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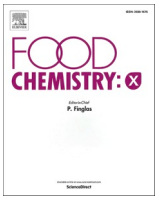

Evaluating modified atmosphere with variable CO_2-O_2 concentrations for *Tribolium castaneum* management and quality preservation in Rice storage

Xue Dong, Ming Yang, Peian Tang *

College of Food Science and Engineering, Nanjing University of Finance and Economics/ Collaborative Innovation Center for Modern Grain Circulation and Safety, NO.3 Wenyuan Road, Xixia District, Nanjing 210023, Jiangsu, China

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ABSTRACT

This study explored the efficacy of CO_2-MA with variable CO_2-O_2 concentrations (TA: 2 % O_2 + 35 % CO_2, TB: 14 % O_2 + 35 % CO_2, TC: 21 % O_2 + 35 % CO_2, TD: 21 % O_2 + 60 % CO_2) in controlling *Tribolium castaneum* and preserving rice quality. TD exhibited the highest efficacy, achieving rapid and complete pest mortality within 48 h, particularly in the resilient pupal stage, and nearly 100 % mortality under grain-embedded conditions within 10 days. Quality assessments revealed that TD effectively mitigated lipid oxidation by reducing aldehydes and alcohols, major contributors to rice aging and off-flavors while maintaining acceptable enzymatic activities and VOC profiles. Principal component analysis confirmed that TD minimized oxidative stress and preserved desirable sensory attributes more effectively than hypoxic treatments. These findings presented an advancement in sustainable grain storage, with tailored CO_2-O_2 ratios to achieve desirable outcomes as a sustainable strategy for long-term rice storage.

1. Introduction

Postharvest loss is a crucial challenge for global food security, with up to one-third of the world's grain lost annually during storage, largely caused by insect infestation (Mesterházy, Oláh, & Popp, 2020). The red flour beetle, *Tribolium castaneum* (Herbst), a destructive pest, damages grain by creating irregular holes in the kernels, leading to severe economic losses by reducing both the quantity and quality of stored grains. Current pest management strategies heavily rely due to insect infestation on chemical fumigants, which are cost-effective and efficient for preserving commodities. However, many fumigants have been phased out owing to unfavourable properties. For instance, methyl bromide, once widely used, was banned since it caused the depletion of the ozone layer (Nayak, Daglish, Phillips, & Ebert, 2020). Phosphine, though commonly used, has led to serious risks of pest resistance (Chen, Schlipalius, Opit, Subramanyam, & Phillips, 2015). Sulfuryl fluoride and hydrogen cyanide are costly and face limited regional acceptance (Aulicky, Stejskal, Dlouhy, & Liskova, 2015). Thus, alternative sustainable solutions are needed to control grain storage pests and preserve grain quality.

Modified atmosphere (MA) storage offers a promising and residue-free approach, creating environments with altered O_2 and CO_2 levels to suppress pest activity, microbial growth, and enzymatic degradation by disrupting their respiratory and metabolic processes (Bhattarai et al., 2023; Carvalho et al., 2019; Opio & Photchanachai, 2018). However, prolonged exposure to low-oxygen MA has increased adaptability in some storage pests, highlighting the potential of CO_2-enriched atmospheres as an effective complementary strategy. High CO_2 concentrations have been shown to achieve rapid and effective control across various pest species and developmental stages (Kumar, Vijay, Subbarao, & Chandra, 2022). Additionally, studies demonstrated the synergistic effects of depleted O_2 combined with elevated CO_2 in effectively controlling stored grain pests (Navarro et al., 2020). Despite these promising findings, studies on the effect of varying O_2 and CO_2 concentrations remain limited.

CO_2-MA are not only valuable for pest elimination but also for maintaining food quality. CO_2-enriched environments could slow down lipid oxidation and suppress enzymatic activity, effectively preserving

Abbreviations: AAT, alcohol acyltransferase; AMS, α-amylase; ANOVA, one-way analysis of variance; CAT, catalase; CO_2-MA, CO_2-modified atmosphere; EI, electron impact; FAVs, fatty acid values; HSD, Honest Significant Difference; KOH, potassium hydroxide; LT_{50}, median lethal time; LT_{99}, lethal time of 99 %; MDA, Malondialdehyde; NIST, National Institute of Standards and Technology; PCA, Principal component analysis; PLS-DA, Partial least squares discriminant analysis; PUFAs, polyunsaturated fatty acids; RIs, retention indices; TBA, thiobarbituric acid; VIP, variable importance in projection; VOC, volatile organic compounds.

* Corresponding author.
E-mail address: tangpeian@163.com (P. Tang).

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the sensory and nutritional attributes of stored grains (Qu et al., 2022). However, different ratios of CO_2 and O_2 could cause contrasting effects on food quality. For example, while a controlled O_2/CO_2 atmosphere could extend the shelf-life of postharvest broccoli and prevent off-flavors, unsuitable ratios have been shown to lead to undesirable flavor changes (Li, Zhang, Guo, & Nian, 2014).

Existing studies often focus on chemical fumigants or single atmospheric compositions. However, the effects of varying O_2-CO_2 combinations on pest control and key quality indicators in grain are elusive. To address these challenges, we designed a study to evaluate the effects of different modified atmosphere treatments with varying CO_2 and O_2 levels. We hypothesize that increased CO_2 concentration combined with optimized O_2 levels will enhance pest control efficacy while minimizing rice quality deterioration during storage. Fatty acid values (FAV) and malondialdehyde (MDA) were selected as key indicators of rice rancidity and storage stability due to their roles as markers of lipid hydrolysis and peroxidation (Hu et al., 2023). **Catalase (**CAT) is an antioxidant enzyme reflects the grain's ability to mitigate oxidative stress by neutralizing reactive oxygen species (ROS). **α-amylase (**AMS) is an *endo*-enzyme that hydrolyzes straight-chain starch into glucose and maltose and hydrolyzes branched-chain starch into glucose, maltose, and dextrin, influencing grain texture and eating quality (Huang et al., 2020). Volatile organic compounds (VOCs) were chosen to evaluate changes in aroma profiles and metabolism, critical for consumer acceptance and quality evaluation. These indicators directly address the study's focus on storage-related spoilage and quality changes. Thus, this study aims to comprehensively assess the efficacy of four CO_2-MA treatments on managing *Tribolium castaneum* across developmental stages and their impact on rice quality, including lipid degradation, enzymatic activity, and VOC profiles.

2. Material and methods

2.1. Insects Rearing

The Red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae), used for bioassays, was collected from the grain storage ecological region of Yunnan, China and maintained for several generations in the Laboratory of Food Storage and Transportation at Nanjing University of Finance and Economics, China. The insects were reared on a wheat flour/yeast mixture (19:1 ratio) in 1 L jars sealed with mesh lids at the optimal condition of 29 ± 1 °C, 65 ± 5 % RH, and a constant 0:24 (L:D) h photoperiod. Approximately 200 adults (three days old) laid eggs in wheat flour within 3 days, after which they were transferred to another jar. Early instar larvae hatched within 7 to 10 days. While late-instar larvae pupated 21 to 24 days. Pupae were regularly separated, and adults were sieved from the flour 2 to 3 days after emergence. For laboratory bioassay, third instar larvae (early instar), eighth-instar larvae (late instar), three-day-old pupae and adults were used to assess mortality under different conditions.

2.2. Raw rice samples

Rice samples of the Fengliangyou (indica rice) variety were harvested in 2022 from Nanjing, Jiangsu province, China. After harvesting, the samples were stored at −5 °C for one week to eliminate the remaining insect eggs. Then the pest-free samples were dried to a moisture content of 12.7 %, stored at 4 °C, and equilibrated to room temperature before quality measurements.

