mushrooms. Since focusing on one class provides only partial information, the entire volatilome must be considered. To better distinguish the species, multivariate analysis was employed in the next section to integrate all volatile data and better resolve differences among the species.

Volatile Profiles as Fingerprints for Discriminating Indonesian Culinary Mushrooms

This research successfully identified hundreds of diverse volatiles. In general, volatile profiles play a central role in the formation of characteristic aroma attributes in food commodities, including mushrooms, as they reflect the combined output of multiple metabolic pathways rather than isolated compounds. Previous studies have shown that mushrooms with distinct volatile fingerprints can be differentiated at the species or genotype level, even when morphological similarities exist, highlighting the discriminatory value of volatilomics in complex food matrices [7,10,24]. At the same time, many of the volatiles contributing to discrimination are also key drivers of mushroom aroma perception, linking chemical variation directly to sensory-relevant characteristics [11,26]. However, because aroma arises from the interaction of multiple volatile classes, reliance on a single compound group often provides incomplete discrimination, particularly among closely related species. Consequently, integrated volatile profiling (volatilomics) has emerged as an effective approach to capture both species-specific chemical signatures and aroma-related diversity, supporting applications in mushroom characterization, authentication, and quality evaluation [5-6,11].

Metabolomics-based research generates high-dimensional datasets that require multivariate data analysis to extract meaningful patterns and relationships. In this study, PCA was first applied as an unsupervised exploratory method to visualize inherent similarities and differences among the volatile profiles of 13 Indonesian culinary mushrooms without prior class assignment [35]. The PCA model with ten principal components explained 84.3% of the total variance, reflecting the chemical complexity of mushroom volatilomes. Although the first two principal components accounted for a relatively modest proportion of variance (13.8 and 13.4%), the overall model performance (R^2X and Q^2 values > 0.4) confirmed its adequacy for exploratory interpretation, consistent with previous recommendations for complex biological datasets [35-36].

The PCA score plot (Fig. 9(a)) revealed five major clusters driven by similarities in volatile composition rather than taxonomic family or morphological classification. These results were consistent with previous research, where mushroom samples clustered according to non-volatile chemical profiles [13]. Mushrooms traditionally grouped by morphology (e.g., oyster-, ear-, or button-type mushrooms) did not cluster

