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Original Article

Dynamic profiling of volatile organic compounds in IR64 rice infested with *Sitophilus oryzae* (Linnaeus): An exploratory HS-SPME-GC-MS study for initial biomarker screening

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ABSTRACT

This study examines the volatile organic compounds (VOCs) as potential indicators of the presence of rice weevils, *Sitophilus oryzae* (Linnaeus), in IR64 rice grains. Conventional detection methods have limitations because pest infestation is detected after signs of pest damage have appeared. Meanwhile, identification of VOCs resulting from host-pest interactions can be used as chemical indicators in the early detection of infestation. This experiment used a sample unit of 10 g of rice in a vial infested with 10 adults S. oryzae, observed over five weekly intervals. VOCs from the sample headspace were extracted using headspace solid-phase microextraction (HS-SPME) and then analyzed using gas chromatographic-mass spectrometry (GC-MS). The results showed a shift in VOC composition, with the peak-area abundance of aldehyde compounds originating from plants decreasing while hydrocarbon compounds originating from insects became dominant. Aldehyde compounds, for example hexanal, decreased from 3.76×10^8 in the control to 1.12×10^8 in the fourth week, while hydrocarbon compounds, such as 3,6-dimethylundecane, increased from 5.67×10^7 to 2.82×10^8. The volatile compounds that most affected changes in the VOC profile include hydrocarbon compounds with high VIP scores and positive $\log_2$FC, such as 3,6-dimethylundecane (1.68 and 2.31) and pentadecane (1.40 and 1.97), while aldehyde compounds with high VIP scores and negative $\log_2$FC values include dodecanal (1.60 and -1.60) and hexanal (1.49 and -1.79). These aldehyde and hydrocarbon compounds with high VIP > 1 and $\log_2$FC are thought to have the strongest contribution to changes in the VOC profile due to *S. oryzae* infestation and may be preliminary information for developing an early detection model in rice storage. However, further verification is needed, including experimental replication across several rice varieties and storage conditions.

Key words: chemical fingerprinting, chemometric analysis, early detection, postharvest storage, stored grain pests, volatilomics

INTRODUCTION

The quality and supply of cereal crops worldwide are significantly affected by the presence of insects and warehouse pests. The main pest on rice that causes economic losses is the rice weevil, *Sitophilus oryzae* (Ardakani & D'Amico 2020; Arouna et al. 2021). In tropical regions with inadequate storage conditions, these insect pests cause yield loss of 10% to 30% (Babendreier et al. 2020). In Cameroon, *S. oryzae* is the dominant pest, accounting up to 81.9% of observed warehouse pests, and has resulted in a 19.9% decrease in seed mass (Mapiemfu-Lamare et al. 2023). In Indonesia, rice is a staple food for the general population, and post-harvest pests pose a threat to national food security (Mastuti et al. 2020; Simanjuntak & Farida 2025). Damage and grain weight loss caused by *S. oryzae*´range from 5% to 25.65%, depending on rice variety, storage duration, and insect population density (Syahrullah et al. 2019). Therefore, alternative scientific methods are needed to detect early the presence of *S. oryzae* before severe damage occurs.

In the early stages of *S. oryzae* larval infestation on rice, symptoms cannot be detected because the insect feeds inside of the grain. Conventional detection methods such as visual examination and screening are less effective (Marsadi & Yuliadhi 2025; Doherty et al. 2023). Currently, non-destructive and sensitive detection techniques are still limited and require further development. Monitoring volatile compounds through chemical analysis using gas chromatography can be used to detect the pests presence early, by identifying changes in aroma before physical damage becomes visible (Liu et al. 2024; Tanaka et al. 2021). Considerable amount of research has been conducted utilizing this technology to analyze volatile compounds in various plants (Guan et al. 2024; Xu et al. 2021).

Volatile organic compounds (VOCs) are carbon-based chemical compounds that readily vaporize at room temperature. Rice produces VOCs from lipid oxidation, enzymatic activity, and interactions with insects or microbes (Hou et al. 2025; Tian et al. 2023). In healthy rice, the VOCs produced are generally the result of plant lipid oxidation, especially aliphatic aldehydes such as hexanal. These compounds are produced as a result of endogenous lipooxygenase that degrades polyunsaturated fatty acids, including linoleic acid (Gao et al. 2021). VOCs can be used as an early indicator of oxidative damage and to assess cereal quality in storage (Ye et al. 2024). Dynamic concentrations of VOCs can reflect biological changes under different storage conditions.

The presence of *S. oryzae* on rice grains changes the composition of VOCs in the surrounding environment. Insects feed on lipids in the endosperm of rice grains, which leads to a decrease in aldehyde levels. The biological activity of insects triggers an increase in aliphatic hydrocarbons and methyl-chain alkanes from their cuticles (Liu et al. 2024). The chemical compounds produced by these insects function to prevent the drying of plants and serve as a means of communication between insects. The change in the composition of these volatile compounds reflects an increase in insect metabolic activity.