2.3. CO_2-MA treatments

A total of 30 insects from each group were placed in petri dishes (90 mm × 15 mm) for mortality assessment (without grain but with added feed to prevent starvation). These petri dishes were placed in packaging boxes (20 cm × 14 cm × 6.5 cm) for treatments. The semi-automatic gas-modified packaging machine (Model GQ-1D400, Shanghai Gangqing Machinery Manufacturing Co., Ltd., Shanghai, China) was utilized for MAs packaging. The samples were subjected to MA treatments: TA (2 % O_2 + 35 % CO_2), TB (14 %O_2 + 35 %CO_2), TC (21 %O_2 + 35 % CO_2), TD (21 % O_2 + 60 % CO_2) and CK (control, 100 % air), at various exposure times (3, 6, 12, 24, 36, 48, 72, 96, 120 and 168 h), with three replicates. A CO_2 concentration of 35 % was selected based on its recommended efficacy for pest control according to the standard LS/T1213–2022 (National Food and Strategic Reserves Administration of China, 2022). Treatments TA, TB, and TC were designed to evaluate the effects of introducing different oxygen levels under the same CO_2 concentration. TD, with 60 % CO_2 and normal oxygen (21 %), was included to assess the impact of high CO_2 in a normoxic environment. This experimental design enables a systematic comparison of oxygen and CO_2 effects on pest control and grain quality.

In addition, 30 insects with 500 g paddy rice were placed in the same packaging and treated under the above conditions. The mortality of insects in paddy rice was assessed at 10, 15 and 20 days. Each group was repeated in triplicate. Following 30 days of MA storage, quality parameters of paddy rice were determined. Gas concentration within each package was monitored by a portable gas analyzer (OXYBABY M+ O_2/CO_2, WITT-GASETECHNIK GmbH & Co KG, Germany). All treatments were conducted at 29 ± 1 °C and 65 % RH.

2.4. Detection of mortality of Tribolium castaneum under application of CO_2-MA treatments

All developmental stages of *Tribolium castaneum* of the treated and untreated groups were retrieved at the end of the exposure from their respective treatment conditions and the petri dishes with insects were placed in the climate chamber at 29 ± 1 °C and 65 % RH for further observation. The mortality rate of insects was assessed at 72 h after CO_2-MA treatments. The insects were considered as dead without movement when gently stimulated with a brush. The mortality was calculated based on a comparison of the number of dead insects between treated and control samples.

2.5. Determination of quality parameters of paddy rice

2.5.1. Fatty acid values (FAV) and malondialdehyde (MDA) content

FAV in samples was determined following the method described by (Wang, Hu, Mugambi Mariga, Cao, & Yang, 2018) with slight modifications. Briefly, 50 mL of anhydrous ethanol was added to the 10.00 *g* ground rice sample and centrifuged at 3000 rpm for 10 min to extract free fatty acids, followed by filtration. 10 mL filtrate was diluted with 50 mL of distilled water, and 3 to 5 drops of phenolphthalein-ethanol solution was added as an indicator for the titration endpoint. The solution was titrated with a standard potassium hydroxide (KOH) solution. FAV was then calculated based on the volume of KOH required to neutralize the free fatty acids in 100 g of rice sample. The results were expressed as mg of free fatty acids per 100 g of rice (mg/100 g).

MDA content was measured by a colorimetric assay based on its reaction with thiobarbituric acid (TBA), forming a brownish-red compound with peak absorbance at 532 nm. Firstly, rice samples were cleaned of impurities and hulls, and then finely ground using a cryogenic ball mill to obtain 0.1 g of rice powder (accurate to 0.001 g). This powder was mixed with an extraction solution according to the protocol provided in the MDA assay kit (Solarbio Science & Technology, Beijing, China). The mixture was then centrifuged at 8500 rpm for 10 min, the supernatant was collected and then incubated in a water bath at 100 °C for 60 min. After cooling with running water, 200 μL of the supernatant was transferred into a 96-well plate, and the absorbance at 532 nm and 600 nm was measured using a SpectraMaxM2e microplate reader (Molecular Devices, San Jose, CA).

2.5.2. Catalase (CAT) and α-amylase (AMS) activity

The CAT activity assay was determined using the CAT assay Kit (Solarbio Science & Technology, Beijing, China). Finely ground 0.1 g (accurate to 0.001 g) of rice flour was mixed with 1 mL of extraction solution in an ice bath. The mixture was centrifuged at 8500 rpm for 10 min at 4 °C, and the supernatant was collected. The absorbance was measured at 240 nm by a SpectraMaxM2e microplate reader, with each sample analyzed in triplicates. The amount of enzyme that decomposes 1 nmol of H_2O_2 per minute is considered one unit (U) of enzyme activity. CAT activity was then expressed as U per gram of protein (U/g protein).

AMS activity was assessed by an AMS assay kit (Jiancheng Bioengineering Institute, Jiangsu, China). 0.001 g of rice was combined with phosphate buffer (0.1 M, pH 7.0–7.4) at a 1:4 (g/mL) ratio. The mixture was ground at 50 Hz for 30 s per session, repeated 2 to 3 times, and then centrifuged at 3500 rpm for 10 min. The supernatant was collected and absorbance was measured at 660 nm using a UV spectrophotometer (Shanghai Youke Instrumentation Co., Ltd., Shanghai, China).

2.6. GC–MS conditions and VOC analysis

For VOC analysis, 4.0 g of milled rice kernels were combined with 20 μL of an internal standard (3-octanol, 50 μg/L) in a 20 mL SPME vial sealed with a screw cap. A three-phase SPME fiber (50/30 μm) with a 2 cm combination coating of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Sigma-Aldrich Trading Co.Ltd. Shanghai, China) was utilized inserting for VOC collection. Analysis was conducted with an Agilent GC–MS (7890 A-5975C, Agilent Technologies Co., Palo Alto, CA, USA) system equipped with an Agilent HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm film thickness, 5 % phenyl, and 95 % dimethylpolysiloxane stationary phase). The method is based on Hu et al. (2023) with minor modifications. Ultra-high purity helium (Air Liquide, China) was the carrier gas at a constant flow rate of 1.0 mL/min. Samples were injected in splitless mode at 250 °C desorption temperature for 5 min. The temperature program started at 30 °C for 4 min, ramped at 5 °C/min to 250 °C, and then held at 250 °C for 3 min. The GC–MS system operated in electron impact (EI) ionization mode at 70 eV, scanning a mass range of 50–400 amu. Ion source, MS quadrupole, and transfer line temperatures were set at 230 °C, 250 °C, and 280 °C, respectively.

The identification of volatiles was performed firstly by comparing the mass spectra with the National Institute of Standards and Technology (NIST) mass spectral Library (matching score > 70) and by experimentally obtained Kovats retention indices (RIs) from C6–C40 alkane standards with those in the NIST MS library. RIs were calculated as follows:

$RI = 100n + 100 \times \frac{t-t_n}{t_{n+1}+t_n}$ where t (min) is the corrected retention time of the detected compounds, t_n and t_{n+1} (min) are retention times of n-alkanes with n and n+1 carbon atoms, respectively. The relationship between them is $t_n < t < t_{n+1}$. Experiments were performed in triplicate.

2.7. Statistical analysis

The experiment was conducted using a completely randomized design. Time-mortality curves, median lethal time (LT_{50}) and lethal time of 99 % (LT_{99}) were generated by Probit analysis. One-way analysis of variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) were performed to identify statistical differences among different treatments ($p < 0.05$) using SPSS (Version 27.0 IBM Corporation, USA). All data were expressed as mean ± standard deviation based on three independent replicates. Principal component analysis (PCA) and Partial least squares discriminant analysis (PLS-DA) with variable importance in projection (VIP) were performed using MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/, accessed on 10 September 2024). Figures were generated using Origin software (Version 2021b, Origin Lab, Northampton, MA, USA).

