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Potential volatile markers of brown rice infested by the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae)

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A R T I C L E I N F O

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Chemical compounds studied in this article:
1-Octen-3-ol (PubChem CID: 18827)
1-Hexanol (PubChem CID: 8103)
3-Octanone (PubChem CID: 246728)
Allyl cyanide (PubChem CID: 8009)
Trimethylamine (PubChem CID: 1146)
2-Propenenitrile (PubChem CID: 7855)
Ammonia (PubChem CID: 222)
Methylpropanal (PubChem CID: 6561)

A B S T R A C T

The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) could cause significant grain loss by feeding internally on seeds. In this study, we tried to analyze the volatile compounds in non-infested and *S. oryzae*-infested brown rice during different storage periods to identify potential markers in *S. oryzae*-infested brown rice and facilitate pest monitoring during brown rice storage. Headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC–MS) and headspace-gas chromatography-ion mobility spectrometry (HS-GC-IMS) were used to identify the volatile compounds. On the basis of GC–MS and GC-IMS data, a reliable method to distinguish between non-infested and *S. oryzae*-infested brown rice was discovered using partial least squares-discriminant analysis (PLS-DA). 1-Octen-3-ol, 1-hexanol and 3-octanone were co-selected as potential markers because their variable importance in projection (VIP) was greater than 1 in both models. The current study's findings lay a foundation for further research on the brown rice infestation mechanism and safe storage monitoring.

Introduction

The issue of grain security has always been the primary concern of our country and this concern has only increased as we face recent issues with food supply. The COVID-19 outbreak in late 2019 and the Russia-Ukraine war in early 2022 both had a very substantial impact on grain security globally, affecting the prices of wheat, rice and other grains. With more than half of the world's population consuming rice as a staple food, it is the second-most widely cultivated cereal crop in the world (Sun et al., 2022). Therefore, the safe storage of grains is crucial because it will allow us to store enough grain to deal with the possibility of a price increase brought on by some issues in the future. However, as the world's population continues to grow, carbon dioxide emissions far exceed the balance, leading to global warming (Adler et al., 2022). The gradual increase in temperature is more conducive to the breeding of pests in grain storage. Storage pests damage and loss is one of the most common difficulties in grain storage. Statistics show that grain storage losses might account for up to 50 % of the total harvest, resulting in a loss of several billion dollars globally (Luo, Olsen, Liu, & Zhang, 2022). Thus, the task of grain storage security is arduous.

Rice is usually stored and transported in the form of brown rice. Brown rice is made by immediately hulling rough rice, which is mainly made up of about 90 % w/w endosperm. In addition, it also contains 6 %–7% w/w bran layer and 2 %–3% w/w embryo (Liu et al., 2021). However, because the brown rice had lost its exterior layer after being hulled, it was more susceptible to pest infestation during the storage period, which could subsequently affect the rice's quality. The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) is a tough pest to manage on stored grains because it feeds internally in seeds (Jalaeian, Mohammadzadeh, Mohammadzadeh, & Borzoui, 2021). *S. oryaze* infestation can reduce the quality and quantity of stored grains. The ideal conditions for *S. oryaze* development are 25–27 ℃ and 60–70 % RH, with a 24–36-day life cycle from egg to adult (Abdel-Hady, Ramadan, Lü, & Hashem, 2021). The effect of storage temperature, processing methods, and different cultivars on rice volatile compounds has been the subject of extensive investigation (Choi & Lee, 2021; Sun

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et al., 2022; Tian et al., 2022). Previous studies have shown that the characteristic volatile compounds identified in insects or insect-infested stored grains can be used as a marker for discrimination. Tanaka, Magariyama, & Miyanoshita (2020) found that isopentenols were the biomarkers in brown rice samples infested by moths and polysulfides were the biomarkers in brown rice samples infested by *S. zeamais*. Niu, Hardy, Agarwal, Hua, & Ren (2016) found that 2-ethyl-2,5-cyclohexadiene-1,4-dione can be a biomarker in flour samples infested by *T. castaneum*. In our early study, we found that 1-pentadecene can be used as a volatile biomarker for the detection of *T. castaneum* infested brown rice under different temperatures (Tian et al., 2022). However, little data is known about the function of volatiles on *S. oryaze*-infested brown rice under different storage periods. Thus, more research into the finding and identification of these compounds in *S. oryaze*-infested brown rice versus non-infested brown rice could help with the early detection of the *S. oryaze* infestation.