In chemical ecology, interactions between host plants and insects drive changes in VOC emissions. These volatile compounds can indicate the health of rice grains and the presence of pests. Rice undergoes lipid degradation, resulting in a decrease in volatile aldehyde compounds, while insects produce hydrocarbon compounds due to their physiological activity. The increase in hydrocarbon compounds also corresponds to the increase in insect populations. Monitoring both compound classes simultaneously over time can provide a comprehensive assessment of VOC dynamics in *S. oryzae* infested rice. Previous studies have documented compounds such as 1-octen-3-ol, 1-hexanal, and 3-octanone increasing at the beginning of *S. oryzae* infestations in wheat seeds (Liu et al. 2024). Meanwhile, concentrations of long-chain hydrocarbons, such as pentadecane and hexadecane, were also detected to increase in the affected grains. However, most of the research that has existed has only emphasized a single-time-point comparisons between healthy and infected grains rather than longitudinal analyses across multiple observation periods.

An additional challenge is that certain VOC signals may come from insects and microbes, which complicates their interpretation. In this study, microbial activity was minimized by using clean rice grains and maintaining dry storage conditions; however, VOCs derived from microbes cannot be eliminated. This study focused on monitoring changes in plant-derived compounds (e.g., aldehydes) and insect-derived compounds (e.g., hydrocarbons), consistent with previous research, with the modification of observations multiple time points. Several studies have simultaneously examined the decrease in plant-derived VOCs, such as hexanal, and the increase in insect-derived hydrocarbons, such as 3,6-dimethylundecane, over time in stored rice. Monitoring of these compound ratios can facilitate the development of more effective early-warning chemical assays for pest detection.

Liu et al. (2024), Tanaka et al. (2021), and Tian et al. (2023) studies have shown that VOCs can be used as indicators of insect infestations in cereal crops. However, most studies have compared infested and healthy samples at a single point in time. Identifying VOC from a single compound as a candidate biomarker is challenging because many VOCs originate from multiple sources. To address this gap, the present study examined longitudinal changes in the VOCs profile of rice grains over successive weeks of *S. oryzae* infestation. The main objective was to identify which volatile compounds show the most pronounced changes and could serve as potential indicator of *S. oryzae* infestation. This study addresses two questions: (1) How do VOC types change weekly during *S. oryzae* infestation? (2) Which volatile compounds are the most reliable indicators of pest presence relative to previous studies?

MATERIALS AND METHODS

Experimental design and sample preparation

This study was designed and conducted under controlled laboratory conditions using rice grains infested with *Sitophilus oryzae*. The experimental medium was the IR64 rice variety, which is commonly consumed in the Indonesian market, and *S. oryzae*, which had been reared to the second filial generation (F2) and identified based on the morphology of the inner genitalia of adult insects. Samples were prepared by purposive sampling: each sample consisted of 10 g of rice grains in a 22 ml vial infested with 10 adult *S. oryzae*. A longitudinal design was established with the following infestation time intervals: (A) week 0 (control or no infestation), (B) week 1, (C) week 2, (D) week 3, and (E) week 4. Each vial sample unit was an independent, non-replicated sample for each time interval. During the storage period, up to the fourth week of infestation, all samples were stored at room temperature (25–30 °C) and relative humidity of 70–75% in ventilated vials. This study aimed to analyze the chemical compounds resulting from *S. oryzae* adult infestation in rice; sex-based comparisons between male and female individuals were not made, and insects were not removed from the vials after oviposition. Data were obtained through headspace solid-phase microextraction (HS-SPME) and then gas chromatography–mass spectrometry (GC–MS) analysis (detailed below) in February 2023 at the Flavor Analysis Laboratory, Rice Crop Assembly and Modernization Center (Balai Perakitan dan Modernisasi Pertanian Tanaman Padi/ BRMP Padi), Sukamandi, Subang, West Java, Indonesia.

VOCs extraction and GC-MS analysis

Headspace solid-phase microextraction (HS-SPME) and analyse with gas chromatographic-mass spectrometry (GC-MS). Each sample unit was incubated at 80 °C for 30 minutes to transfer volatile compounds into the head chamber (Figure 1). At these temperatures, VOCs can be captured while minimizing degradation of the aroma profile (Gao et al. 2021). Once equilibrium was achieved, DVB/CAR/PDMS-COATED 50/30 μm SPME fibers extracted a broad spectrum of compounds, from light aldehydes to long-chain hydrocarbons (Tian et al. 2023). Calibration was performed using internal standards, and compound identification analysis was based on mass spectral matching with NIST libraries and retention time verification. This methodology follows established, comprehensive approaches to food chemical profile analysis (Gao et al. 2021; Hou et al. 2025; Zhou et al. 2023).

In this study, the generalizability of the results is limited due to the lack of biological replication. Therefore, data analysis focused on describing trends rather than statistical inference. Descriptive analyses, including compound summary distributions and multivariate analyses such as principal component analysis (PCA), were performed to identify patterns and differentiate samples based on infestation duration. Each compound was assessed using a variable importance in projection (VIP) score, calculated using a Partial Least Squares (PLS)-based chemometric approach, to determine its influence on VOC pattern separation over the entire infestation duration. Fold change analysis measured the difference in peak area between control and infested samples. Results were visualized using PCA plots, heatmaps, and linear regression trend graphs, consistent with recent volatilomics research on pattern recognition and biomarker candidates (Liu et al. 2024). VOC data obtained from HS-SPME-GC-MS analysis were processed using MetaboAnalyst for multivariate chemometric analysis. Prior to multivariate analysis, the data were pre-processed to mitigate the influence of differences in signal magnitude transformed with Log10(Area + 1) followed by auto-scaling (z-score) to create clustering patterns with colour visualization.