3. Results

3.1. Effect of CO_2-MA treatments on the mortality rates across developmental stages of Tribolium castaneum

The mortality rates of *Tribolium castaneum* varied significantly across early instar larvae, late instar larvae, pupae, and adults under different CO_2-MA treatments and exposure times (Fig. 1). Probit analysis was conducted to calculate the LT_{50} and LT_{99} values for each development stage of *Tribolium castaneum*, providing a more detailed assessment of treatment efficacy (Table S1). For early instar larvae, TA (2 % O_2 + 35 % CO_2) and TD (21 % O_2 + 60 % CO_2) reached complete mortality within 48 h, while TB (14 % O_2 + 35 % CO_2) and TC (21 % O_2 + 35 % CO_2) resulted in mortality rates of 96.67 ± 3.33 % and 98.89 ± 1.92 %, respectively, even after 168 h of exposure (Fig. 1a). LT_{50} values for TA and TD (7.431 h and 7.389 h, respectively) were markedly shorter than those of TB (31.251 h) and TC (22.123 h). TB had the longest LT_{99} values of 207.821 h among all treatments (Table S1). Late instar larvae had longer exposure times to achieve complete mortality. TA and TD reached 100 % mortality within 72 h, whereas TB required 168 h, and TC achieved 98.89 ± 1.92 % mortality after 168 h (Fig. 1b). LT_{50} values were 5.591 h for TA, 22.777 h for TB, and 8.166 h for TD, respectively, with TC exhibiting the longest LT_{50} values of 36.020 (Table S1). Pupae showed the highest tolerance to all treatments. TA achieved complete mortality after 96 h, and TD required 48 h, while neither TB nor TC achieved complete mortality within 168 h, with maximum mortality rates of 97.78 ± 3.85 % (Fig. 1c). LT_{50} values for TA, TB, TC, and TD were 23.921 h, 36.229 h, 38.029 h, and 10.288 h, respectively (Table S1). Adults also demonstrated considerable resistance, with TA and TD requiring 72 h to achieve complete mortality, while TC required 168 h. TB-treated adults showed the highest tolerance, with a mortality rate of 97.78 ± 1.92 % after 168 h (Fig. 1d). LT_{50} was the shortest for TD (7.770 h) and TA (10.426 h), followed by TC (45.607 h) and TB (59.467 h) (Table S1). Overall, TD emerged as the most effective treatment across all developmental stages, achieving rapid and complete mortality. TA (2 % O_2 + 35 % CO_2) was slightly less effective but still demonstrated strong insecticidal activity. TB and TC were the least effective, particularly for the more resistant pupal and adult stages.

3.2. Efficacy of CO_2-MA treatments against Tribolium castaneum in paddy rice

The insecticidal efficacy of CO_2-MA treatments within paddy rice storage was conducted to assess treatment performance under more practical, grain-embedded scenarios. The effectiveness of CO_2-MA treatments in controlling adults of *Tribolium castaneum* in paddy rice was evaluated over 10, 15, and 20 days (Fig. 2). At all-time points, TA, TB, TC, and TD significantly increased adult mortality compared to the control. After 10 days of MAs, TA and TD achieved mortality rates of 100 % and 98.89 ± 1.92 %, respectively, while TB and TC reached mortality rates of 83.33 ± 3.33 % and 78.89 ± 5.09 %, respectively. When the treatment period was extended to 15 days, the mortality rate in TD reached 100 %, while TB and TC remained slightly slower. By day 20, all treatments achieved maximum mortality, demonstrating that TD and TA were the most effective treatments for *Tribolium castaneum* management in rice storage.

3.3. Effect of CO_2-MA storage on quality parameters of paddy rice

CO_2-MA treatment completely killed *Tribolium castaneum* adults in paddy rice within 20 days across all treatments. To assess the potential impact of CO_2-MA treatment on rice quality during extended storage, the exposure time was extended to 30 days.

3.3.1. FAV and MDA content of paddy rice

Lipid degradation was assessed through FAV and MDA content. TA

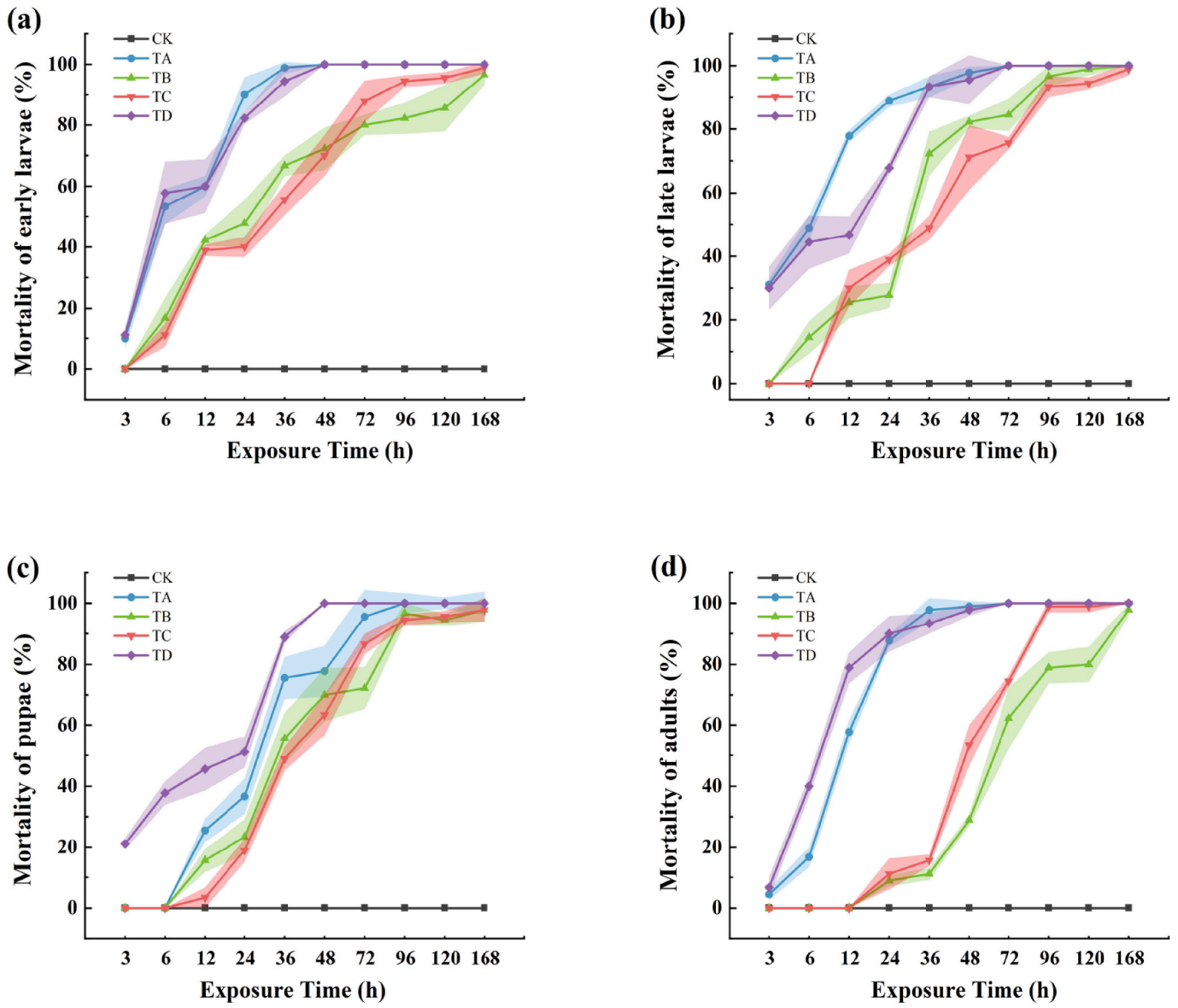

Fig. 1. Mortality rates of *Tribolium castaneum* at different developmental stages exposed to CO_2-MA treatments for various exposure times. (a) early instar larvae, (b) late instar larvae, (c) pupae, and (d) adults. CK: 100 % air; TA: 2 % O_2 + 35 % CO_2; TB:14 % O_2 + 35 % CO_2; TC: 21 % O_2 + 35 % CO_2; and TD: 21 % O_2 + 60 % CO_2. Shaded regions represent standard deviations of three replicates.

and TD treatments significantly increased FAV compared to CK (18.81 ± 1.47 mg/100 g), reaching 27.54 ± 0.70 mg/100 g and 25.68 ± 0.54 mg/100 g, respectively, indicating higher lipid degradation. In contrast, TB and TC maintained FAV levels closer to CK at 20.2 ± 1.02 mg/100 g and 20.73 ± 0.48 mg/100 g, respectively, suggesting that TB and TC were more effective at retarding lipid degradation during the 30-day treatment compared to TA and TD (Fig. 3a). MDA content, a marker of lipid peroxidation, was significantly elevated in TA-treated samples (194.47 ± 2.43 nmol/g), whereas TB, TC, and TD treatments exhibited lower MDA levels of 173.59 ± 2.28 nmol/g, 164.98 ± 3.39 nmol/g, and 167.26 ± 1.29 nmol/g, respectively, with TC showing the most effective inhibition of lipid peroxidation (Fig. 3b).

