Metabolic mechanism of nitrogen modified atmosphere storage on delaying quality deterioration of rice grains

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ABSTRACT

Nitrogen modified atmosphere was an effective way to control pest infestation in grains. In this study, the quality changes of rice during nitrogen modified atmosphere packaging storage (N2-MAPS) were monitored. An un-targeted metabolomics method was used to detect the rice metabolites and explore the mechanism of N2-MAPS for delaying rice deterioration. In this study, two rice species were studied under N2-MAPS and conventional storage at 30 °C for 150 days. The quality changes of rice during storage were monitored. The results showed that N2-MAPS could retard the increase of fatty acid value and amylose content, and defer the decrease of enzyme activities. And N2-MAPS had no significant influence on texture characteristics of rice. The metabolomics results suggested some metabolites and pathways were affected by N2-MAPS and revealed that N2-MAPS could protect rice cells from oxidative damage, maintain cell integrity and stability by regulating the metabolism to delay the rice deterioration.

Introduction

Rice (Oryza sativa L.) is one of the most important grains in the world, which is used as staple food to supply protein, starch, lipid and essential elements to human body (Xie et al., 2020). Due to the seasonality of production, but the continuity of consumption, harvested rice grains must be stored for an extended duration under good conditions to maintain its quality and avoid pest or mold infection (Shad & Atungulu, 2020).

If storage conditions were not appropriate, rice seed may lose its quality properties. It was reported that high-temperature would accelerate lipid rancidity and quality deterioration of rice seeds (Zhao et al., 2021). This was due to that the higher the storage temperature, the higher the metabolic activity of the rice, leading to rapid deterioration (Wang, Feng, Fu, et al., 2020). At present, common rice storage methods included conventional storage (CS), low-temperature storage and modified atmosphere (MA) storage. Conventional stored rice was vulnerable to pests and fungal infestations, resulting in considerable quality deterioration (Ahmad et al., 2019; Quesille-Villalobos et al., 2019; Tang, Shao, Tang, & Zhou, 2018). Low-temperature storage was more beneficial to maintain rice quality, e.g. the germination percentage and growth attributes of primed rice seedlings (Wang, He, Peng, et al., 2018). It could massively reduce fungal growth and decrease in pest incidence rate. The MA storage was an effective way to extend storage time and retard rice deterioration (Sun et al., 2019). Gas composition was one of the most important abiotic conditions affecting the growth of fungi and pests. Nitrogen filling, carbon dioxide filling and vacuuming were three common MA storage method to provide an hypoxia environment.

MA was widely used in the storage of grains, fruits, vegetables and other agricultural products. Studies had reported that N2-MA storage can effectively delay the deterioration of grain quality and protect its internal enzyme scavenging system (Wang, Zhang, & Lu, 2018). Related research had also found that vitamin E and polyphenol in grains could be protected during N2-MA storage (Moncini et al., 2020). Vunduk et al. demonstrated that MA storage could effectively inhibit the activity of...
α-glucosidase, retard the oxidation of ascorbic acid, and improve the protection of citric acid, total phenolics and total antioxidant capacity in agricultural products (Vunduk, Kozarski, Djekic, Tomasevic, & Klaus, 2021). Perez-Perez et al. applied the MA technology to chickpea storage, and found that the antioxidant capacity and phenolic compounds of chickpea were more stable in N2-MA storage due to prevention of its oxidative degradation (Perez-Perez et al., 2021). Lang et al. also demonstrated that nitrogen MA storage can reduce the degradation of phenolic compounds in black rice during storage (Lang et al., 2019).

All the above quality protection by MA storage was related to the internal metabolism changes exposed to hypoxia stress, which can be investigated by metabolomics. Metabolomics was a useful technology for the analysis of all small-molecule metabolites in organisms and could be used to study the response of organisms to environment (Silva et al., 2021). Targeted and non-targeted metabolomics were two approaches of metabolomics research methods. Targeted metabolomics mainly involved multiplex analysis of known metabolites, including the use of standards for absolute qualitative and quantitative analysis of substances to be tested. Its shortcoming was the limited coverage of substances. In contrast, non-targeted metabolomics was to collect as much information of substances as possible and had a broader coverage of substances. Non-targeted metabolomics analysis was employed by Wang et al. to study stored brown rice, which showed that palmitoleic acid, cholesterol, linoleic acid and lauric acid were four key metabolites in lipid metabolism during storage (Wang, Feng, Zhang, et al., 2020).