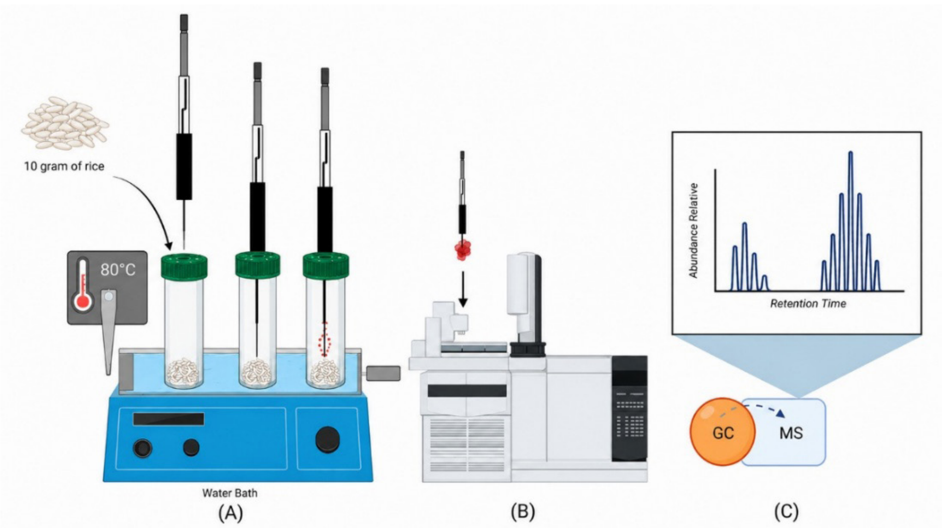

Figure 1. Headspace solid-phase microextraction (HS-SPME) gas chromatography-mass spectrometry (GC–MS) sampling scheme. A: extraction stage, in which the fiber was exposed to VOCs in the sample headspace; B: SPME extraction and thermal desorption in the GC injector; and C: VOC separation, analysis, and identification.

The hierarchical clustering process was carried out to see the similarities and differences in VOC content patterns between different samples and compounds, thus facilitating comparisons between VOC variables.

RESULTS

VOC chromatographic profile and chemical composition dynamics

The VOC profile of IR64 grains rice infested with *S. oryzae* was analyzed using GC-MS across different infestation durations. Across all observation samples, 210 volatile compounds were identified, including aldehydes, alcohols, ketones, hydrocarbons and aromatic compounds. Each sample unit showed changes in compound composition, reflecting the VOC profile resulting from the interaction between the rice grain host and insect pests.

The control sample (A), representing uninfestated rice, contained 147 compounds dominated by aldehydes such as hexanal, nonanal, octanal and benzaldehyde – the natural VOCs of rice grains derived from lipid oxidation. In week one (B), the number of compounds increased to 153, aldehydes began to decline but remained dominant, while alcohols (1-octen-3-ol, 1-hexanol) and hydrocarbons began to appear. By week two (C), the number of compounds increased to 172, and a metabolic transition occurred, with hydrocarbons such as 3,6-dimethylundecane and pentadecane beginning to dominate over aldehydes. In the third week (D), the number of compounds decreased slightly to 167. The dominance of long-chain hydrocarbons, such as alkanes and alkenes, became more apparent, and aromatic compounds began to increase. In the fourth week (E), the sample showed the highest diversity with 173 compounds. VOCs were dominated by hydrocarbons and aromatic compounds, while aldehyde levels became very low.

A comparison of peak area abundance of major compounds across observation time intervals of *S. oryzae*-infested IR64 rice grains is presented in Table 1. The SPME-GC-MS analysis revealed two main trends in VOC peak-area abundance. Aldehyde compounds such as hexanal showed overall decrease in the peak area, from 3.76×10^8 in the control sample to 1.12×10^8 in the fourth week infestation sample. In contrast to hexanal, acetaldehyde peak-area abundance showed an increasing trend from 1.83×10^8 in week 0 to 3.71×10^8 in week 1, then remained stable during continued infestation. Meanwhile, hydrocarbon compounds such as 3,6-dimethylundecane increased from 5.67×10^7 to 2.82×10^8 and 1-pentadecene increased from 7.69×10^6 to 3.01×10^7. This increase in hydrocarbon compounds was most evident in the third- and fourth-week samples, making these compounds the most dominant constituents of the VOC profile. Meanwhile, the alcohol compound 1-octen-3-ol fluctuated in peak-area abundance, ranging from 2.46×10^7 to 3.80×10^7.