At present, HS-SPME-GC–MS is a well-established analytical method for studying volatile compounds (Zhang et al., 2020). GC mainly uses the differences in polarity, boiling point and adsorption properties of substances to realize the separation of mixtures. After the vaporization of the sample to be analyzed in the gasifier, it is carried into the chromatographic column by the carrier gas. The retention time of the mixture on the chromatographic column is long or short, and the outflow sequence is first and then, so as to achieve the effect that the mixture is separated on the chromatographic column. GC–MS has been widely used in the identification of volatile compounds in rice. 2-AP is the most important volatile compound in aromatic rice (Dias, Hacke, Bergara, Villela, Mariutti, & Bragagnolo, 2021). J. Zhang et al. (2022) have used the volatile compounds detected by GC–MS to judge whether rice was infested by mold. Different volatile compounds were detected by GC–MS to distinguish rice with different processing methods. The results showed that 16 characteristic volatiles could be used as markers in raw rice and 22 other volatiles could be used as markers in cooked rice (Zhao et al., 2022). GC–MS analysis of volatile compounds in black rice samples with varying milling degrees showed that the relative content of total volatile compounds in non-milled black rice was higher, while the content of volatile compounds in milled black rice was significantly lower (Choi & Lee, 2021). An emerging and popular technique for analyzing volatile compounds is HS-GC-IMS. It is a two-dimensional measurement based on GC and IMS that can detect volatile fingerprints of samples with less pretreatment (Cavanna, Zanardi, Dall'Asta, & Suman, 2019). When compared to GC–MS, this technique has a much higher sensitivity and requires less pretreatment. The GC-IMS technique could be used to rapidly detect fungal infection in rice and peanut (Gu, Chen, Wang, & Wang, 2021; Gu, Chen, Wang, Wang, & Huo, 2020). Ethyl acetate is a common volatile compound detected by GC-IMS in brown rice flour and noodles (Sun et al., 2022). GC-IMS technology can quickly and intuitively identify the volatile components in green wheat samples and clearly classify the samples according to the differences in volatile components (Zhang, Zhang, Gao, Zhuang, Feng, & Xu, 2021). Yang et al. used GC-IMS technology to evaluate volatile compounds in several quinoa samples, detecting 120 peaks in total and identifying 61 compounds. Different quinoa samples can be clearly distinguished by OPLS-DA (Yang et al., 2021). Fan, Jiao, Liu, Jia, Blanchard, & Zhou (2021) used GC–MS and GC-IMS to analyze volatile compounds in sorghum. GC–MS identified 40 volatile compounds and GC-IMS detected 26 volatile compounds that were not detected in GC–MS. The research showed that the two methods combined can provide a more comprehensive understanding of the volatile compounds in sorghum.

In order to improve the separation of volatile compounds, this study used GC–MS in combination with GC-IMS as an alternative separation approach. Using GC–MS and GC-IMS, we have comprehensively explored the volatile compounds in *S. oryaze*-infested brown rice and each corresponding non-infested brown rice during different storage periods. PLS-DA was used to test the ability to distinguish between *S. oryaze*-infested and non-infested brown rice based on their volatile compounds. On the basis of PLS-DA models, the VIP value more than 1 was used to identify the potential markers in *S. oryaze*-infested brown rice. This study aimed to quickly identify the *S. oryaze*-infested brown rice and serve as a platform for further study on infestation mechanisms and safe storage monitoring.

Materials and methods

Materials preparation

In this study, Suijing 18, a variety of 2021 late japonica rice planted in the northeast of China, was selected and hulled with a rice huller (JLG-II) to obtain brown rice samples. *S. oryzae* was reared in the incubator in complete darkness at 28 ± 2 °C and 70 ± 5 % R.H. All adults of the same age were chosen for the experiment.

A 10 weeks' simulated infestation experiment was conducted in a jar (80 cm × 100 cm) with 100 mesh gauze. 200 g of brown rice and 20 adults of *S. oryzae* were placed in a jar marked as the experimental group (S-2w, S-4w, S-6w, S-8w, and S-10w). Only 200 g of brown rice was placed in the same jar marked as the control group (C-2w, C-4w, C-6w, C-8w, C-10w). Both the experimental and control groups were triplicated and kept under the same conditions as described above.

HS-SPME-GC–MS analysis

Brown rice (8 g) and 10 μL 2-octanol (0.1 mg/mL, 99.99 %) were put in a 20 mL headspace container, which was then placed in a 70 °C water bath for 30 min. The volatile compounds were extracted by exposing the fiber (50/30 μm DVB/CAR/PDMS, Supelco) to the samples for 70 min. GC–MS (QP2010-plus, Shimadzu Co., Japan) was equipped with a Rxi-5 MS column (30.0 m × 0.25 mm, 0.25 μm). Following these steps, the chromatographic column was programmed to increase from the initial temperature of 45 °C to 250 °C at a rate of 5 °C/min. Both processes are held for 5 min. Helium (purity of 99.99 %) was used as a carrier gas and its flow rate was set at 1 mL/min. The temperatures of the ion source and the interface are 200 °C and 250 °C, respectively. The full scan is carried out at a speed of 1666 amu/s, and the scanning range is 45–500 amu.

Qualitative analysis of volatile compounds was accomplished by comparison with the standard atlas libraries (NIST08 and NIST08s). The ratio of peak areas was used for quantitative analysis and the calculation formula is as follows:

$$C = \frac{S_1 \times m_2}{S_2 \times m_1} \times 1000$$

Where C is the content of volatile compounds (μg/kg), S_1 is the chromatographic peak area of the compound to be tested, S_2 is the chromatographic peak area of the internal standard, m_1 is the sample mass (g), and m_2 is the internal standard mass (μg). The results were displayed as mean ± standard deviation of triplicate.

HS-GC-IMS analysis

GC-IMS analysis was carried out on an instrument of FlavourSpec® (G.A.S. Dortmund Company) with an MXT-5 capillary column (15 m × 0.53 mm). Brown rice (5 g) was placed in a 20 mL headspace container and incubated at 70 °C at a speed of 500 rpm for 15 min. The temperature of the injector was set at 85 °C and the injection volume was 500

Table 1
The conditions of gas chromatography.