This study evaluated the influences of different storage conditions, N2-MAPS and CS, on rice quality. Compared with CS, N2-MAPS could delay rice deterioration. However, there were few studies reported on the mechanism of N2-MAPS in maintaining rice quality. Since rice in N2-MAPS faced hypoxic environment, its physiological metabolism was inevitably affected. We hypothesized that some pivotal rice metabolic pathways were altered. Therefore, in order to better understand the metabolic changes of rice under hypoxic conditions, metabolomics was employed to investigate the mechanism of N2-MAPS in delaying rice quality deterioration.

Materials and methods

Rice materials

Rice Yongjing 68A (YJ) was an ordinary-quality Japonica rice harvested in August 2019, Zhejiang Province, China, and rice Jiahe 218 (JH) was a high-quality Japonica rice harvested in October 2019, Zhejiang Province, China.

Rice storage

Two storage conditions, N2-MAPS and CS, were used in our experiment. CS: rice samples were stored in transparent bags made of polyamide and polyethylene (PA/PE). N2-MAPS: rice samples were stored in PA/PE bags with nitrogen. The concentration of residual oxygen in the storage was determined using a gas detector (MIC-600, Shenzhen Yiyuntian Technology Co., Ltd, detection range 0 ~ 100 %, accuracy 0.1 %). The concentration of residual oxygen in the packaging was determined once a week and kept lower than 1.5 ± 0.1 % during the whole storage. All the bags with rice were put in an incubator with 30 °C ± 1 °C and 65 % ± 5 % RH for 150 days. The rice samples were taken every 30 days for quality determination.

Determination of rice quality

Fatty acid value (FAV)

FAVs were determined according to the Chinese National Standard GB/T 20569-2006. 50 mL anhydrous ethanol was first added to 10.00 g ground rice sample, and the mixture was shaken for 10 min and then filtered. 25 mL filtrate was mixed with 50 mL distilled water, and 3 drops of phenolphthalein indicator was added. The diluted filtrate was then titrated with potassium hydroxide standard titration solution. It was repeated 3 times for each sample. The FAV was expressed as the amount of potassium hydroxide required to neutralize the free fatty acids (FA) in 100 g rice sample.

Amylose content

Amylose content was determined according to the Chinese National Standard GB/T 15683-2008. 10.00 g of milled rice sample was defatted by reflux extraction using 85 % methanol solution to prepare the defatted sample. Then the defatted sample was dispersed in 1 M NaOH, using iodine as chromogenic reagent and measuring absorbance at 720 nm. The determination for each sample was carried out in triplicate.

Peroxidase (POD) activity

The POD activity was measured using the POD kit (kit no. A084-3-1, Nanjing jiancheng Bioengineering Institute, China), which was based on guaiacol oxidation. The POD activity (U/g) was recorded by the absorbance at 420 nm. The POD activity for each sample were repeated 3 times.

Catalase (CAT) activity

CAT activity was measured using the method of Mi et al. (Mi, Gregorich, Xu, McLaughlin, & Liu, 2018). CAT could catalyze hydrogen peroxide to produce water and oxygen. Excessive hydrogen peroxide was added into the reaction solution, and the excess hydrogen peroxide was titrated with potassium permanganate. The CAT activity was expressed by the consumption of potassium permanganate. The CAT activity for each sample were carried out in triplicate.

Texture characteristics of rice

Texture Analyzer (TA.XT plus, Stable Micro Systems, UK) was used to detect the texture characteristics of rice. P/36R probe was applied to measure cooked rice at pre-test speed of 5 mm/s, test speed of 0.5 mm/s and post-test speed of 5 mm/s. The texture characteristics of each rice sample were determined for six times.

Determination of metabolites in rice

Metabolites extraction

Rice sample of 20 ± 1 mg was mixed by 1000 μL 75 % methanol (including internal L-2-Chlorophenylalanine). The mixture was ground for 4 min and sonicated in an ice water bath for 5 min, and then placed in the refrigerator at −40 °C for 1 h. The mixture was stand 1 h and then was centrifuged at 12000 rpm for 15 min at 4 °C. The supernatant was used for gas chromatography coupled with a time-of-flight mass spectrometry (GC-TOF-MS) and ultra high-performance liquid chromatography coupled with Q-Exactive Mass spectrometry (UHPLC-QE-MS) determination. 100 μL of each sample was mixed to prepare QC sample.