Table 1. Comparison of peak area abundance of key compounds from IR64 rice infested by *Sitophilus oryzae* across weekly observation intervals

Compound name	Class	Sample A (W-0)	Sample B (W-1)	Sample C (W-2)	Sample D (W-3)	Sample E (W-4)	Trend pattern	Source (Origin)
Acetaldehyde	Aldehydes	1.83×10^8	3.71×10^8	3.19×10^8	3.33×10^8	3.53×10^8	Increasing	By-product of carbohydrate fermentation (Mitsuya et al. 2009)
Hexanal	Aldehydes	3.76×10^8	1.38×10^8	2.49×10^8	1.90×10^8	1.12×10^8	Overall decreasing	Host lipid oxidation due to insect feeding damage (Cao et al. 2024)
3,6-dimethyl-Undecane	Alkanes	5.67×10^7	1.03×10^8	1.67×10^8	1.61×10^8	2.82×10^8	Overall increase	*Sitophilus* cuticular fingerprint (Liu et al. 2024)
1-Pentadecene	Alkenes	7.69×10^6	8.64×10^6	1.51×10^7	9.08×10^6	3.01×10^7	Sharp increase	Adult insect pheromone (Cai et al. 2022)
1-Octen-3-ol	Alcohols	2.53×10^7	2.92×10^7	3.80×10^7	2.92×10^7	2.46×10^7	Fluctuating	Host lipid degradation and fungal activity (Tian et al. 2023)

Noted: - Compiled data from samples A–E and literatures.
- Values represent integrated GC-MS peak areas expressed as arbitrary units (a.u.).

PCA analysis: Separation patterns and infestation gradient

The PCA score plot visualizes the separation of VOC profiles among rice grain samples at various *S. oryzae* infestation durations (Figure 2). PC1 explained 75.67% of the data variance, capturing the main changes in VOC profiles from the control stage through subsequent infestation durations. PC2 explained 13.09% of the data variance. Sample A appeared separated from the other samples in the lower right corner, indicating the most distinct VOC profile. Sample B was positioned in the upper right corner, showing differences primarily along the PC2 component. Samples C, D, and E were positioned on the left side of the plot, indicating greater similarity in VOC profiles compared to Samples A and B. The PC1 value decreased from 24.874 in the control sample (A) to -16.490 in the fourth-week *S. oryzae* infestation sample (E). This reflects a shift in dominance from plant-derived compounds to insect-based compounds. The PC2 values were more variable, accounting for a smaller proportion of the total variance.

PCA loading plot: Identification of dominant compounds

The PCA loading plot shows the contribution of each volatile compound variable to the formation of the principal component. High loading values, whether positive and negative, indicate that these variables play a dominant role in distinguishing sample characteristics. The positive PC1 region was associated with the VOC profile characteristic of the control and early infestation samples, whereas the negative PC1 region was associated with the VOC profile of the later infestation stages. Meanwhile, the PC2 values (positive and negative) reflect secondary variation not captured by PC1. Compounds positioned close together on the plot exhibit similar pattern.

As shown in Figure 3, short-chain aldehyde compounds (hexanal, hexenal, (E)-2-hexenal, heptanal) were positioned on the negative side of PC1 and the positive side of PC2. These compounds are associated with early lipid oxidation in control rice and early-stage infestation, and their levels tend to decrease as infestation progresses. The compounds 3,6-dimethylundecane, nonanal, decane, undecane, and octane were positioned near the center toward the positive side of PC1, indicating a stable, moderate contribution. Meanwhile, 1-pentadecene was positioned on the positive side of PC1 with high loading on PC2, suggesting an important role in differentiating secondary variation among advanced infestation samples.

Heatmap: VOC grouping and reorganization patterns

Heatmap analysis shows changes in the discrimination pattern and dendrogram of VOCs in *S. oryzae*-infested rice grains over the five observation periods. The detected compounds are divided into seven main chemical classes: aldehydes, hydrocarbons, alcohols, esters, ketones, acids, and other compounds (Figure 4). The distribution of these compounds changes over time. Aldehydes are generally higher in the early observation periods, while hydrocarbons become more dominant in later periods.

Other chemical classes also contribute to changes that occur over time. Ketone compounds, including

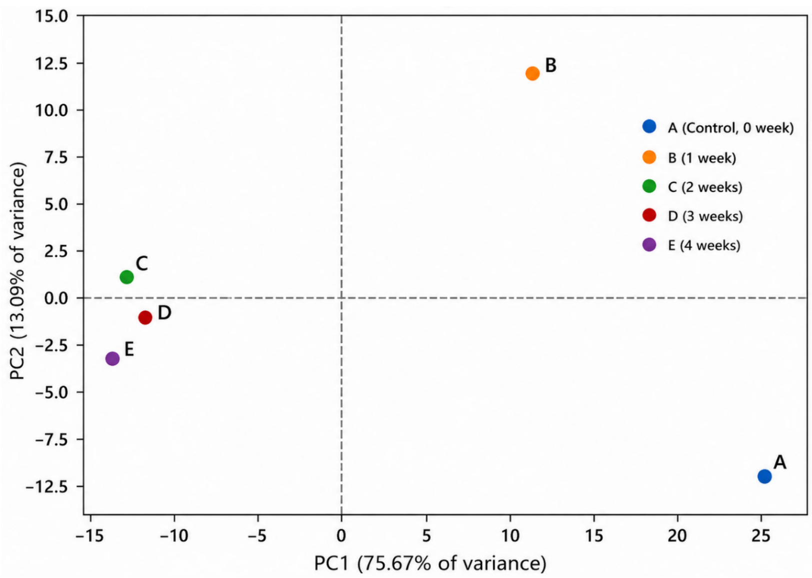

Figure 2. Principal component analysis (PCA) score plot showing the separation of IR64 rice infested by *Sitophilus oryzae* across weekly observation intervals.