3.3.2. *The CAT activity and AMS activity of paddy rice*

CAT activity, an indicator of the oxidative stress response, significantly decreased across four CO_2-MA conditions compared to CK (69.45 ± 2.52 U/g) ($p < 0.05$). TA and TB treatments, exposed to low-oxygen conditions, displayed lower declines with values of 50.84 ± 2.23 U/g and 58.55 ± 3.30 U/g, respectively. Conversely, TC and TD showed more pronounced reductions, with values of 27.97 ± 3.24 U/g and 13.36 ± 1.00 U/g, respectively (Fig. 3c). TD exhibited the lowest CAT activity ($p < 0.05$).

AMS activity, associated with starch preservation, also decreased significantly under CO_2-MA treatments. The most significant reduction was observed in TD-treated rice (0.15 ± 0.04 U/g, a 61.54 % reduction compared to CK) (Fig. 3d). AMS activity levels in TA, TB, and TC were 0.19 ± 0.02 U/g,0.18 ± 0.06 U/g, and 0.29 ± 0.05 U/g, respectively, showing no significant differences among these treatments ($p < 0.05$).

3.4. *Effect of CO_2-MA treatments on total VOCs profiles*

Fifty-nine volatile compounds in rice kernels were identified and qualified by HS-SPME-GC–MS, including 26 heterocyclics, 16 esters, 5 alcohols, 3 ketones, 2 aldehydes, 2 heterocycles, and 1 other (Table 1). The diversity and quantity of VOCs varied among treatments. A total of 56 VOCs were identified in TA and 55 in TB treatment, while 55 and 54 volatiles were identified in TC and TD treatments, respectively (Fig. 4a). Additionally, Hypoxia treatments (TA and TB) resulted in an overall

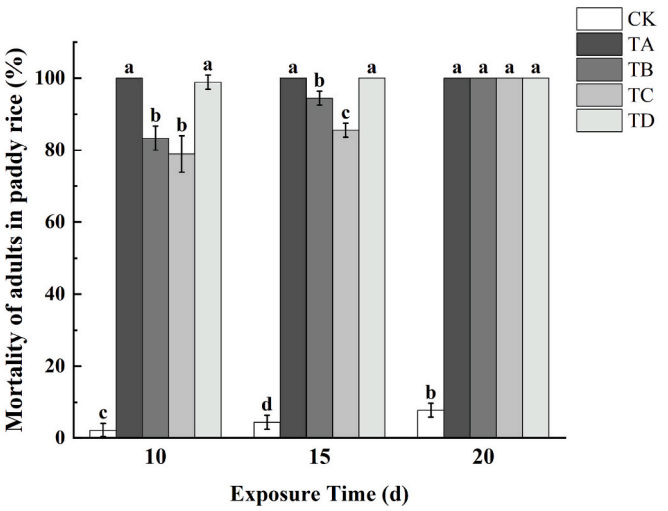

Fig. 2. Mortality rates of adult stages of *Tribolium castaneum* by CO_2-MA treatments in different storage phases (Day10, Day15 and Day20) with three replicates. CK: 100 % air; TA: 2 % O_2 + 35 % CO_2; TB:14 % O_2 + 35 % CO_2; TC: 21 % O_2 + 35 % CO_2; and TD: 21 % O_2 + 60 % CO_2. Means followed by different letters in each column are significantly different accroding to Tukey's HSD test ($p < 0.05$).

increase in VOC levels compared to CK, while normoxia and hypercapnia treatments (TC and TD) led to a decrease (Fig. 4b). TA exhibited the highest VOC concentration (1867.67 ng/kg), dominated by esters (1149.91 ng/kg) and heterocyclics (219.6 ng/kg). TB (1287.94 ng/kg) also showed high ester content (849.14 ng/kg) but lower alcohol content (47.11 ng/kg) than TC and TD. TC (1123.55 ng/kg total VOCs) had the lowest levels of heterocyclics (98.00 ng/kg), aldehydes (40.37 ng/kg) and ketones (27.23 ng/kg), although its ester content (761.74 ng/kg) was higher than that in TD-treated rice (605.97 ng/kg). TD treatment, with the lowest levels of VOCs (1060.05 ng/kg), also minimized lipid oxidation byproducts, including alcohols (63.67 ng/kg) and aldehydes (45.53 ng/kg), indicating superior quality preservation.

3.5. Comparison of VOCs in paddy rice at different CO_2-MA treatments Using Multivariate Analysis

Metabolites with over 50 % missing values were excluded, and the data were transformed using square root scaling and mean centering. PCA was performed to evaluate variations in rice treated with various CO_2-MA conditions. The first two components (PCs) explained 76.8 % of the total variance, with PC1 accounting for 50.3 % and PC2 for 26.5 % (Fig. 5). Control samples clustered separately from the treated groups, indicating a unique volatile profile compared to MA treatments. TA and TB formed separate clusters, with TA displaying the largest deviation from CK along PC1, suggesting significant alterations under hypoxia. Additionally, TC and TD formed distinct clusters, with TD located at the most distant position from control and hypoxia-treated samples.

PLS-DA was also utilized to differentiate rice samples at different CO_2-MA conditions (Fig. S1). The VIP scores derived from the PLS-DA analysis reveal the key volatile compounds of rice under various CO_2-MA treatments. For the hypoxia treatments (TA and TB), 9 VOCs with VIP scores above 1.4 were selected, including linoleic acid methyl ester, nonanoic acid methyl ester, palmitic acid methyl ester, 2-heptanone, methyl stearate, 5-methyl-undecane, 2,6-dimethyl-undecane, 4-methyl-3-heptanol and nonadecane (Fig. 6a). Linoleic acid methyl ester and nonanoic acid methyl ester displayed the highest VIP scores, indicating their significant role in distinguishing VOCs in the low-oxygen treatments. Under normoxia treatments (TC and TD), 8 VOCs