GC-TOF-MS analysis

The supernatant above was dried in a vacuum concentrator. Then 30 μL methoxymation hydrochloride (20 mg/mL in pyridine) was added. The mixture was shaken well and placed in an oven at 80 °C for 30 min. 40 μL BSTFA (1 % TMCS, v/v) was added to it and incubated at 70 °C for 1.5 h. After cooling to room temperature, all samples were analyzed by gas chromatograph (7890A, Agilent Technologies Inc., USA) coupled with a time-of-flight mass spectrometer (PEGASUS HT, LECO Inc., USA).

Capillary column (DB-5MS, 30 m × 250 μm × 0.25 μm, Agilent Technologies Inc., USA) was used to separate the samples. Helium was used as carrier gas with front inlet purge flow 3 mL/min, gas flow rate through the column 1 mL/min. The initial temperature was maintained at 50 °C for 1 min, then increased to 310 °C at a rate of 10 °C/min, and then at 310 °C for 8 min. The injection, transfer line, and ion source temperatures were 280 °C, 280 °C and 250 °C, respectively. The energy...
Fig. 1. The changes of FAV of JH (a) and YJ (b), the changes of amylose content of JH (c) and YJ (d), the changes of POD activity of JH (e) and YJ (f), the changes of CAT activity of JH (g) and YJ (h) during storage. $N_2$ denoted nitrogen modified atmosphere packaging storage ($N_2$-MAPS). CS denoted conventional storage (CS). * There was a significant difference between $N_2$-MAPS and CS.
Table 1

| Texture characteristics | Storage time (d) | JH-N₂ | JH-CS | YJ-N₂ | YJ-CS |
|--------------------------|-----------------|-------|-------|-------|-------|
| Hardness                 | 0               | 1314 ± 34 | 1308 ± 3 | 1308 ± 6* | 1314 ± 34 |
|                         | 30              | 1329 ± 30 | 1402 ± 30 | 1648 ± 30 | 1444 ± 30 |
|                         | 60              | 1730 ± 60 | 1862 ± 60 | 1714 ± 60 | 1526 ± 60 |
|                         | 90              | 1918 ± 90 | 1931 ± 90 | 1832 ± 90 | 1811 ± 90 |
|                         | 120             | 1991 ± 120 | 2011 ± 120 | 1862 ± 120 | 1836 ± 120 |
|                         | 150             | 2217 ± 150 | 2176 ± 150 | 1958 ± 150 | 2008 ± 150 |
| Guminess                 | 0               | -97.8 ± 0.00 | -97.8 ± 0.00 | -94.0 ± 0.00 | -94.0 ± 0.00 |
|                         | 30              | -77.3 ± 0.00 | -69.0 ± 0.00 | -86.7 ± 0.00 | -77.3 ± 0.00 |
|                         | 60              | -60.8 ± 0.00 | -68.3 ± 0.00 | -55.4 ± 0.00 | -56.2 ± 0.00 |
|                         | 90              | -54.2 ± 0.00 | -53.7 ± 0.00 | -46.2 ± 0.00 | -46.0 ± 0.00 |
|                         | 120             | -45.0 ± 0.00 | -39.1 ± 0.00 | -44.2 ± 0.00 | -42.9 ± 0.00 |
|                         | 150             | -37.9 ± 0.00 | -29.5 ± 0.00 | -45.0 ± 0.00 | -41.1 ± 0.00 |
| Cohesiveness             | 0               | 0.56 ± 0.64 | 0.55 ± 0.63 | 0.55 ± 0.63 | 0.55 ± 0.63 |
|                         | 30              | 0.65 ± 0.64 | 0.63 ± 0.63 | 0.63 ± 0.63 | 0.63 ± 0.63 |
|                         | 60              | 0.63 ± 0.62 | 0.62 ± 0.60 | 0.59 ± 0.59 | 0.59 ± 0.59 |
|                         | 90              | 0.63 ± 0.63 | 0.63 ± 0.61 | 0.61 ± 0.63 | 0.61 ± 0.63 |
|                         | 120             | 0.65 ± 0.67 | 0.67 ± 0.67 | 0.67 ± 0.67 | 0.67 ± 0.67 |
|                         | 150             | 0.69 ± 0.68 | 0.67 ± 0.68 | 0.67 ± 0.68 | 0.67 ± 0.68 |
| Chewiness                | 0               | 738 ± 26 | 738 ± 26 | 729 ± 26 | 55 ± 26 |
|                         | 30              | 857 ± 30 | 904 ± 30 | 1087 ± 30 | 956 ± 30 |
|                         | 60              | 1086 ± 60 | 1156 ± 60 | 984 ± 60 | 849 ± 60 |
|                         | 90              | 1207 ± 90 | 1264 ± 90 | 1109 ± 90 | 1136 ± 90 |
|                         | 120             | 1289 ± 120 | 1243 ± 120 | 1144 ± 120 | 1113 ± 120 |
|                         | 150             | 1526 ± 150 | 1417 ± 150 | 1240 ± 150 | 1259 ± 150 |
| Resilience               | 0               | 0.31 ± 0.21 | 0.29 ± 0.21 | 0.29 ± 0.21 | 0.29 ± 0.21 |
|                         | 30              | 0.39 ± 0.21 | 0.38 ± 0.35 | 0.38 ± 0.35 | 0.35 ± 0.35 |
|                         | 60              | 0.21 ± 0.20 | 0.20 ± 0.20 | 0.20 ± 0.20 | 0.20 ± 0.20 |
|                         | 90              | 0.21 ± 0.21 | 0.19 ± 0.21 | 0.19 ± 0.21 | 0.21 ± 0.21 |
|                         | 120             | 0.22 ± 0.21 | 0.22 ± 0.19 | 0.22 ± 0.19 | 0.19 ± 0.19 |
|                         | 150             | 0.24 ± 0.21 | 0.23 ± 0.15 | 0.21 ± 0.15 | 0.15 ± 0.15 |