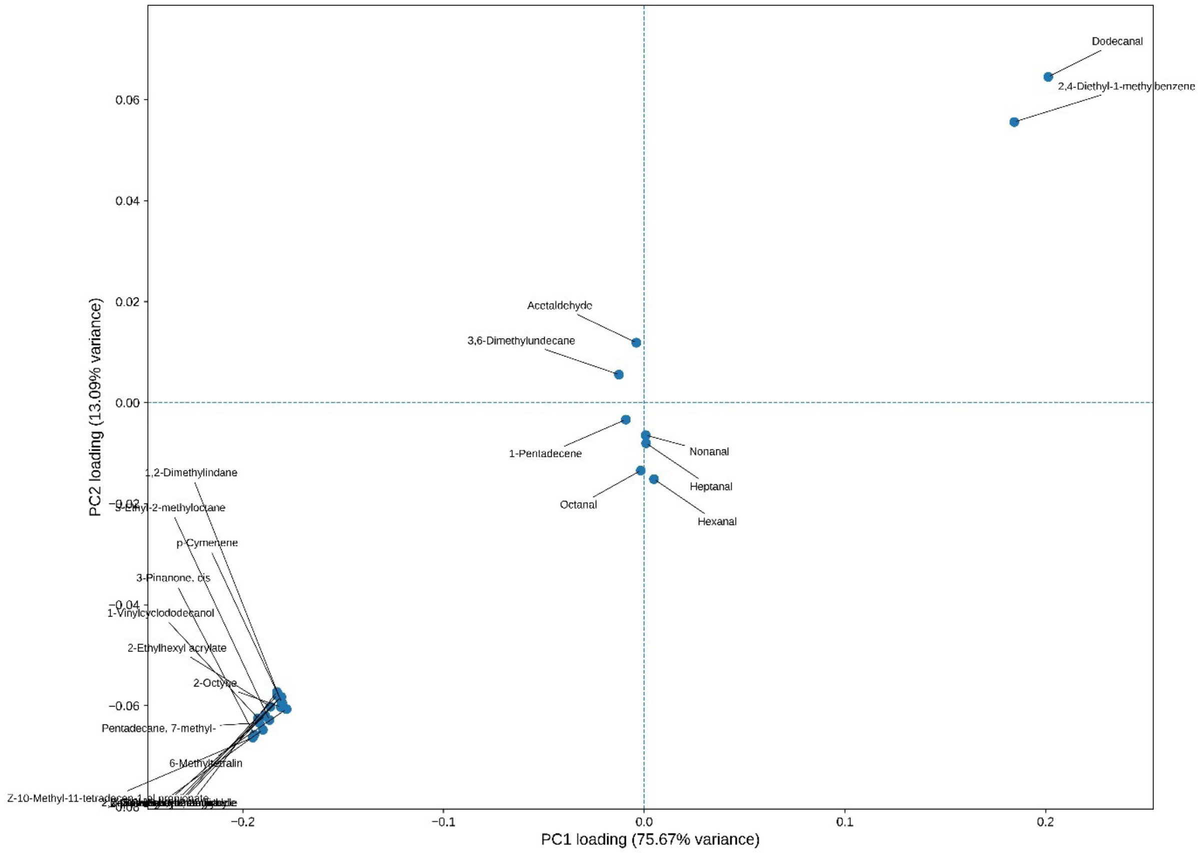

Figure 3. Principal component analysis (PCA) loading plot illustrating the contribution of individual volatile compounds of IR64 rice infested by *Sitophilus oryzae* to the sample separation.

2-heptanone and 6-methyl-5-hepten-2-one, exhibit changes during the infestation developmental stage. These compounds are likely related to lipid oxidation and secondary metabolic processes occurring within rice tissues. However, the presence of these compounds should be considered as indicators of changes in biochemical activity, rather than as specific markers of *S. oryzae*. This is due to the fact that ketone compounds can originate from a variety of different biological and chemical pathways.

Alcohols also exhibited variation over time, but their patterns of variation were less consistent than those of the major hydrocarbon and aldehyde groups. This variability is consistent with possible contributions from plant-derived metabolic processes and microbial activity. Esters, represented in the dataset by compounds such as 2-ethylhexyl acetate and methyl salicylate, were smaller but still recognizable components in the VOC profiles. The presence of these compounds suggests changes in volatile metabolism during storage and pest infestation. Similarly, acids such as acetic acid and hexanoic acid played a role in differentiating the chemical properties of the samples. These compounds likely reflect changes in fatty acid metabolism, as well as microbial activity or fermentation processes as environmental conditions changed during storage.

Overall, the heatmap analysis results indicate that changes in VOC compound structure associated with infestation are not limited to a single chemical class. Instead, these changes occur simultaneously across several different compound groups. Aldehydes and hydrocarbons exhibit opposite patterns of change, while ketones, alcohols, esters, and acids contribute to the formation of more complex chemical patterns. This interpretation aligns with the results of PCA and chromatographic analyses, which indicate gradual changes in VOC compound structure, rather than changes confined to a single compound class.