Time (min: *sec*)	Drift gas (mL min^{-1})	Carrier gas (mL min^{-1})	Recording
00: 00	150	2	rec
02: 00	150	2	–
10: 00	150	10	–
20: 00	150	100	stop

μL. The conditions of gas chromatography are shown in Table 1. The temperatures of the chromatographic column and IMS were set to 60 °C and 45 °C, respectively. The migration gas, nitrogen, with a purity of 99.99 % is selected to migrate at a rate of 150 mL/min. The standard RI and drift time in the GC-IMS library were used for the characterization of volatile compounds. All of the analyses were carried out in triplicate.

Statistical analysis

With SPSS software (ver. 22.0, Chicago, USA), the GC–MS analysis data were examined using one-way analysis of variance (ANOVA) and Duncan's multiple range tests. The changes of volatile compounds in brown rice under different infestation time were further compared and analyzed. The instrumental analysis program for GC-IMS includes Laboratory Analytical Viewer (LAV) and GC-IMS Library Search. Both analysis programs can be used to analyze samples from various perspectives. PLS-DA is a discriminant classifier, which uses regression methods to solve classification problems (Reale, Biancolillo, Foschi, Di Donato, Di Censo, & D'Archivio, 2022). PLS-DA was performed in the SMICA 14.1 (Umea, Sweden) software. Based on PLS-DA model, the method of VIP value greater than 1 was used to identify the characteristic volatile compounds in samples.

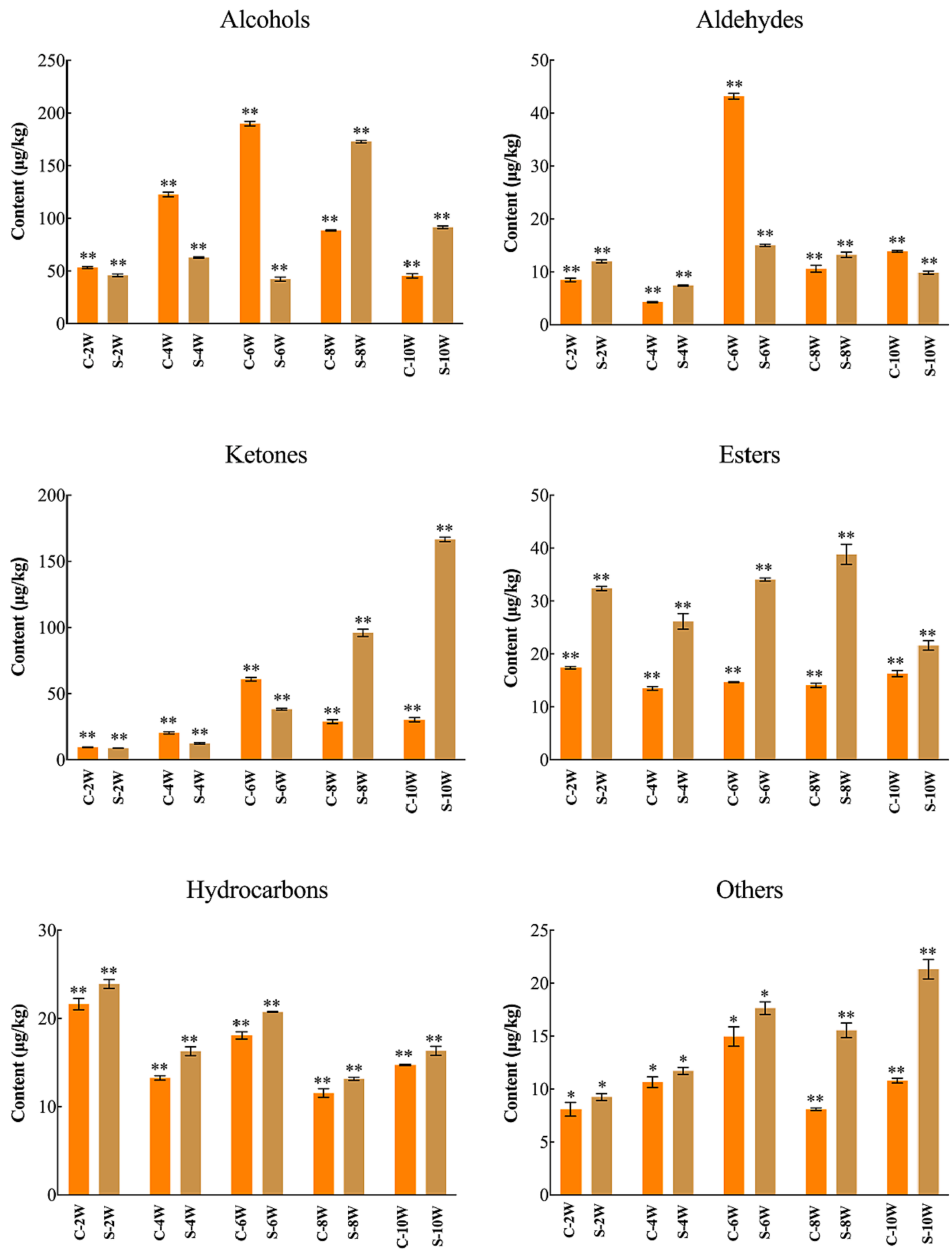

Fig. 1. Volatile compound variation in *S. oryaze*-infested and non-infested brown rice during different periods. C presenting non-infested brown rice and S presenting *S. oryaze*-infested brown rice. * in the same period indicate significant differences at $p < 0.05$ and ** in the same period indicate significant differences at $p < 0.01$.