Different letters (a, b) indicated significant differences in the same rice species at the same storage time.

N₂ denoted nitrogen modified atmosphere packaging storage (N₂-MAPS).

CS denoted conventional storage (CS).

The significance analysis of rice quality indexes was carried out by SPSS (version 20.0, SPSS Inc., USA). ANOVA analysis with the Tukey’s multiple comparison testing was used to confirm the significant differences at a level of p-value (p) < 0.05. Origin (version 9.1, Origin Lab, USA) was used to draw charts.

Results and discussion

Rice quality

FAV usually indicated the degree of lipid hydrolysis in grains. It was an important standard index for judging grain quality. It can be observed that the fatty acid values of rice samples showed an increasing trend along with storage in both storage conditions (Fig. 1 a-b). Amylose content of rice was an important determinant to the edible and cooking quality of rice. The lower the amylose content, the softer and stickier the cooked rice, with better taste. It can be seen from Fig. 1 c and d that amylose contents increased gradually with the increase of storage time. After 150 days of storage, the amylose contents of rice samples (JH and YJ) in N₂-MAPS were significantly lower than that in CS.

POD and CAT were two enzymes related to seed antioxidant. POD was a family of oxidoreductases, which can eliminate reactive oxygen species (ROS) in cells and reduce cell damage (Sheng, Liu, Song, Xu, & Zhu, 2021). CAT was a marker enzyme of PODs and one of the most important antioxidant enzymes in cells. In our experiment, the activities of both POD and CAT displayed a decreasing trend along with storage, showing that the antioxidant activity of rice decreased during storage (Fig. 1 e-h). The texture characteristics of rice were important indexes for evaluating rice quality. Six indexes of hardness, guminess, chewiness, cohesiveness, chewiness, adhesion and resilience were measured (as shown in Table 1). The results showed that different storage conditions had little effect on rice texture characteristics.

In summary, after 150 days’ storage, the FAV and amylose content of...
rice in N$_2$-MAPS was lower than that in CS, which indicated that N$_2$-MAPS could retard the deterioration of rice quality. The two antioxidant enzymes of rice, POD and CAT, exhibited higher activities in N$_2$-MAPS than CS, which indicated that N$_2$-MAPS could slow down the oxidation of rice. Therefore, N$_2$-MAPS was a better storage method for rice.