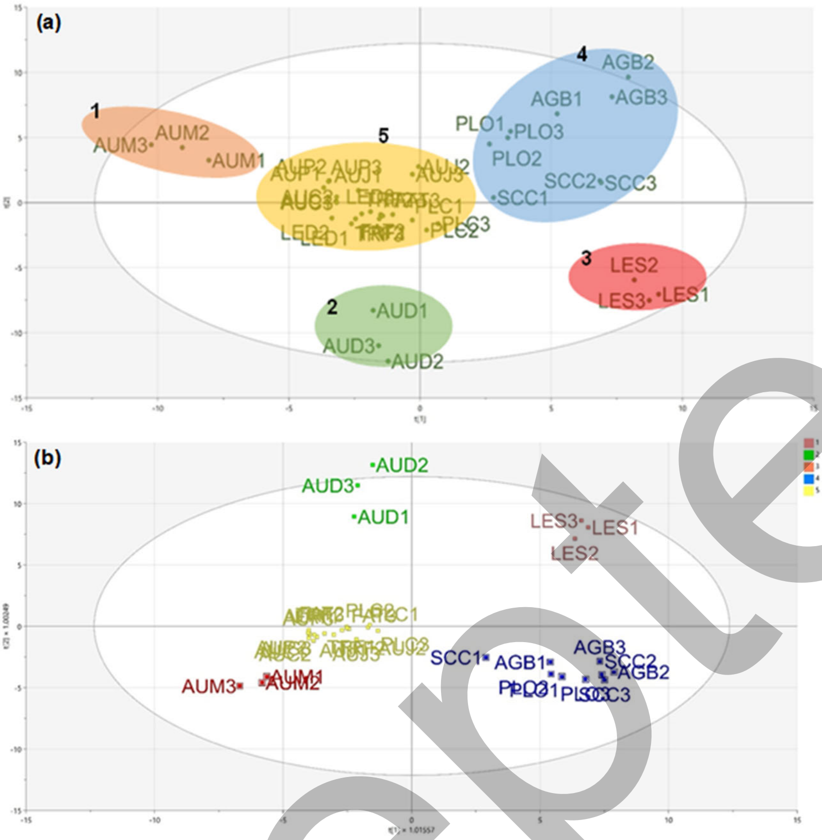

Fig 9. Score plot of (a) PCA and (b) OPLS-DA of volatile compounds from 13 Indonesian culinary mushrooms, showing five clusters marked in different colors (for group class see Table 2)

together, indicating that external appearance does not reliably reflect volatile chemistry. Instead, two ear mushroom species, AUM and AUD, formed distinct clusters, suggesting unique volatile fingerprints. This finding highlights the biological relevance of volatilomics for chemical-based discrimination and aligns with previous metabolomics studies showing that mushroom classification based on chemical composition may differ from that based on morphology. Two species of ear mushrooms, AUM and AUD, were grouped in different clusters (cluster 1 and 2, respectively). Two Lentinus species, LES and LED, were also separated in clusters 3 and 5, respectively. Similarly, two species of oyster mushrooms, PLO and PLC, were distinctly grouped in clusters 4 and 5, respectively. In contrast, mushrooms with different genera, such as PLO AGB, and SCC, clustered at the right of the quadrant (blue cluster), while AUP, AUJ, AUC, LED, TRF, FAT, and PLC clustered in the same group (cluster 4). Similarly, cluster 5 comprised distinct mushrooms across different genera and morphologies (Fig. 9(a)).

To further enhance discrimination and identify volatiles driving group separation, OPLS-DA was applied using five predefined classes derived from PCA clustering (Fig. 9(b)). The OPLS-DA model demonstrated good explanatory and predictive ability (R^2Y and Q^2 > 0.4), confirming its robustness. Importantly, OPLS-DA enabled the identification of key volatile markers responsible for class separation, providing practical insight into species-specific chemical signatures. Unique chemical markers for each group were selected using VIP and Y-coefficient values (Table 2). AUM (class 1) was associated with aldehydes, including octanal and benzaldehyde. Benzaldehyde was known for the sweet, almond notes, while octanal contributed a citrus-like, fatty aroma [10]. AUD (class 2), characterized by the presence of acetic acid and 2-hexylester, contributed to a sharp, vinegar-like odor as

Table 2. Chemical marker of 13 Indonesian culinary mushrooms based on OPLS-DA analysis

Class	Sample	Chemical marker
1	AUM	Octanal, benzaldehyde
2	AUD	Acetic acid, 2-hexylester acetic acid
3	LES	3-Octanol, 1-octen-3-ol, 2-methyl-1-butanol
4	PLO, AGB, SCC	Chrysanthemyl alcohol, dimethyl disulfide, isolongifolene, methyl isovalerate, eucalyptol, α-pinene, benzenacetaldehyde
5	AUJ, AUC, AUP, FAT, LED, TRF, PLC	Furfural, decane, β-elemene, 3-carene, 4-methyl-3-hexanone, 3-ethyl-5-methylene-heptane, α-guaiene

well as a fruity, green note. LES (class 3) was characterized by C8 alcohols linked to typical mushroom-related aroma, including 3-octanol, 1-octen-3-ol, and 2-methyl butanol. 3-octanol and 1-octen-3-ol were recognized as volatiles responsible for the mushroom-like note, while 2-methyl-1-butanol contributed to the onion aroma [11].