Results and discussion

Analysis of volatile compounds by GC–MS

Forty-eight volatile compounds were detected in brown rice samples, including 7 alcohols, 7 aldehydes, 7 ketones, 11 esters, 15 hydrocarbons and 1 other type of compound (Table S1). The total content of alcohols, aldehydes, ketones, esters, hydrocarbons and other types of compounds differed significantly between non-infested and *S. oryaze*-infested brown rice (Fig. 1).

Among the alcohols, we observed that the total amount of alcohols in the brown rice decreased in the early stages of *S. oryzae* infection, but by the eighth week, the total amount of alcohols in the *S. oryaze*-infested brown rice was higher than that of the non-infested brown rice. The contents of 1-heptanol, 1-octanol and 1-hexacosanol decreased with the extension of infestation time. 1-Hexanol and 2-ethyl-1-hexanol were not detected from 6 weeks to 10 weeks. With the extension of infestation time, some of the original herbal, green, citrus and waxy aroma gradually disappeared. In this study, 1-octen-3-ol was generated in all periods, which gave an earthy aroma to *S. oryaze*-infested brown rice. Zhang et al. (2022) stored japonica rice with a moisture content of 16 % in an incubator at 30 ℃ for one month. Samples were withdrawn every-five days for testing and analysis. 1-Octen-3-ol can be detected at each stage of storage, and the content of 1-octen-3-ol was increased significantly with the storage time. Gao, Li, Pan, Fan, Wang, & Qian (2021) stored rice bran at 37 ℃ and 75 % relative humidity for two weeks and take samples at 0, 7 and 14 days respectively for testing and analysis. The content of 1-octen-3-ol at 0, 7 and 14 days is 26.6 ± 3.5 μg/kg, 87.6 ± 1.5 μg/kg and 133.3 ± 22.7 μg/kg, respectively. Previous studies have shown that 1-octen-3-ol can be produced from unsaturated fatty acids via an enzymatic reaction involving some microbial participation (Lee, Lim, Chang, Hurh, & Kim, 2018).

Among the aldehydes, rich amounts of nonanal and decanal were found in our study. Nonanal is formed by the oxidative decomposition of fatty acids, and decanal is the secondary product of oleic acid. It has been reported that when the concentration of these two compounds is low, they produce a pleasant odor, while a nasty odor is transmitted at an excessive concentration (X. Zhang et al., 2020). In our results, the contents of nonanal and decanal in *S. oryaze*-infested brown rice were all higher than its corresponding non-infested brown rice (Table S2). The increase of these two compounds indicates that *S. oryzae* infestation will increase unpleasant odor and make the original aroma of brown rice disappear.

Among the ketones, the content of 6,10-dimethyl-5,9-undecadien-2-one and 6,10,14-trimethyl-2-pentadecanone significantly decreased with the extension of storage periods, which indicated that the floral aroma was gradually disappearing. By contrast, the content of 3-octanone, 2-octanone and 2-heptadecanone significantly increased with the extension of storage periods, which may be due to the continuous oxidation of fatty acids in brown rice with the infestation of *S. oryzae*, resulting in the increase of ketone content. 6-methyl-5-hepten-2-one and (R,S)-5-ethyl-6-methyl-3E-hepten-2-one were only detected in the second week. Previous research has identified 6-methyl-5-hepten-2-one as a characteristic volatile compound in rice yellowing and is related to pyruvic acid in the glycolysis pathway (Liu et al., 2021).

The total contents of esters and hydrocarbons in *S. oryzae*-infested brown rice were all extremely higher than in non-infested brown rice. Most esters have medium odor intensity and provide a waxy aroma while hydrocarbons generally have no aroma. Esters are formed by the esterification reaction between alcohols and acyl-coenzyme A, which results from the fatty acid lipoxygenase pathway and amino acid metabolisms, respectively, or by the esterification of aldehydes (Hu, Lu, Guo, & Zhu, 2020). Therefore, the reason for the significant difference in esters between *S. oryaze*-infested brown rice and non-infested brown rice may be that *S. oryzae* infestation seriously damaged the lipid membrane of brown rice, increasing lipid oxidation products. In addition, some studies have revealed that short-chain alkanes are byproducts of lipid degradation (Verma & Srivastav, 2020). Studies have also confirmed that the exfoliated epidermis of pests during infestation contains *n*-alkanes and linear alkenes (Prasantha, Reichmuth, Adler, & Felgentreu, 2015).

Analysis of volatile compounds by GC-IMS

In order to obtain more comprehensive information, GC-IMS analysis was also used to analyze the volatile compounds of *S. oryaze*-infested and non-infested brown rice. The difference between GC-IMS analysis and GC–MS analysis is that the former provides total information based on two-dimensional separations, whereas the latter does not (X. Zhang et al., 2020). Differential plots were formed using topographic plot deduction to successfully distinguish different information between non-infested and *S. oryaze*-infested brown rice during different periods (C-2 W and S-2 W as the background, respectively). Based on signal intensity, the red spot indicates higher content of a compound, whereas the blue spot indicates lower content of a compound in the samples (Fig. 2 A). Comparison of matching drift times, retention times, and retention indexes were used to verify the volatile compounds in the samples. The samples' consistency was determined using non-supervised PCA. Each signal displayed in the sample topographic plot was treated as a separate variable. The score plot of the PCA analysis is shown in Fig. 2 B. The first and second principal components (PC1 and PC2) accounted for 37.6 % and 24.1 % of the total components, respectively. On the score plot, the non-infested brown rice samples were gathered on the right side, whereas the *S. oryaze*-infested brown rice samples were gathered on the left side. This finding showed that using GC-IMS data, non-infested and *S. oryaze*-infested brown rice could be reliably categorized.