VIP and fold change analysis: Exploration analysis of biomarker screening

Variable importance in projection (VIP) and fold change analyses were used to identify the key volatile compounds associated with *S. oryzae* infestation level in rice grains. However, because this experiment lacked replication, the VIP and fold change results should be interpreted as exploratory findings – useful for observing trends or narrowing down candidate variables – rather than as absolute conclusions. A VIP value above 1 indicates volatile compounds with the strongest effect, while the $\log_2$-fold change ($\log_2$FC) describes the quantitative change between the initial

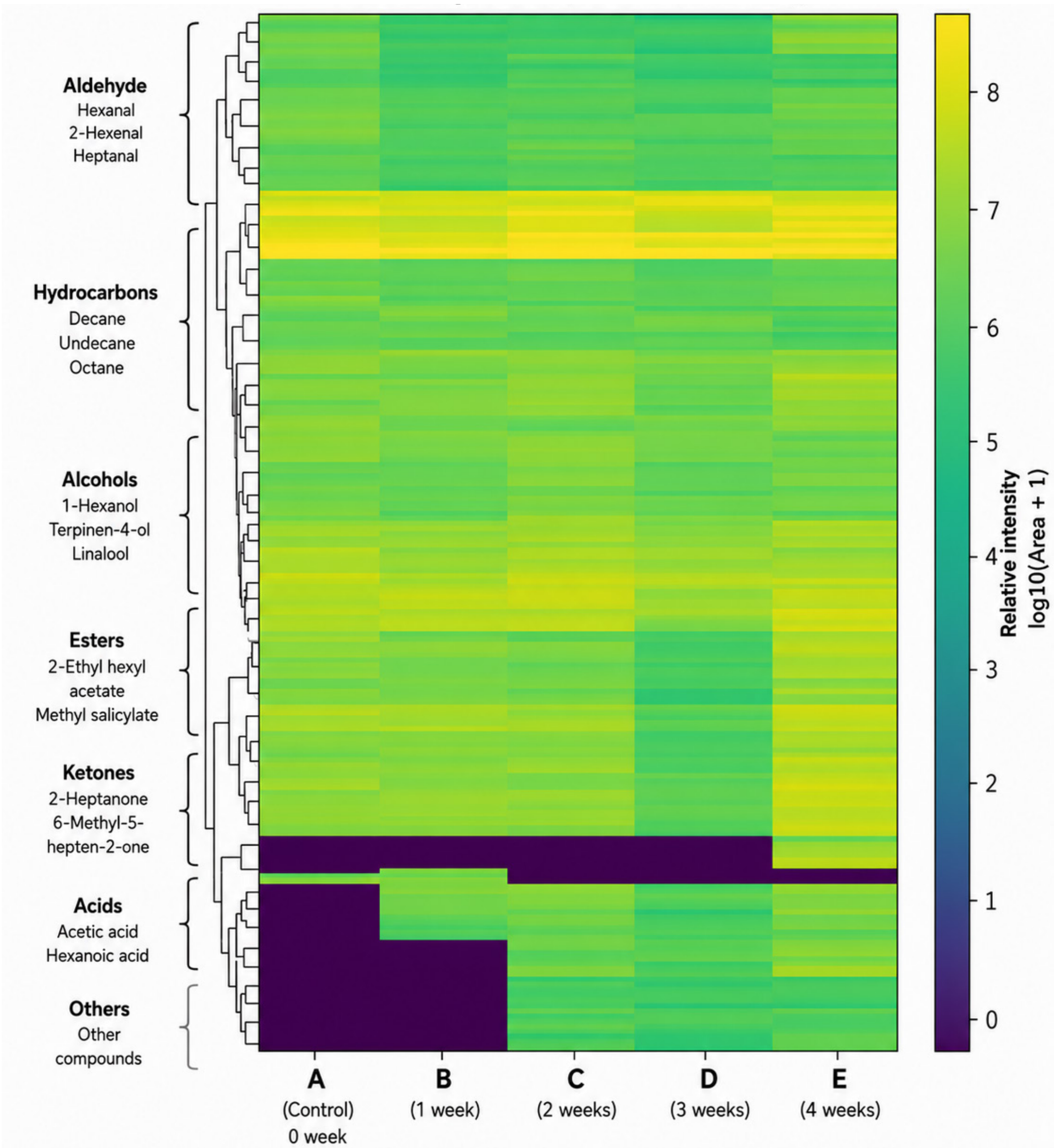

Figure 4. Heatmap visualization and hierarchical discrimination dendrogram of volatile organic compounds (VOCs) of IR64 rice infested by *Sitophilus oryzae*.

(control) and final (infested) conditions. In this study, VIP and fold change analysis were performed to identify the main volatile compounds contributing to the profile of *S. oryzae*-infested rice grains (Table 2).

Compounds with high VIP scores and positive log_2FC values were predominantly hydrocarbons, including 3,6-dimethylundecane (VIP score of 1.68, log_2FC of 2.31) and pentadecane (1.40 and 1.97), which are likely derived from insect metabolism and serve as important markers of insect infestation in the host. Several aromatic compounds also had high VIP scores and positive log_2FC values, however, these likely reflect natural aromatic variation among rice varieties rather than infestation-related changes. On the other hand, plant-derived compounds from such as aldehydes had high VIP scores with negative log_2FC values, namely dodecanal (1.60 and -1.60) and hexanal (1.49 and -1.79), which likely result from lipid oxidation of rice grains driven by the activity of other organisms, including insects and other microbes. The negative log_2FC value of dodecanal indicates a decrease in its abundance from the control to the fourth week of infestation. However, its position on the PCA loading plot is determined by its covariance structure across all samples rather than by the E/A fold change alone.