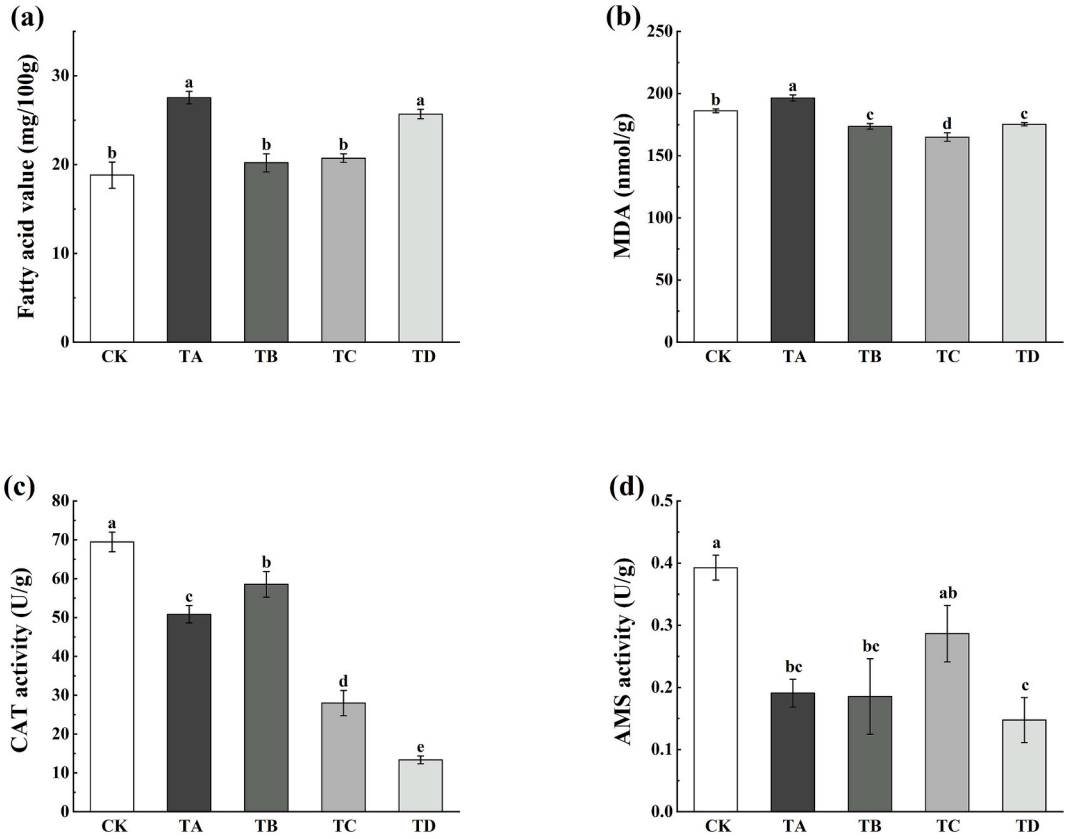

Fig. 3. Effect of 30-day CO_2-MA storage on quality parameters of paddy rice: (a) FAV; (b) MDA; (c) CAT activity; (d) AMS activity. Data represent means of three replicates with standard error bars. Bars with different letters (a–d) indicate significant difference according to Tukey's HSD test ($p < 0.05$).

Table 1
Volatile organic compounds detected in indica rice (Fengliangyou) samples by SPME-GC–MS (ng/kg).