Since the database matched by GC-IMS is not complete at present, only some ion peaks can be qualitatively determined. There were 12 kinds of unknown compounds in non-infested brown rice and 24 kinds of unknown compounds in *S. oryaze*-infested brown rice (Fig. 2C). By comparing the identified compounds in non-infested and *S. oryaze*-infested brown rice samples, we found that there were 14 new volatile compounds in *S. oryaze*-infested brown rice, which were methylpropanal, styrene, thiophene, pyrrole, aniline, dimethyl trisulfide, methional, cyclopentanone, methyl isovalerate, ammonia, 2-propenenitrile, trimethylamine, allyl cyanide and pentyl acetate. Among them, thiophene and pyrrole are five-membered heterocyclic compounds. Thiophene is composed of four carbon atoms and one sulfur atom, while pyrrole contains four carbon atoms and one nitrogen atom. Previous studies have shown that thiophene, pyrrole and other heterocyclic compounds are the main volatile compounds produced during rice aging (J. Xu, Liu, & Zhang, 2021). Tanaka et al. found discovered dimethyl trisulfide and dimethyl disulfide in brown rice infested with S. zeamais (Tanaka et al., 2020), which was consistent with our findings.

For volatile compound analysis, GC–MS analysis could produce reliable qualitative and quantitative data, but GC-IMS has the advantage of speed. GC-IMS analysis could be a simple and time saving technique for recognizing *S. oryaze*-infested brown rice. By using both GC–MS and GC-IMS analysis, the types of detected compounds were slightly different. Hydrocarbons can be detected in GC–MS, but GC-IMS has no response to such compounds. GC-IMS can detect some heterocyclic compounds that were not detected in GC–MS. As a result, by analyzing volatile compounds using two different methodologies simultaneously, more comprehensive information can be obtained.

Discrimination of non-infested and S. oryaze-infested brown rice by PLS-DA

Based on the data obtained by GC–MS and GC-IMS, the PLS-DA models were carried out to distinguish the non-infested and *S. oryaze*-infested brown rice during different periods. There are a total of 30 samples in the PLS-DA model (five periods × two treatments ×