**Metabolite analysis of rice under different storage conditions**

The metabolite profiles of rice were investigated and a total of 677 metabolites were identified, including amino acids, carbohydrates, FAs, polyphenols, flavonoids, organic acids, alcohols, inorganic acids and others.

**Principal component analysis (PCA)**

As an unsupervised multivariate statistical analysis method, PCA was usually applied to explore differences in rice between N$_2$-MAPS and CS. The identified metabolites of YJ and JH by GC-TOF-MS and UHPLC-QE-MS were analyzed by PCA to evaluate overall differences between the rice stored in different conditions (Fig. 2 a). The graph was plotted based on a confidence interval level of 0.95. PC 1 and PC 2 represented the first and second principal components, respectively.

**Orthogonal projections to latent structures-discriminant analysis (OPLS-DA)**

In order to better discriminate the statistically significant differences in rice between N$_2$-MAPS and CS, the supervised statistical method OPLS-DA was introduced. The OPLS-DA score plots were shown in Fig. 2 b and c. From Fig. 2 b and c, it can be seen that the two species of rice stored in N$_2$-MAPS and CS were well differentiated and all the samples were in 95 % confidence interval.

**Identification of key metabolites in rice**

In order to display the overall distribution of metabolites more clearly, the volcano plots with the $-\log_{10}(p)$ of various substances and $\log_{2}(FC)$ were shown in Fig. 2 d and e. Based on the statistical analysis
results, metabolites satisfying with VIP > 1 (OPLS-DA) and \( p < 0.05 \) (Student’s t-test) were screened out. 131 metabolites were distinguished in JH-N\(_2\) vs JH-CS group, and 93 metabolites were differentiated in YJ-N\(_2\) vs YJ-CS. In order to better visualize the results, heat maps were used to show metabolite differences within groups and the top 50 differential metabolites of \( p \)-value in separate groups are shown in Fig. 2 f and g.

**Overview of differential metabolites**

A total of 37 metabolites with the same changing trend in both rice species were displayed in Table 2. It was shown that compared to CS, most amino acids, monosaccharides, and unsaturated FAs were down-regulated, while most soluble carbohydrates were up-regulated in rice samples for N\(_2\)-MAPS.

**Overview of pathway analysis**

To further analyze the relationship among the different metabolites, screened metabolites were projected to KEGG pathway database. Bubble maps were used to visualize the differences of metabolic pathways between N\(_2\)-MAPS and CS (shown in Fig. 3). Each bubble represented the identified metabolic pathway. Fig. 3 showed that N\(_2\)-MAPS mainly affected JH and YJ quality through the same pathways, C5-Branched dibasic acid metabolism, starch and sucrose metabolism, glycerophospholipid metabolism, glycerolipid metabolism and glycine, serine and threonine metabolism.

**Rice metabolism**

From the above analysis, it can be seen that there were significant metabolic differences of rice between the N\(_2\)-MAPS and the CS. To further highlight the relationship of the metabolites involved in rice metabolism, Fig. 4 summarized the relationships of key metabolites and metabolic pathways.

**Amino acid metabolism**

4-aminobutyric acid (GABA) can improve the resistance of rice seeds to unfavorable storage environment and delay the aging process of seeds (Zhu et al., 2022). It was also reported that GABA may accumulated under adverse environments (Chang et al., 2020). Therefore, GABA could be considered as a signaling metabolite or a stress marker to modify the defense system of plant faced with adverse storage environments. In our study, rice samples under N\(_2\)-MAPS exhibited significantly decreased GABA level compared to rice under CS (shown in Fig. 4). It was suggested that rice in CS stimulated more GABA to enhance the defense ability of rice seeds to unfavorable environments.

Glutamic acid is the precursor to produce GABA catalyzing by glutamate decarboxylase. The contents of glutamic acid in rice under N\(_2\)-MAPS were significantly higher compared to that under CS (shown in Fig. 4). It may be due to that more glutamic acid in rice under CS was converted to GABA to withstand adverse storage conditions.