Chemical groups	Compounds[a]	RT [b] (min)	RI [c] (lib)	RI [d] (cal)	CO_2-MA treatment				
					CK	TA	TB	TC	TD
Hydrocarbons	1,3-dimethylbenzene	9.170	862	866	1.55 ± 0.01	2.04 ± 0.28	0.69 ± 0.17	n.d.	5.58 ± 3.27
	undecane	17.021	1100	1099	13.71 ± 1.41	10.81 ± 8.01	12.58 ± 1.19	13.18 ± 1.47	16.35 ± 3.05
	5-methyl-undecane	17.090	1156	1095	n.d.	1.16 ± 2.02	n.d.	6.22 ± 1.21	n.d.
	3-methyl-undecane	18.716	1159	1152	9.91 ± 1.97	2.24 ± 0.20	2.86 ± 0.96	2.2n.d.0.32	4.16 ± 2.08
	Naphthalene	19.154	1169	1177	17.91 ± 1.98	24.83 ± 1.75	17.67 ± 4.19	17.15 ± 2.35	16.28 ± 1.70
	Dodecane	19.531	1171	1168	1.98 ± 0.26	5.03 ± 0.28	4.78 ± 0.76	5.90 ± 0.53	5.00 ± 0.69
	2,6-dimethyl-undecane	20.026	1200	1198	19.76 ± 1.15	28.87 ± 0.61	23.67 ± 2.85	25.08 ± 1.60	29.56 ± 2.63
	1-iodo-tridecane	20.424	1214	1206	0.21 ± 0.01	1.45 ± 0.18	3.32 ± 0.50	4.17 ± 0.15	3.42 ± 0.84
	Tridecane	22.280	1294	1276	2.11 ± 0.11	8.54 ± 0.27	6.00 ± 1.79	n.d.	5.95 ± 1.28
	5-butyl-nonane	22.822	1300	1300	13.23 ± 2.79	21.21 ± 2.07	18.61 ± 3.84	22.05 ± 0.96	24.05 ± 3.49
	2-Bromo-dodecane	23.780	1505	1332	2.29 ± 0.28	5.34 ± 0.39	4.49 ± 1.05	6.39 ± 0.60	3.84 ± 1.77
	3-methyl-tridecane	24.031	1372	1331	1.7n.d.0.51	3.42 ± 0.13	2.31 ± 0.70	2.45 ± 0.24	2.48 ± 0.36
	1-Tetradecene	24.842	1370	1372	2.03 ± 0.60	3.29 ± 0.05	4.05 ± 0.59	4.60 ± 0.13	12.98 ± 7.68
	Tetradecane	25.250	1392	1391	1.92 ± 0.39	3.36 ± 0.13	3.11 ± 0.43	2.04 ± 0.21	1.44 ± 0.85
	beta-santalene	25.453	1400	1399	7.83 ± 0.41	10.08 ± 0.50	7.16 ± 1.86	8.47 ± 0.45	7.32 ± 1.68
	1-Pentadecene	27.010	1456	1453	5.70 ± 0.21	7.43 ± 1.20	6.43 ± 1.83	7.48 ± 0.30	5.78 ± 1.94
	Pentacosane	27.760	1493	1491	2.61 ± 0.21	2.45 ± 0.31	3.81 ± 0.68	2.26 ± 0.53	1.54 ± 0.24
	3-methyl-pentadecane	27.937	1500	1498	5.70 ± 0.69	6.78 ± 0.25	4.49 ± 1.51	4.39 ± 0.65	4.61 ± 2.93
	Hexadecane	29.611	1570	1567	1.47 ± 0.04	1.66 ± 0.05	1.17 ± 0.35	1.70 ± 0.33	2.65 ± 0.95
	2,6,10-trimethyl- pentadecane	30.435	1600	1598	5.75 ± 0.70	7.56 ± 0.16	3.76 ± 1.49	3.50 ± 0.26	5.72 ± 1.90
	1-Heptadecene	31.384	1649	1634	0.63 ± 0.01	4.65 ± 0.12	n.d.	0.33 ± 0.19	0.47 ± 0.17
	Heptadecane	32.359	1692	1689	0.97 ± 0.04	1.49 ± 0.18	n.d.	2.20 ± 0.56	n.d.
	3-methyl-heptadecane	32.524	1700	1692	10.06 ± 3.58	10.03 ± 1.59	4.50 ± 2.53	2.4 ± 0.63	5.95 ± 1.08
	Octadecane	34.046	1771	1768	1.12 ± 0.03	n.d.	n.d.	n.d.	n.d.
	Nonadecane	34.648	1800	1798	21.44 ± 23.17	10.59 ± 2.58	4.92 ± 2.95	2.21 ± 0.31	5.78 ± 0.5
	Eicosane	36.669	1900	1899	20.29 ± 19.74	10.19 ± 0.85	3.80 ± 2.07	2.41 ± 0.47	13.96 ± 7.86
	1,3-dimethyl- benzene	38.594	2000	2000	4.60 ± 0.60	6.81 ± 0.27	3.37 ± 1.27	2.00 ± 0.38	5.37 ± 4.8
Esters	Hexanoic acid methyl ester	11.294	924	903	206.31 ± 11.9	272.07 ± 5.25	171.97 ± 5.96	167.25 ± 9.74	200.59 ± 89.25
	Heptanoic acid methyl ester	14.671	1026	1024	25.36 ± 1.86	44.08 ± 6.25	35.61 ± 5.28	25.12 ± 6.54	30.61 ± 13.29
	Octanoic acid methyl ester	17.823	1128	1116	7.00 ± 0.84	141.07 ± 1.39	73.45 ± 1.71	71.33 ± 5.2	112.63 ± 48.65
	Nonanoic acid methyl ester	20.762	1227	1215	83.02 ± 3.70	168.7 ± 3.89	127.99 ± 5.84	87.23 ± 3.27	128.53 ± 53.68
	Isopropyl salicylate	23.039	1320	1285	5.67 ± 0.10	15.35 ± 2.76	11.56 ± 3.20	11.54 ± 1.45	12.88 ± 2.53
	Decanoic acid methyl ester	23.520	1328	1314	13.75 ± 1.98	22.18 ± 2.09	17.54 ± 3.08	15.15 ± 1.01	9.8 ± 7.42
	Dodecanoic acid methyl ester	28.566	1527	1517	12.93 ± 2.94	18.61 ± 4.77	13.40 ± 4.31	11.02 ± 4.52	7.59 ± 1.22
	Tridecanoic acid methyl ester	30.911	1631	1617	0.42 ± 0.02	1.43 ± 0.56	0.57 ± 0.46	0.24 ± 0.04	2.65 ± 0.95
	Methyl jasmonate	31.527	1655	1644	0.21 ± 0.09	n.d.	n.d.	n.d.	n.d.
	Methyl tetradecanoate	33.096	1727	1715	43.33 ± 8.63	20.21 ± 9.25	51.78 ± 4.42	46.71 ± 2.34	13.94 ± 7.69
	Pentadecanoic acid methyl ester	35.208	1812	1814	1.34 ± 0.02	1.44 ± 0.63	0.71 ± 0.32	0.58 ± 0.3	1.22 ± 1.75
	Methyl palmitoleate	36.803	1932	1901	2.59 ± 0.10	4.20 ± 0.50	2.27 ± 1.09	2.3 ± 0.1	1.79 ± 1.08
	Palmitic acid methyl ester	37.224	1926	1921	235.99 ± 42.74	370.67 ± 34.79	281.06 ± 7.11	270.61 ± 31.51	64.9 ± 30.46
	Linoleic acid methyl ester	40.263	2092	2087	n.d.	10.9 ± 6.30	10.93 ± 1.12	11.04 ± 0.41	n.d.
	Oleic acid methyl ester	40.610	2103	2105	35.86 ± 1.48	51.01 ± 0.81	38.02 ± 5.46	39.57 ± 2.86	7.93 ± 4.37
	Methyl stearate	40.974	2130	2124	1.41 ± 0.12	2.26 ± 0.01	9.94 ± 2.04	1.24 ± 0.56	1.04 ± 0.58
Aldehydes	Nonanal	17.182	1102	1098	38.68 ± 4.95	39.86 ± 0.93	32.03 ± 0.35	29.91 ± 1.46	31.7 ± 11.53
	Decanal	20.221	1200	1200	5.45 ± 0.56	2.61 ± 0.19	4.77 ± 0.38	6.95 ± 0.11	4.96 ± 2.75
	Apricolin	24.595	1362	1360	3.86 ± 0.08	5.70 ± 1.57	3.95 ± 0.48	3.51 ± 0.09	8.77 ± 4.92
Ketones	2-Heptanone	10.072	889	868	11.5 ± 2.90	68.11 ± 14.25	26.77 ± 5.19	n.d.	n.d.
	3-Octanone	13.419	984	988	13.09 ± 2.06	31.94 ± 7.28	14.47 ± 5.06	13.21 ± 0.31	23.32 ± 24.84
	2-Nonanone	16.817	1095	1087	19.59 ± 4.50	47.88 ± 7.95	18.35 ± 4.49	4.74 ± 1.81	15.32 ± 4.78
	Hexahydropseudoionone	25.614	1408	1401	2.62 ± 0.21	n.d.	1.94 ± 0.33	3.36 ± 1.46	1.78 ± 1.03
	Geranyl acetone	26.854	1434	1447	2.79 ± 0.15	4.72 ± 0.65	4.26 ± 0.71	3.71 ± 0.09	2.77 ± 0.47
	Hexahydrofarnesyl acetone	35.598	1847	1845	2.56 ± 2.06	2.54 ± 0.45	1.87 ± 0.90	2.21 ± 0.31	2.34 ± 1.55
Alcohols	2-Heptanone	10.072	889	868	11.50 ± 2.90	68.11 ± 14.25	26.77 ± 5.19	n.d.	n.d.
	3-Octanone	13.419	984	988	13.09 ± 2.06	31.94 ± 7.28	14.47 ± 5.06	13.21 ± 0.31	23.32 ± 24.84
	2-Nonanone	16.817	1095	1087	19.59 ± 4.50	47.88 ± 7.95	18.35 ± 4.49	4.74 ± 1.81	15.32 ± 4.78
	Hexahydropseudoionone	25.614	1408	1401	2.62 ± 0.21	n.d.	1.94 ± 0.33	3.36 ± 1.46	1.78 ± 1.03
	Geranyl acetone	26.854	1434	1447	2.79 ± 0.15	4.72 ± 0.65	4.26 ± 0.71	3.71 ± 0.09	2.77 ± 0.47
	Hexahydrofarnesyl acetone	35.598	1847	1845	2.56 ± 2.06	2.54 ± 0.45	1.87 ± 0.90	2.21 ± 0.31	2.34 ± 1.55
	2-Heptanone	10.072	889	868	11.50 ± 2.90	68.11 ± 14.25	26.77 ± 5.19	n.d.	n.d.
	1-Hexanol	9.417	867	867	23.03 ± 1.11	44.77 ± 6.71	18.9 ± 4.18	18.38 ± 2.56	26.35 ± 7.65
	1-Octen-3-ol	13.223	986	982	4.63 ± 0.26	6.52 ± 0.39	5.51 ± 1.04	9.15 ± 0.42	7.22 ± 0.21
	4-methyl-3-heptanol	14.459	997	1018	5.21 ± 0.41	n.d.	n.d.	2.09 ± 0.41	n.d.
	1-Octanol	19.254	1169	1158	13.07 ± 0.84	16.92 ± 2.12	11.4 ± 1.56	7.53 ± 0.77	13.84 ± 2.00
Heterocyclics	2-pentyl-furan	13.536	996	992	98.27 ± 4.30	199.01 ± 19.98	120.74 ± 18.20	87.08 ± 7.4	96.42 ± 10.71
	2-n-Heptyl furan	19.848	1182	1192	9.20 ± 1.23	20.59 ± 1.59	12.93 ± 3.33	10.92 ± 0.99	11.77 ± 1.84

[a] Only identified compounds are shown the content. n.d. means not detected; [b] Retention time; [c] Retention index by searching NIST library;
[d] Retention index calculated by C7 − C40 alkanes external standards.

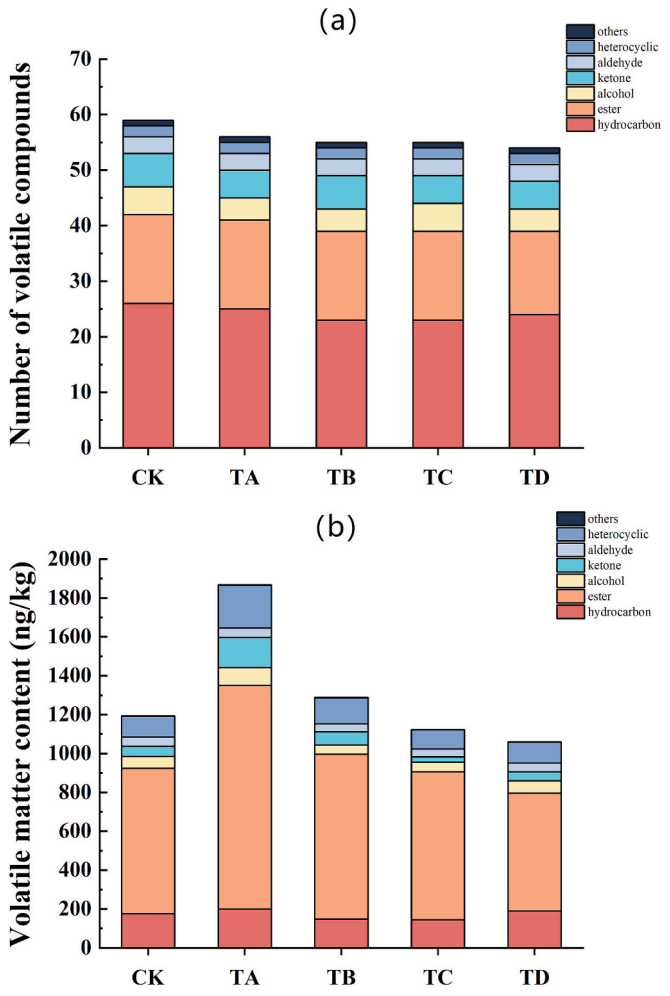

Fig. 4. Volatile compound profiles of rice stored for 30 days under various CO_2-MA conditions: (a) total numbers of VOCs and (b) total VOCs content.