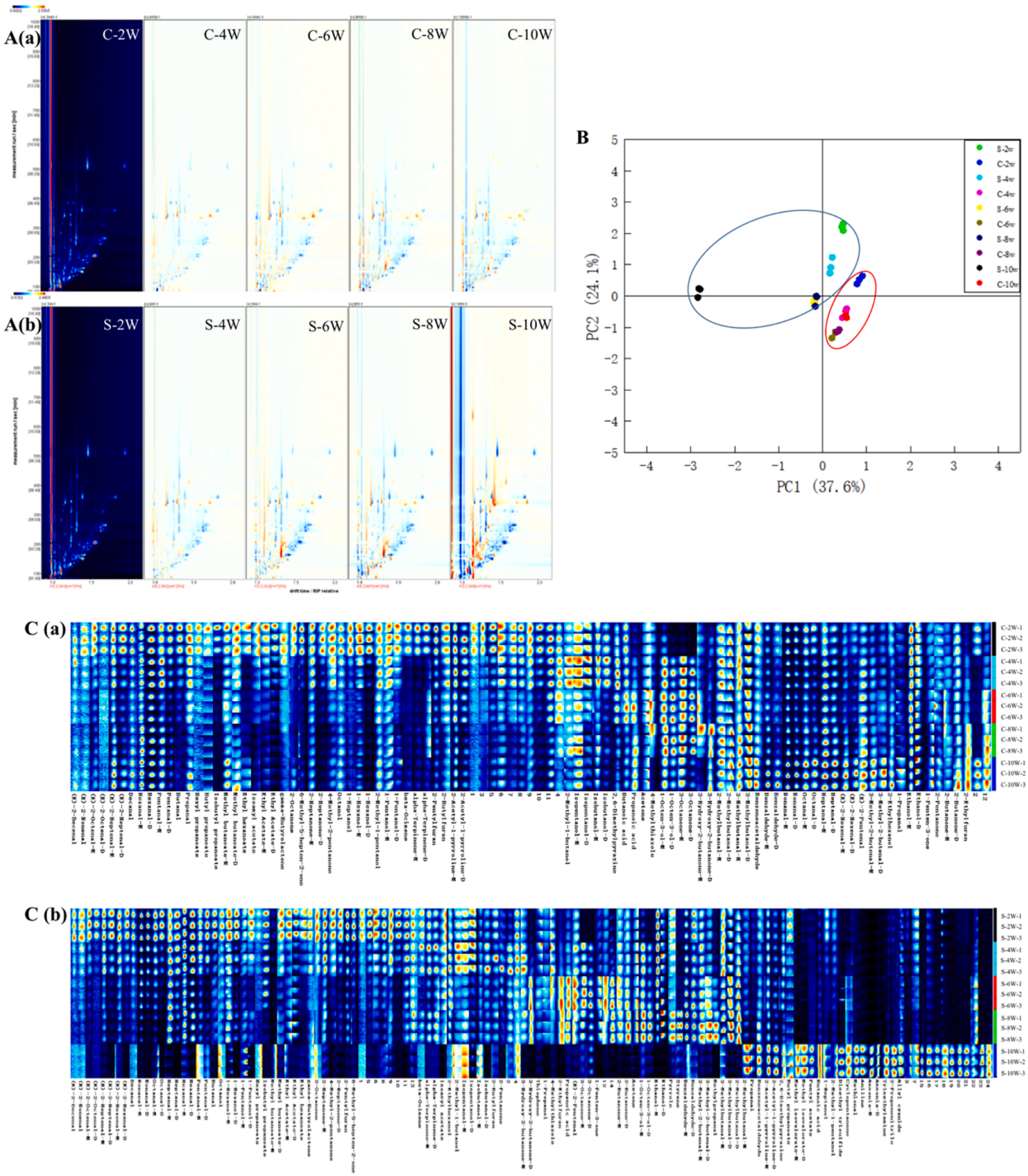

Fig. 2. (A) Topographic plot of (a) non-infested and (b) brown rice samples during different storage periods. (B) An overview of volatile fingerprints of non-infested and *S. oryaze*-infested brown rice samples by PCA score plot. (C) GC-IMS fingerprint of (a) non-infested and (b) *S. oryaze*-infested brown rice during different storage periods.

triplicate). Distinctive volatile compounds were discovered using a VIP value greater than 1. Fig. 3 shows the models' distinguishing capability.

In the GC–MS PLS-DA model, the first and second principal components (PC1 and PC2) accounted for 59.4 % and 19.2 %, respectively. All the *S. oryaze*-infested brown rice samples were located on the lower side of the score plot. In the GC-IMS PLS-DA model, PC1 and PC2 accounted for 55.3 % and 20.8 %, respectively. All the *S. oryaze*-infested brown rice samples were gathered on the score plot's left side. This indicates that both of these volatile detection methods can better determine whether brown rice is infested or not.

The statistically validated parameters of these PLS-DA models were utilized to compare their performance. It is widely accepted that $R^2(X)$,

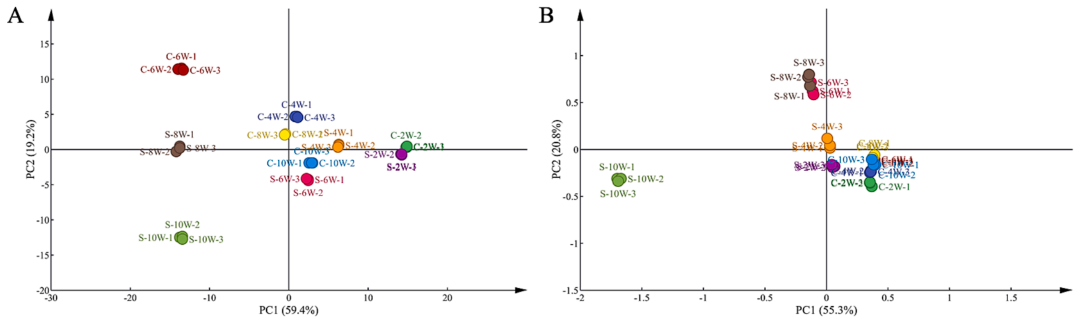

Fig. 3. PLS-DA score plot of (A) GC–MS data and (B) GC-IMS data. C presenting non-infested brown rice and S presenting *S. oryaze*-infested brown rice. The numbers 1、2 and 3 presenting three parallel test.

R^2(Y) and Q^2 are related to the stability, interpretation ability and predictability of the model, respectively (Hao et al., 2022). In the PLS-DA model, the higher R^2 (Y) and Q^2, the higher the performance of the model. The threshold value of Q^2 is greater than 0.5, indicating that the model has good predictability (Rivera-Pérez, Romero-González, & Garrido Frenich, 2022). In the GC–MS PLS-DA model, R^2(X) was 0.998, R^2(Y) was 0.993, and Q^2 was 0.984. These values were determined to be 0.989, 0.981 and 0.92 in the GC-IMS PLS-DA model. Both models were reliable and accurate. The larger the VIP value, the more conducive it is to discrimination. The volatile compounds with VIP > 1 could be used as characteristic compounds in these two models to discriminate between non-infested and *S. oryaze*-infested brown rice. 14 types of characteristic volatile compounds were screened in the GC–MS PLS-DA model (Table 2), while 31 types were screened in the GC-IMS PLS-DA model (Table 3).