Glycine acted as endogenous plant antioxidant to protect cell membrane from ROS (Bohnert, Nelson, & Jensen, 1995). When exposed to excessive ROS, seed survival depended on the strength of endogenous antioxidant defense barriers (Diez, Traikov, Schmeisser, Das Adhikari, & Kurzchalia, 2021). Glycine can serve as a provider of reductive equivalents to maintain the cellular antioxidant system, helping to maintain the antioxidant capacity of cells after stress (Yang et al., 2017). Moreover, glycine was a constituent amino acid of endogenous antioxidant
glutathione. Glutathione also played an important role in cellular anti
oxidant mechanism. It was demonstrated that high levels of glycine, as
the reducing agent of the tricarboxylic acid cycle. It was also demonstrated that high levels of

VIP denoted the variable importance in the projection from OPLS-DA.

Table 2

| Metabolite name | JH VIP | p   | YJ VIP | p   | Regulating trend |
|-----------------|--------|-----|--------|-----|-----------------|
| stearidonic acid| 1.8797 | 2.00E-05 | 1.781 | 5.00E-03 | Down |
| gamma-linolenic acid| 1.9349 | 3.00E-06 | 1.715 | 7.80E-03 | Down |
| 13s-hydroxystearidonic acid| 1.9023 | 9.00E-06 | 2.154 | 3.00E-05 | Down |
| 9-oxocholesterol| 1.9372 | 3.00E-06 | 1.74 | 7.40E-03 | Down |
| 5-(2-hydroxyethyl)-4-methylthiazole| 1.8135 | 7.00E-05 | 2.038 | 1.00E-04 | Up |
| alpha-dimorphemic acid| 1.9371 | 2.00E-06 | 1.948 | 1.20E-03 | Down |
| gluconolactone| 2.0011 | 4.00E-08 | 2.278 | 1.00E-08 | Down |
| l-iditol| 2.0011 | 2.00E-01 | 1.00E-01 | 1.00E-01 | Down |
| trehalose 6-phosphate| 1.9788 | 1.00E-03 | 1.578 | 2.10E-02 | Down |
| prostaglandin E1| 1.7104 | 7.00E-04 | 1.252 | 4.81E-02 | Down |
| 1-trans-alpha-alanine-2-carboxycyclopentanecarboxylic acid| 1.3336 | 3.01E-02 | 1.515 | 3.93E-02 | Down |
| d-glutamic acid| 1.6287 | 9.00E-05 | 1.539 | 1.60E-02 | Up |
| l-gulose| 1.4171 | 1.28E-02 | 2.054 | 1.00E-04 | Down |
| oxopropene| 1.9003 | 1.00E-05 | 1.958 | 1.50E-03 | Up |
| ni-2,3-dihydro-2-oxo-1-b-isole-3-acetic acid| 1.2189 | 3.94E-02 | 1.493 | 2.91E-02 | Down |
| sorbitol| 2.0321 | 3.00E-07 | 2.245 | 2.00E-07 | Down |
| 2-phenylethyl beta-d-glucopyranoside| 1.8911 | 3.00E-05 | 1.594 | 2.30E-02 | Down |
| ethanolamine| 1.6625 | 2.00E-12 | 2.073 | 3.00E-04 | Down |
| gluconic lactone 1| 2.0309 | 3.00E-04 | 2.152 | 1.00E-05 | Down |
| glycerol 2| 1.7526 | 2.00E-07 | 2.234 | 3.00E-06 | Down |
| glycine 1| 1.9231 | 1.10E-03 | 2.258 | 3.00E-07 | Down |
| 4-amino-butryic acid 1| 1.7933 | 9.00E-12 | 2.138 | 4.00E-05 | Down |
| stearic acid| 1.3109 | 3.00E-04 | 1.66 | 1.39E-02 | Up |
| kojibiose| 1.5050 | 6.00E-05 | 1.624 | 1.22E-02 | Up |
| glycerol-3-phosphate| 1.8254 | 3.00E-06 | 1.922 | 1.70E-03 | Up |
| octadecenal acid| 1.6142 | 7.00E-04 | 1.992 | 9.00E-04 | Down |
| 2-isopropylphenyl methylocarbamate| 2.1010 | 2.81E-02 | 2.331 | 2.00E-04 | Down |
| d-tocotrienol| 2.1807 | 5.80E-03 | 1.447 | 4.28E-02 | Up |
| d-glyceric acid| 1.9701 | 7.00E-05 | 1.662 | 1.26E-02 | Down |
| palmitinol 2| 2.0311 | 1.30E-03 | 2.232 | 5.00E-07 | Down |
| d-glutaric acid| 2.0028 | 2.00E-08 | 1.985 | 7.00E-08 | Down |
| trehalose 6-phosphate| 1.9788 | 1.00E-06 | 1.525 | 2.40E-02 | Up |
| 3-cloroerytroseine| 1.2719 | 1.00E-06 | 1.737 | 1.61E-02 | Down |
| 6-o-actilasturostatin| 1.5209 | 3.00E-11 | 1.696 | 7.80E-03 | Up |
| 2-deoxy-d-galactose 1| 2.0268 | 3.00E-12 | 1.95 | 4.00E-04 | Down |
| mannotose 1| 1.6928 | 2.00E-07 | 1.963 | 8.00E-08 | Up |
| 2,2-dimethylbutyric acid| 1.9037 | 1.25E-02 | 2.075 | 2.00E-04 | Down |