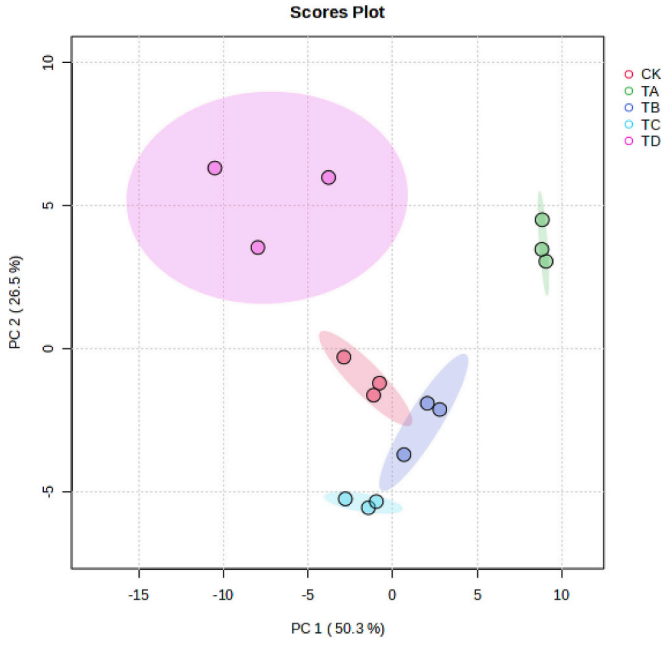

Fig. 5. PCA scores plot for separation of rice stored for 30 days with different CO_2-MA treatments (CK, TA, TB, TC and TD).

were prominent, including palmitic acid methyl ester, oleic acid methyl ester, methyl tetradecanoate, octanoic acid methyl ester, nonanoic acid methyl ester, tridecane, 3-methyl-2-heptanone and 4-methyl-3-heptanol (Fig. 6b). Some VOCs, such as palmitic acid methyl ester and 2-heptanone, contributed to both hypoxia and normoxia treatments but displayed distinct trends in their concentrations. Palmitic acid methyl ester exhibited a positive correlation with low oxygen concentrations but its concentration decreased with increasing CO_2 levels. This suggested that low oxygen levels may enhance lipid oxidation, whereas elevated CO_2 concentration could mitigate this effect. Similarly, 2-heptanone increased under TA and TB treatments but decreased under TC and TD treatments. Conversely, 4-methyl-3-heptanol consistently decreased in TA and TB treatments and also decreased in TC and TD treatments, showing a negative correlation with CO_2 levels.

4. Discussion

4.1. Efficacy of CO_2-MAs in managing *Tribolium castaneum*

The results demonstrated the high efficacy of CO_2-MA in controlling *Tribolium castaneum* across various developmental stages, with noticeable variations in mortality rates depending on CO_2 and O_2 concentrations. Among treatments, TD (21 % O_2 + 60 % CO_2) was the most effective, causing rapid and complete mortality across all stages, followed by TA (2 % O_2 + 35 % CO_2). These findings are consistent with previous studies demonstrating that elevated CO_2 levels (>60 %) or reduced O_2 (<3 %) could effectively eliminate stored grain pests by creating an inhospitable environment (Cheng, Lei, Ahn, Liu, & Zhu-Salzman, 2012). The enhanced efficacy of TA compared to TB and TC highlighted the critical role of hypoxia in increasing CO_2 toxicity. Hypoxic conditions, such as TA (2 % O_2), could intensify CO_2-induced respiratory inhibition by forcing insects to shift from aerobic to anaerobic pathways, leading to impaired ATP production and disruptions in vital physiological processes such as feeding and protein synthesis (Levy-De la Torre et al., 2022). Studies suggest that hypoxia combined with hypercapnia induces earlier mortality than hypoxia alone (Huang, Wang, Jian, Zhang, & Liu, 2023). Elevated CO_2 concentrations, like those in TD, could exacerbate respiratory inhibition, inducing severe metabolic and physiological disruptions, leading to eventual insect mortality, which is consistent with studies suggesting that CO_2 could achieve rapid mortality across all stages of *Tribolium castaneum* (Hashem, Khalifa, & Ahmed, 2021).

Different developmental stages responded differently to CO_2-MA treatments. Early and late instar larvae were more susceptible than pupae and adults to all treatments, likely due to their structural vulnerability and immature detoxification mechanisms (Guan et al., 2018; Zhou et al., 2024). Pupae, with their reduced metabolic activity (Mehmood et al., 2018), demonstrated higher resilience to CO_2-induced stress, but even they reached 100 % mortality under TD within 96 h, comfirming its efficacy against the most resilient stages. The results further validated the practicality of CO_2-MAs for pest control under grain-embedded conditions. In these scenarios, TD and TA treatments achieved near-complete adult mortality within 10 days, and all treatments reached maximum efficacy by day 20. These findings confirmed the applicability of CO_2-MAs as an effective pest management solution for practical storage scenarios.

4.2. Impact of CO_2-MA on Rice quality

CO_2-MA also significantly influenced rice quality, particularly lipid degradation and enzyme activity, which are key indicators of grain spoilage. Lipid degradation, a crucial factor for grain quality, was assessed by measuring the value of free fatty acids that are susceptible to oxidative degradation, leading to potential spoilage (Hu et al., 2023). Elevated FAV levels in TA and TD indicated accelerated lipid hydrolysis under hypoxic and hypercapnic conditions at 30-day MA storage,

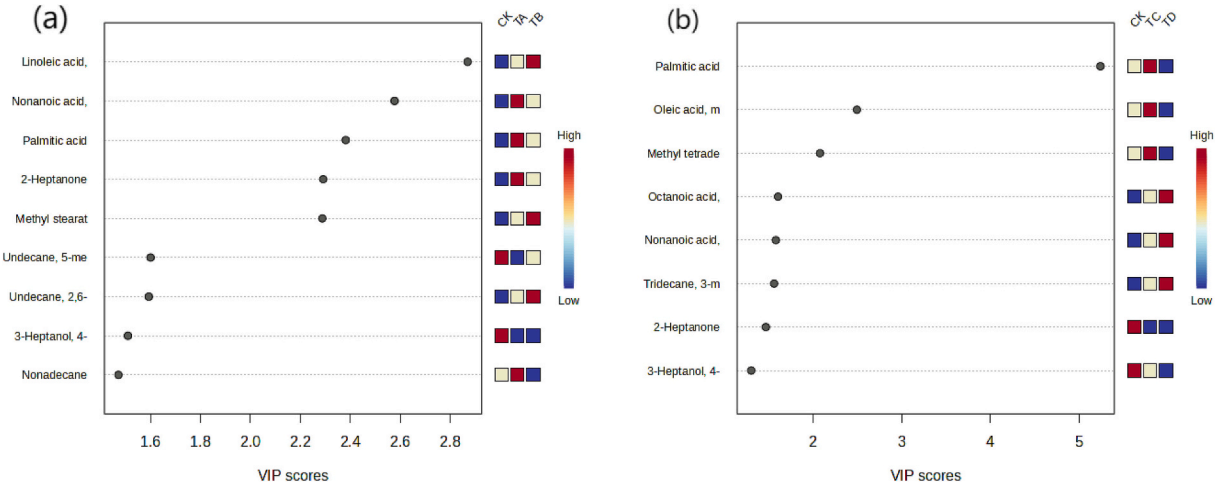

Fig. 6. Variable importance in projection (VIP) scores for VOCs in rice stored for 30 days under CO_2-MA treatments derived from OPLS-DA analysis. (a) VIP scores for low-oxygen and CO_2-MA treatments (TA and TB) and (b) VIP scores for normoxia CO_2-MA treatments (TC and TD) (VIP >1.4). Red indicates a higher abundance of volatile compounds, while blue signifies a lower abundance. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