Three co-selected markers to distinguish *S. oryaze*-infested brown rice were 1-octen-3-ol, 1-hexanol and 3-octanone. Brown rice is rich in vitamins, proteins, lipids and other nutrients, while lipid and lipid degrading enzymes are mostly located in bran and germ (Wei, Guo, Liu, Wang, Xu, & Chen, 2022). During the storage of brown rice, the lipid is first hydrolyzed and degraded by lipase, leading to the release of free fatty acids, which will be further decomposed into volatile compounds such as aldehydes and ketones (Hu, Lu, Guo, & Zhu, 2020). The infestation of *S. oryaze* destroys the lipid membrane of brown rice, accelerating the degradation of lipid in brown rice, and then produce some unpleasant odor during storage. With the extension of storage time, the content of 1-octen-3-ol increased from 5.76 ± 0.40 μg/kg to 168.67 ± 0.97 μg/kg, a significant increase of 29 times. It has been described as having a strong earthy smell and is thought to be the main source of the unpleasant smell of brown rice in previous studies (Griglione et al., 2015). Linoleic acid is first transformed to 10-hydroperoxide by enzyme catalysis and then degraded into 1-octen-3-ol by internal lyase (Lee et al., 2018). 1-Hexanol, with an herbal odor, has been discovered to be derived from the metabolism of polyunsaturated fatty acids. 1-Hexanol is a product of the lipoxygenase pathway, which is formed by linoleic acid (M. Xu, Jin, Lan, Rao, & Chen, 2019). With the extension of storage periods, other new products are produced by the lipoxygenase pathway at the same time, resulting in a decrease in 1-hexanol. 3-octanone has an herbal aroma, and its content increased significantly with the extension of storage periods. The autoxidation of fatty acids, particularly unsaturated fatty acids, produces ketones (Fan et al., 2021). In addition, allyl cyanide, trimethylamine, 2-propenenitrile, ammonia and methylpropanal were exclusively found by GC-IMS in *S. oryaze*-infested brown rice, and their VIP were greater than 1, indicating that they can be used as characteristic volatile compounds in *S. oryaze*-infested brown rice to monitor pest infestation. Among them, the contents of 2-propenenitrile, allyl cyanide, trimethylamine and ammonia showed an upward trend, with the highest content occurring in the tenth week. The final product of amino acid metabolism is ammonia. Ammonia can be detected in insect feces of diptera, neuropteran, dictyopetra and odonatan (Weihrauch & O'Donnell, 2021). Trimethylamine is a simple amine in volatile compounds, which is related to the decomposition process of corpses (Li et al., 2018). From the GC-IMS fingerprint, we found that the content of trimethylamine was the highest at the 10th week of *S. oryaze* infestation, which may be due to the death of some adult of *S. oryaze* at the 10th week, leading to the increase of trimethylamine content. Furthermore, some volatile compounds detected in *S. oryzae*-infested brown rice samples have been reported to be toxic. Both 2-propenenitrile and allyl cyanide contain toxic cyano groups (Panda et al., 2017).

Conclusions

To summarize, this study uses GC–MS and GC-IMS analytical methods to observe the changes of volatile compounds in non-infested and *S. oryaze*-infested brown rice. Based on the data gathered, the results revealed that the total content of alcohols, aldehydes, ketones,

Table 2
Characteristic volatile compounds (VIP greater than 1) in the GC–MS PLS-DA model.

No.	R.T.	Compound name	VIP value	Concentration(mean ± SD)				
				2 W	4 W	6 W	8 W	10 W
1	12.496	1-Octen-3-ol	2.19	5.76 ± 0.4e	44.03 ± 1.01c	38.33 ± 2.05d	168.67 ± 0.97a	90.51 ± 1.24b
2	12.733	3-Octanone	1.78	ND	10.19 ± 0.75d	19.32 ± 0.47c	59.64 ± 2.34b	91.31 ± 2.08a
3	12.865	2-Octanone	1.74	ND	ND	17.13 ± 0.19c	35.05 ± 0.37b	74.66 ± 1.33a
4	8.324	1-Hexanol	1.70	21.96 ± 1.36a	3.02 ± 0.26b	ND	ND	ND
5	14.302	2-Ethyl-1-hexanol	1.69	4.92 ± 0.2b	7.23 ± 0.08a	ND	ND	ND
6	39.803	Hexadecanoic acid, ethyl ester	1.58	11.27 ± 0.66b	10.36 ± 1.44b	16.14 ± 0.54a	14.58 ± 1.23a	9.54 ± 0.95b
7	13.644	1,3-Dichloro-benzene	1.50	9.25 ± 0.32e	11.72 ± 0.33d	17.65 ± 0.6b	15.55 ± 0.69c	21.32 ± 0.92a
8	31.081	Hexadecane	1.26	5.8 ± 0.01a	ND	2.04 ± 0.05c	ND	2.68 ± 0.18b
9	33.6	2,6,10-Trimethyl-dodecane	1.20	2.44 ± 0.04a	1.33 ± 0.04c	1.19 ± 0.08d	1.99 ± 0.06b	ND
10	16.763	Undecane	1.10	ND	2.45 ± 0.13d	5.78 ± 0.3b	3.07 ± 0.09c	6.98 ± 0.43a
11	43.09	Linoleic acid ethyl ester	1.09	1.46 ± 0c	1.46 ± 0.1c	3.53 ± 0.15b	4.09 ± 0.16a	ND
12	22.97	Nonanoic acid, ethyl ester	1.08	5.04 ± 0.25a	2.84 ± 0.18b	1.74 ± 0.49 cd	2.1 ± 0.18c	1.27 ± 0.08d
13	23.435	2,6,10,15-Tetramethyl- heptadecane	1.06	0.42 ± 0.02c	0.55 ± 0.03b	0.6 ± 0.02b	1.59 ± 0.13a	0.36 ± 0.02c
14	38.24	Hexadecanal	1.03	ND	ND	1.03 ± 0.1a	0.51 ± 0.06c	0.84 ± 0.06b

Table 3
Characteristic volatile compounds (VIP greater than 1) in the GC-IMS PLS-DA model.