VIP denoted the variable importance in the projection from OPLS-DA. p denoted the p-value from Student’s t-test.

Lipid metabolism

Lipid hydrolysis was an important part of lipid metabolism, which can produce glycerol and FAs. Glycerol was first oxidized to form glyceraldehyde, which then isomerizes to dihydroxyacetone and glycerate. Glycerate is then phosphorylated to form glycerol-3-phosphate (G3P) in glycerolipid metabolism. G3P can then be further phosphorylated to produce fructose-1,6-bisphosphate and dihydroxyacetone phosphate. Dihydroxyacetone phosphate is then converted to dihydroxyacetone and glycerol-3-phosphate. Glycerol-3-phosphate is then used in the synthesis of dihydroxyacetone phosphate and glycerol.

The synthesis of dihydroxyacetone phosphate and glycerol-3-phosphate is essential for the synthesis of lipids, carbohydrates, and amino acids. Glycerol-3-phosphate is also an important precursor for the synthesis of fatty acids. Fatty acids are essential biomolecules in plant cellular metabolism and can affect cell membrane fluidity and metabolic energy storage during different stresses (Liu et al., 2020). The synthesis of fatty acids can be regulated by transcription factors, which can be induced by different environmental stresses.

Different storage conditions were investigated in CS, the rice accumulated more GABA, glycine, and phenylalanine to protect itself from adverse environments and defense environmental stress. It can be seen that rice maintained the balance of the antioxidant system by increasing amino acid synthesis.

In general, the metabolism difference of amino acids in rice under different storage conditions were obvious. In CS, the rice accumulated more GABA, glycine, tyrosine, and phenylalanine to protect itself from adverse environments and defense environmental stress. It can be seen that rice maintained the balance of the antioxidant system by increasing amino acid synthesis.
Stearic acid was a long-chain saturated fatty acid, which was usually formed by oxidation of polyunsaturated FAs. Stearic acid and its derivatives played an important role in plant defense mechanisms. Studies have shown that increased levels of stearic acid could help plants improve their tolerance to heat stress and protect cells from damage (Lakhssassi et al., 2017). Compared with CS, the content of stearic acid in rice was significantly up-regulated in N$_2$-MAPS, which indicated that N$_2$-MAPS could protect the functional integrity of rice cell membrane (shown in Fig. 4).

Linolenic acid was the main fatty acid in all crops. The accumulation of linolenic acid could lead to the ROS accumulation in plant cells. And excessive ROS would cause oxidative stress damage to plant cells and affect seed vigor (Yu et al., 2020). Singh et al. illustrated that linolenic acid was present in higher level in injured plants than in healthy, intact plant cells (Singh et al., 2015). The down-regulation of linolenic acid in rice under N$_2$-MAPS suggested that N$_2$-MAPS could protect cells from damage by ROS, maintain cell integrity and activity, slow down the deterioration of rice quality, and prolong the storage life of rice by inhibiting the accumulation of linolenic acid in alpha-Linolenic acid metabolism.

Thus, CS led to up-regulation of FAs, which indicated that rice underwent more intense oxidation reaction in CS. Bita et al. reported that increasing the saturation level of FAs appeared to be critical for maintaining membrane stability (Bita & Gerats, 2013). In our study, up-regulation of saturated fatty acids (stearic acid) and down-regulation of unsaturated fatty acids (linolenic acid, alpha-dimorphhecolic acid, stearidonic acid, 13s-hydroxyoctadecadienoic acid, etc.) in rice in N$_2$-MAPS were observed, indicating that N$_2$-MAPS could protect the cellular integrity of rice.