Table 2. Exploration analysis of the top 20 volatile organic compounds (VIP + fold change) of IR64 rice infested by *Sitophilus oryzae*

No	Compound	Class	VIP score	Fold change (E/A)	log_2FC	Tren
1.	3,6-Dimethylundecane	Hydrocarbon	1.68	4.97	2.31	Increasing
2.	Naphthalene, 2-methyl-	Aromatic	1.66	6.42	2.68	Increasing
3.	Cumene, p-ethyl-	Aromatic	1.62	8.11	3.02	Increasing
4.	Cyclopentadecane	Cycloalkane	1.58	7.84	2.97	Increasing
5.	Biphenyl	Aromatic	1.55	6.73	2.75	Increasing
6.	m-Propyltoluene	Aromatic	1.65	5.96	2.58	Increasing
7.	2,2-Dimethyl-3-octanone	Keton	1.72	5.41	2.44	Increasing
8.	6-Methyltetralin	Aromatic	1.72	5.18	2.37	Increasing
9.	1-Heptanol	Alcohol	1.58	0.42	-1.25	Decreasing
10.	Linalyl acetate	Ester	1.61	0.37	-1.43	Decreasing
11.	Dodecanal	Aldehyde	1.60	0.33	-1.60	Decreasing
12.	Hexanal	Aldehyde	1.49	0.29	-1.79	Decreasing
13.	2-Octenal	Aldehyde	1.47	0.28	-1.84	Decreasing
14.	1-Pentanol	Alcohol	1.45	0.31	-1.69	Decreasing
15.	Decanal	Aldehyde	1.43	0.35	-1.51	Decreasing
16.	Pentadecane	Hydrocarbon	1.40	3.92	1.97	Increasing
17.	Hexadecane	Hydrocarbon	1.38	3.65	1.87	Increasing
18.	Tetradecane	Hydrocarbon	1.36	3.28	1.71	Increasing
19.	Undecane	Hydrocarbon	1.34	2.94	1.56	Increasing
20.	1-Octen-3-ol	Alcohol	1.32	0.97	-0.04	Fluctuating

VIP: variable importance in projection scores;
Fold Change (E/A): ratio of peak area in sample E relative to sample A;
FC > 1 indicates an increase during advanced infestation stages;
FC < 1 indicates a decrease relative to the control condition;
log_2FC was used to interpret volcano plots and the direction of biological changes;
Compound with VIP > 1 and high $|log_2FC|$ values were considered to have the strongest contribution to the VOC profile separation caused by *Sitophilus oryzae* infestation.

DISCUSSION

Data collected from IR64 rice grains infested with *S. oryzae* using HS-SPME and GC-MS analysis, successfully demonstrated a pattern of change in the composition of VOCs. However, because no replication design was used, this study focused on documenting emerging patterns by detailing the specific characteristics of the candidate volatile compounds identified. In this study, 210 volatile compounds were identified – a substantially more detailed profiles than in previous studies. Zhang et al. (2019) detected 64 volatiles in wheat samples infested by the rice weevil *S. oryzae* using SPME, while Liu et al. (2024) identified 126 volatiles from headspace gas flow coupled with GC-MS in wheat infested by the same insect. Based on the duration of infestation, analysis of the VOC peak areas between treatment samples showed a fairly consistent pattern. This emerging pattern aligns with research by Gao et al. (2021), which showed that VOC profiles in storage warehouses reflect the biological status of host-pest interactions.

Sitophilus oryzae infestation of rice was associated with a decrease in plant-derived aldehyde levels. Aldehyde compounds initially dominated in control samples and early infestation samples. Meanwhile, aliphatic hydrocarbons and aromatic compounds derived from insect metabolism became increasingly prominent as the insects developed and triggered the emergence of other microbes. This compound arises from lipid degradation by fungal activity in rice grains, which is also triggered by insect activity that increases local temperature and humidity. Changes in the microbial population during the storage of rice grains also contribute to changes in the composition of volatile compounds (Lee et al. 2019; Qiu et al. 2024). Therefore, the differences in the patterns of change in the peak-area abundance of acetaldehyde and hexanal, both of which are aldehyde compounds, may be due to differences in the mechanisms by which these compounds are produced. Acetaldehyde serves as an indicator of microbial metabolic activity, whereas hexanal indicates lipid oxidation processes (Cao et al. 2024; Mitsuya et al. 2009). A study by Liu et al. (2024) found that hydrocarbon compounds predominate in wheat infested by *S. oryzae*. In rice, however, this study shows that hydrocarbons not only accumulate but also come to dominate rapidly as infestation duration increases.

The shift from plant-derived compounds to insect-derived compounds reflects changes in the volatile environment. Aldehyde levels declined to their lowest points, while hydrocarbons became the dominant compounds, particularly in the third and fourth weeks. The continued presence of aldehyde compounds indicates that the rice structure remains sufficiently intact to allow for continued lipid oxidation and the production of important volatile compounds (Gao et al. 2021). Hydrocarbon compounds such as 3,6-dimethylundecane, derived from the cuticle, and 1-pentadecenes, a pheromone metabolite of adult insects, increased sharply from the first week of insect infestation, peaking in the fourth week. The increase in hydrocarbon compounds reflects the accumulation of biomass and high insect activity, which can trigger the emergence of other microbes that exacerbate grain damage. These findings are consistent with previous research showing that larval growth in the grain increases the insect-derived cuticular hydrocarbons (Liu et al. 2024). Research by Tian et al. (2023) found that the alcohol compound 1-octen-3-ol can indicate early infestation by *S. oryzae*. However, in this study, the 1-octen-3-ol fluctuated more and was less reliable in distinguishing infestation stages. This may be because the alcohol compound is sensitive to microbial changes and humidity (Tesfaye et al. 2021). The decrease in plant-derived aldehydes represents a novel finding that has not been widely studied. Previous studies have often focused on increases in insect metabolites as an early marker of stress. These findings suggest that a diagnostic method based on trends in these changes could help develop an early detection system for post-harvest pest infestations, distinguishing biological infestations from natural aging.