No.	Compound name	VIP value	RI	RT	DT	CAS	Formula
1	Acetone	2.45	472.5	104.559	1.11177	67-64-1	C_3H_6O
2	Nonanal	2.08	1110.5	508.593	1.47661	124-19-6	$C_9H_{18}O$
3	3-Octanone	2.07	992.1	341.255	1.72949	106-68-3	$C_8H_{16}O$
4	3-Hydroxy-2-butanone	2.00	716.1	172.811	1.33202	513-86-0	$C_4H_8O_2$
5	Ethanol	1.96	458.2	100.783	1.1274	64-17-5	C_2H_6O
6	2-Pentanone	1.55	688.0	161.466	1.36907	107-87-9	$C_5H_{10}O$
7	Hexanal	1.53	791.6	204.411	1.25690	66-25-1	$C_6H_{12}O$
8	alpha-Terpinene	1.52	1025.4	386.42	1.29246	99-86-5	$C_{10}H_{16}$
9	1-Octen-3-ol	1.47	984.2	334.505	1.60377	3191-86-4	$C_8H_{16}O$
10	2-Ethylfuran	1.43	686.4	161.045	1.32850	3208-16-0	C_6H_8O
11	4-Methylthiazole	1.41	812.3	215.136	1.05775	693-95-8	C_4H_5NS
12	2-Methylbutanal	1.37	657.3	153.375	1.40177	96-17-3	$C_5H_{10}O$
13	6-Methyl-5-hepten-2-one	1.36	992.4	341.438	1.18084	110-93-0	$C_8H_{14}O$
14	Octanal	1.30	1006.2	358.902	1.40408	124-13-0	$C_8H_{16}O$
15	3-Methylbutanal	1.28	641.0	149.060	1.40887	590-86-3	$C_5H_{10}O$
16	Propanoic acid	1.28	704.1	167.939	1.10466	79-09-4	$C_3H_6O_2$
17	2-Butanone	1.27	579.7	132.877	1.25108	78-93-3	C_4H_8O
18	Pentanal	1.23	693.5	163.623	1.42735	110-62-3	$C_5H_{10}O$
19	Methyl propanal	1.23	543.7	123.369	1.28891	78-84-2	C_4H_8O
20	2-Propenenitrile	1.23	481.1	106.843	1.08974	107-13-1	C_3H_3N
21	1-Hexanol	1.20	870.0	245.130	1.63818	111-27-3	$C_6H_{14}O$
22	2-Acetyl-1-pyrroline	1.16	922.9	282.688	1.13090	85213-22-5	C_6H_9NO
23	1-Penten-3-one	1.15	666.5	155.802	1.31789	1629-58-9	C_5H_8O
24	3-Methyl-2-butenal	1.14	779.6	198.685	1.36196	107-86-8	C_5H_8O
25	Propanal	1.08	545.0	123.708	1.16863	123-38-6	C_3H_6O
26	Ammonia	1.07	406.0	86.993	0.85355	7664-41-7	H_3N
27	Benzaldehyde	1.05	960.5	314.45	1.15418	122-78-1	C_8H_8O
28	Isobutanol	1.05	620.6	143.666	1.36054	78-83-1	$C_4H_{10}O$
29	Allyl cyanide	1.04	642.6	149.477	1.26214	109-75-1	C_4H_5N
30	Trimethylamine	1.02	566.9	129.478	1.15549	75-50-3	C_3H_9N
31	(E)-2-Hexenal	1.01	847.2	233.27	1.18326	6728-26-3	$C_6H_{10}O$

esters, hydrocarbons, and other types of compounds varied significantly between *S. oryaze*-infested and non-infested brown rice. PLS-DA was used to perform discrimination analysis on *S. oryaze*-infested brown rice during various storage periods using GC–MS and GC-IMS volatile data. Both of these two analysis methods have obtained comprehensive data and achieved substantial distinguishing outcomes. In addition, *S. oryaze*-infested rice could be identified using three co-selected potential volatile markers: 1-octen-3-ol, 1-hexanol and 3-octanone. All of these markers are connected to fatty acid oxygenation. Moreover, allyl cyanide, trimethylamine, 2-propenenitrile, ammonia and methyl propanal were only detected by GC-IMS in *S. oryaze*-infested brown rice and their VIP was greater than 1, which can also be used as characteristic compounds to monitor the pest infestation in brown rice. The current study's findings provide a foundation for further research on the rice infestation mechanism. In the future, we can further explore the relevant genes that regulate the changes of key volatile compounds through omics technology, and further explore the infestation mechanism of brown rice. In addition, we can also design a sensor to monitor the security of storage environment through the changes of volatile compounds through computer technology. Furthermore, GC-IMS technology has the advantages of speed, which may improve infestation process monitoring during brown rice storage so as to ensure the safety of stored grain.

CRediT authorship contribution statement

Xuemei Tian: Conceptualization, Software, Data curation, Investigation, Validation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Fenghua Wu:** Resources, Formal analysis, Writing – review & editing. **Guoxin Zhou:** Formal analysis, Funding acquisition, Writing – review & editing. **Jian Guo:** Resources, Investigation, Formal analysis. **Xingquan Liu:** Funding acquisition, Supervision, Project administration. **Tao Zhang:** Conceptualization, Methodology, Funding acquisition, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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