In contrast, class-level overlap observed across several species (e.g., classes 4 and 5) indicates that some mushrooms share common volatile features, reinforcing the idea that discrimination is strongest when considering multivariate patterns rather than single compounds. Class 4, which consisted of PLO, AGB, and SCC, was characterized by three significant markers, namely chrysanthemyl alcohol, dimethyl disulfide, and isolongifolene. Chrysanthemyl alcohol or chrysanthemol is a monoterpenoid with an alcohol-like odor. Dimethyl disulfide, a common sulphur compound in mushrooms, is a key contributor to sulfuric aroma [10]. Isolongifolene is not typically associated with mushroom odor but has a woody and amber note. Class five, comprising AUP, AUJ, AUC, LED, TRF, FAT, and PLC, was characterized by furfural, decane, and β-elemene. Furfural is a heterocyclic compound with an almond, burnt odor. Decane is a hydrocarbon with no characterized odor, while β-elemene is a sesquiterpenoid known for the woody and herbaceous note [37].

Beyond classification, the identified volatile markers also suggest potential health-related functional relevance. Even though direct evidence of antioxidant activity in mushrooms was limited, several discriminatory compounds reported in this study have been associated with antioxidant activity in previous literature, supporting the hypothesis that volatile composition may contribute to broader biological functionality [38-40]. Macedo et al. [38] reported that 1-octen-3-ol modulated antioxidant enzyme activity in *Drosophila melanogaster*. Previous investigations also reported that methanolic extracts of SCC, AUJ, and AUC exhibited significant *in vitro* antioxidant activity in the ABTS and CUPRAC assays [13]. The chemical markers of mushrooms in class 5, β-elemene and 3-carene, were reported to inhibit ROS formation in human umbilical vein endothelial cells [41] and to exhibit strong scavenging activity against the DPPH radical [39]. An isomer of α-guaiene (another class 5 marker), β-guaiene, also showed antioxidant properties by scavenging ABTS and DPPH radicals [40]. SCC markers, isolongifolene and α-pinene, were also reported to have strong antioxidant activity across multiple assays (DPPH, ABTS) and to enhance the production of antioxidant enzymes in cells [42-43]. While antioxidant activity was not directly assessed here, the convergence between volatile markers and previously reported bioactivities strengthens the practical significance of chemometric discrimination. Overall, the combined PCA and OPLS-DA analyses demonstrated that integrated multivariate evaluation of volatile profiles provide a powerful framework for mushroom discrimination, chemical characterization, and hypothesis generation for future functional studies.

■ CONCLUSION

This study directly demonstrates that Indonesian culinary mushrooms have distinct VOC profiles that can be effectively characterized and discriminated using SPME–GC–MS–based volatilomics combined with multivariate data analysis. Hydrocarbons, terpenoids, alcohols, aldehydes, ketones, and acids were identified as the major volatile classes, and integration of the complete volatilome enabled classification of 13 mushroom species into 5 distinct groups, independent of morphology or taxonomy. OPLS-DA further identified species- and group-specific volatile markers, including key aroma-related compounds such as 1-octen-3-ol and 3-octanol, which impart characteristic mushroom-like and nutty notes; octanal and benzaldehyde, associated with citrus–fatty and almond-like aromas; and acetic acid, which contributes sharp acidic nuances. Although antioxidant activity was not experimentally evaluated in this study, previous literature reports antioxidant or redox-related activities for several identified volatiles, such as 1-octen-3-ol, β-elemene, 3-carene, isolongifolene, and α-pinene, have been reported elsewhere to exhibit antioxidant or redox-modulating activity using *in vitro* or cellular models. Overall, this work provides the first comprehensive volatilomic characterization of Indonesian culinary mushrooms and establishes a robust analytical framework for future studies integrating volatile profiling with sensory and functional bioactivity assessments.

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■ CONFLICT OF INTEREST

The authors declare no competing interests.

■ AUTHOR CONTRIBUTIONS

Adri Nora conducted the experiment, data acquisition and analysis, wrote and revised the manuscript. Donald John Calvien conducted the experiment, data acquisition, and analysis. Hanifah Nuryani Lioe, Mohamad Rafi, and Endang Prangdimurti conducted the data acquisition and analysis, supervised, wrote, and revised the manuscript. Yane Regiyana conducted the experiment, data acquisition, and analysis. Nancy Dewi Yuliana conducted data acquisition and analysis, supervision, wrote, and revised the manuscript. All authors have agreed to the final version of this manuscript.

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