consistent with a stress-induced metabolic shift (Xie, Zhou, Chen, & Xiao, 2021). However, FAV levels remained below 30 mg KOH/100 g, a threshold indicating suitability for indica rice storage even under these conditions (Zhu et al., 2024). This suggested that while lipid metabolism was affected, the treatments were still within an acceptable range for storage quality. The increased MDA content is associated with increased cell membrane permeability, indicative of grain aging and potential quality degradation (Granella, Christ, Werncke, Bechlin, & Machado Coelho, 2018). The MDA content in TA-treated rice reflected increased oxidative stress and membrane damage, whereas the lower MDA levels in TB, TC, and TD-treated rice reflected reduced oxidative degradation, implying that normoxic CO_2-MA protect lipids from oxidative degradation better than hypoxic treatments. The reduction in CAT activity across all CO_2-MA treatments indicated that grains were experiencing oxidative stress, which overwhelms their antioxidant defense systems. Zhao et al. (2024) suggested that low-oxygen-controlled atmosphere conditions could delay the CAT decrease in rice during long-term storage. In contrast, CAT activities in *Agaricus bisporus* were significantly promoted by 95 %–100 % CO_2-MA treatments (Lin et al., 2017). We also observed that CAT activity was significantly promoted by TA compared to CK at 10-day treatment (Table S2). Reduced AMS activity in TA and TB, particularly in TD-treated rice indicated a stress-induced response that could potentially slow down starch degradation, thereby impacting the sweetness and digestibility of rice. Studies have reported that CO_2-MA storage could delay the decline of AMS activity during long-term storage, thereby preserving rice quality by reducing enzymatic degradation and enhancing aroma compound retention (Jie, Shi, & Zhang, 2024). These emphasized the importance of monitoring enzymatic responses during long-term storage to ensure quality preservation.

4.3. CO_2-MA for Rice quality preservation: Insights on VOC profiles

VOCs are critical to rice aroma and flavor, directly influencing consumer acceptance (Akhoundzadeh, Gholami, Masoum, & Moazeni-Pourasil, 2018). Our findings highlighted the role of CO_2-MA treatments in modulating both the quantity and diversity of VOCs in paddy rice. VOCs released via oxidative stress has been substantiated (Dong, Sun, Maker, Ren, & Yu, 2022). Hypoxic treatments (TA and TB) increased VOC levels, particularly esters, ketones and hydrocarbons. These compounds are associated with intensified lipid oxidation and esterification processes, which enhance green, fatty, and fruity aromas but may also contribute to off-flavors if excessive (Tiwari et al., 2020). Conversely, the lower volatile content observed in TC and TD treatments suggested that higher CO_2 concentrations with normal oxygen may reduce lipid oxidation and suppress the formation of VOCs byproducts. Intriguingly, the reduction in the number of VOCs in TA, TB, and TC compared to the control may indicate that CO_2-MA treatments selectively suppress specific metabolic pathways responsible for generating diverse VOCs, signifying that while the total VOC content may increase under certain conditions, the diversity of these compounds could decrease, potentially altering the overall flavor and aroma profile of the stored rice. TC lowered levels of heterocyclics, aldehydes, and ketones. Meanwhile, TD exhibited lower levels of aldehydes and alcohols, contributing to grain quality preservation. Aldehydes, heterocycles and alcohols have been identified as the major VOCs in rice aging (Xu, Liu, & Zhang, 2021). It has been demonstrated that aldehyde contents in rice dramatically increased when subjected to higher temperature (37 °C) and humidity (70 %), resulting in flavor deterioration (Biao et al., 2019). Alcohols, considered markers of carbohydrate hydrolysis, are produced through the further decomposition of aldehydes. Many alcohols have relatively low odor thresholds, contributing substantially to the aroma profiles of rice during the aging process (Ma, Tian, Chen, & Jin, 2020). Heterocyclic, mainly generated through Maillard reaction and lipid oxidation, are also major contributors to rice aroma. Furans, a subgroup of heterocyclics, serve as important markers for aging discrimination, particularly for identifying and classifying long-aged rice (Wang, Chen, & Zhou, 2020). Excessive accumulation of aldehydes, alcohols, or heterocyclic compounds during storage can lead to undesirable flavor changes and reduced sensory quality. However, a decrease in these VOCs under TD conditions may result in a less desirable aroma for consumers, particularly for aromatic rice varieties. Therefore, further optimization may also be required, especially for aromatic rice, to balance pest control and the preservation of desirable sensory characteristics.

PCA and PLS-DA revealed distinct VOC profiles for each treatment, highlighting the unique metabolic responses induced by hypoxia and hypercapnia. These findings align with Liu, Zhao, Li, and Chen (2018), who demonstrated that vacuum levels significantly influenced VOCs in rice. In hypoxic treatments (TA and TB), key compounds such as linoleic acid methyl ester and nonanoic acid methyl ester were identified with high VIP scores, suggesting their role as markers for low-oxygen environments and their potential impact on flavor. Esters, which are crucial for the development of fruity and floral notes, formed via alcohol acyltransferase (AAT) and fermentative metabolism, are often enhanced under stress conditions like hypoxia (White, Blake, Taylor, & Monks, 2016). Similarly, in mangoes, hypoxia also promotes ester formation,

particularly ethyl acetate and ethyl butanoate, both known to influence flavor profiles (Ntsoane, Luca, Zude-Sasse, Sivakumar, & Mahajan, 2019). The increased levels of linoleic acid methyl ester suggest enhanced lipid hydrolysis and oxidative stress-induced degradation of polyunsaturated fatty acids (PUFAs). This phenomenon is consistent with studies indicating that hypoxia accelerates PUFA oxidation, generating products such as aldehydes, alcohols and esters (Mihálik et al., 2015). Nonanoic acid methyl ester, a product of oxidative cleavage of longer-chain lipids, further supports this shift toward oxidative processes (Enferadi Kerenkan, Béland, & Do, 2016). In contrast, normoxic treatments (TC and TD) exhibited distinct VOC profiles, with palmitic acid methyl ester and oleic acid methyl ester as the highest VIP compounds. These esters exhibited a dual trend, increasing under hypoxia but decreasing as CO_2 concentration rose, highlighting the suppressive effect of high CO_2 on lipid metabolism. Oleic acid methyl ester, was predominantly observed in normoxic conditions, signifying the stabilizing effect of oxygen on less oxidation-prone fatty acids.

5. Conclusion

These results emphasized the critical role of optimizing CO_2-O_2 parameters in MA storage to effectively balance pest control and quality preservation in rice. While hypoxic treatments (TA and TB) offer significant short-term benefits for pest management, they also induce oxidative stress, accelerating lipid oxidation and compromising sensory qualities. In contrast, TD (21 % O_2 + 60 % CO_2) demonstrated a more favorable balance, preserving rice quality and aroma while ensuring efficient pest control. These findings suggested that tailored CO_2-O_2 ratios, particularly in the TD treatment, hold promise as a sustainable strategy for long-term rice storage.

Future research should explore the long-term effects of CO_2-MA treatments over extended storage periods, focusing on their influence on rice quality. Additionally, investigating the applicability of CO_2-MA treatments across different rice varieties will provide valuable insights for optimizing MA storage strategies to meet diverse market demands. Integrating advanced lipidomic profiling with sensory analysis across multiple rice varieties and storage durations will further elucidate the potential of CO_2-MA as a sustainable solution for global grain storage challenges.

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CRediT authorship contribution statement

Xue Dong: Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Ming Yang:** Software, Methodology, Investigation, Formal analysis, Data curation. **Peian Tang:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102252.

Data availability

Data will be made available on request.

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