To identify the VOCs pattern of IR64 rice grains infested with *S. oryzae* over time, a multivariate chemometric pattern recognition approach was used. This technique enables visualization of complex data, discovery of hidden trends, and classification of samples based on their chemical profiles. This approach to understanding and managing analytical data uses several key techniques, such as PCA (Inobeme et al. 2022). A limitation of this study is that the data obtained lacked replication, therefore, PCA functioned as a feature-extraction and data-exploration tool to determine the variation patterns among compound variables that most differentiate samples based on infestation duration. PCA processing revealed differences in VOC profiles across each weekly *S. oryzae* infestation sample. The PC1 explained most of the variation and indicated a shift from plant-derived VOCs to VOCs predominantly derived from insect metabolism and activity. Thus, infestation duration is a major factor influencing VOC variation. This is in line with PCA's general suitability for summarizing datasets with substantial variation (Worley & Powers 2016). In volatilomics, PCA is often used because of its ability to identify complex chemical patterns without requiring assumptions about specific data distributions (Liu et al. 2024).

PCA loading plots provide insight into which biochemical factors drive specific differences between samples. This technique identifies changes in VOC types during *S. oryzae* infestation. Volatile compounds with positive loadings, mostly aldehydes are associated with healthy rice grain tissue and early infestation. The hexanal was located on the positive side of PC1 (loading = 0.00486), consistent with its relatively high abundance in the control sample. Dodecanal also showed a positive PC1 loading but with a substantially greater magnitude (0.20122), indicating a stronger contribution to the principal variation captured by PC1. Other aldehydes showed variable loading directions, including positive loadings for heptanal, and nonanal and negative loadings for octanal, decanal, undecanal, benzeneacetaldehyde, and acetaldehyde. This variation indicates that aldehyde compounds did not exhibit a uniform contribution to PC1 and that their PCA loadings were determined by their complete temporal abundance patterns across samples rather than by chemical class or fold-change direction alone. While compounds with negative loadings (3,6-dimethylundecane, nonanal, decane, undecane, octane, and 1-pentadecene) indicate increasing insect activity that changes on a weekly basis during infestation. Consistent with the results of Gao et al. (2021), a shift from plant-based to insect-derived metabolic profiles was observed. Compounds with high absolute loading values in the PCA plot are strong candidate markers for detecting and monitoring infestation levels. Heatmap analysis also showed a discrimination pattern consistent with the PCA results, with volatile compound distribution and VOC profile changes occurring weekly during *S.oryzae* infestation. These VOC changes could be used to sensitively detect post-harvest pests (Liu et al. 2024; Tian et al. 2023). These findings support the hypothesis that VOC profile changes follow a consistent pattern: a decrease in aldehyde compounds due to rice grain lipid decomposition coincided with an increase in hydrocarbon compounds derived from insect metabolic activity. The PCA separation technique, which sort metabolites according to their biological source, could be further developed to measure pest infestation levels. Changes in VOC patterns could also be used to developed an early warning system for insects presence in rice.

Analysis of VIP scores together with fold change results represents a comprehensive approach to identifying potential biomarker candidates (Liu et al. 2024; Chong & Jun 2005. Compounds with high VIP scores and fold change values serve as sensitive and specific indicators of insect infestation. Since the available data lacked replication, VIP scores along with their fold changes should be viewed as preliminary screening tools for candidate biomarkers, not as confirmed results. Hydrocarbon compounds (3,6-dimethylundecane and pentadecane) meet both criteria and may indicate insect infestation, while aldehyde compounds (dodecanal and hexanal) consistently decreased, reflecting reduced variability in plant-derived data. This supports the hypothesis that these volatile compounds may be reliable indicators of pest presence. This method could serve as a preliminary basis for developing advanced sensors, such as electronic-nose devices and machine learning-based monitoring systems. However, while PCA, heatmap, VIP, and fold change analyses collectively support this trend, the results of this study remain preliminary. Future research should incorporate replicated sampling and statistical validation under various conditions to confirm these patterns to establish the relationship between rice grain degradation and insect presence, include targeted observations or improved analyses to access the specific influence of microbes on grains. With such validation, this approach could meaningfully contribute to early-warning strategies for post-harvest pest management.

CONCLUSION

The VOC profile of IR64 rice grains infested by *S. oryzae* shifted progressively over the 0–4-week storage period, from plant-derived aldehyde to hydrocarbon compounds from insect activity. This shift, together with the VIP and fold-change patterns identified in this study, suggests that the changes aldehydes to hydrocarbons peak-area abundance could serve as a preliminary basis for developing an early pest-detection model for *S. oryzae* presence in stored rice, including real-time monitoring approaches such as electronic nose or similar volatile-sensing technologies.

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