Carbohydrate metabolism

As a ubiquitous monosaccharide, mannose can be found in plants and animal glycans and participated in glycolytic pathway. It was reported that mannose, together with raffinose, galactitol and sorbose, were accumulated as an antibacterial agent in most of the stored plant seeds (Tan et al., 2015). Mannose can also enhance the activities of antioxidant enzymes, such as POD, glutathionc reductase, superoxide dismutase, ascorbate POD, dehydroascorbate reductase (Zhao, Zeng, Li, & Peng, 2020). Our experimental results showed that mannose in rice stored at N$_2$-MA was significantly up-regulated, suggesting that N$_2$-MAPS can benefit the antibacterial and antioxidant capacity of rice. In addition, Fig. 1 e and f showed that POD activity of rice stored at N$_2$-MA was higher than that in CS, which verified that rice stored at N$_2$-MA was with higher antioxidant ability.

Sucrose was the main energy substrate involving in multiple biological processes and acted as a signaling regulator of plant in response to various abiotic stresses. Sucrose can improve abiotic stress tolerance and sensitivity of plants (Li et al., 2021). Li et al. showed that sucrose was very important to the growth and development of lily, with coordinating and balancing various carbohydrates (Li et al., 2022). Sucrose acted as a signaling molecule in plants against pathogens and participated in plant defense mechanisms. In our experiment, the sucrose content of rice seeds in N$_2$-MAPS was higher than that in CS (shown in Fig. 4). It indicated that N$_2$-MAPS was favorable to enhance environmental tolerance and extend storage period of rice seeds.

Raffinose was a trisaccharide comprising galactose, glucose and fructose. Previous studies revealed that high level of raffinose contribute to positive seed storability and antioxidant activity (Fuhrs, Specht, Erban, Kopka, & Horst, 2012). In our study, the level of raffinose in rice was higher during N$_2$-MAPS, which also specified that rice stored at N$_2$-MA was with higher antioxidant ability (shown in Fig. 4).

Compared with CS, trehalose-6-phosphate (T6P) was significantly up-regulated, and glucose was down-regulated in rice under N$_2$-MAPS (shown in Fig. 4). T6P produced glucose in the presence of T6P hydrolyase in the starch and sucrose metabolism. Meanwhile, T6P was a signaling molecule, which engaged in the regulation of carbon metabolism, photosynthesis and homeostasis (Gabriel et al., 2021). T6P was known as a stress protective agent in plants, which participated in plant & pathogen interactions and cell-wall modification. Higher levels of T6P can promote starch synthesis, and inhibited the hexokinase activity and regulated glycolysis as well (Wang, Wang, Pan, et al., 2019).

In general, up-regulated mannose in N$_2$-MAPS enhanced the antioxidant capacity of rice by increasing the activity of antioxidant enzymes. With the up-regulated sucrose and raffinose, the antibacterial capacity of rice was improved. And high levels of soluble carbohydrates can protect the membrane system, improve stress resistance and maintain cell stability (Hossain et al., 2020).
Fig. 4. The changes in metabolites mapped to the metabolic pathways in two rice species. The red blocks represented up-regulated metabolites of rice in N$_2$-MAPS compared to that in CS. The blue blocks represented down-regulated metabolites of rice in N$_2$-MAPS compared to that in CS. The gray blocks represented not significant regulated metabolites of rice in N$_2$-MAPS compared to that in CS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Conclusion

The study revealed that N2-MAPS could delay the quality deterioration of rice by regulating its metabolism. The levels of some important metabolites changed significantly. These metabolites were mostly related to the antioxidant system of rice. Through the discussion of the physiological functions of these metabolites, we found that N2-MAPS could enhance the antioxidant activity, protect the cellular integrity and maintain cell stability of rice by regulating the amino acid metabolism, fatty acid metabolism and carbohydrate metabolism to delay the rice deterioration. This study provided better understanding for the influence of gas composition changes in the storage environment on the metabolism of rice grains.

CRediT authorship contribution statement

Chenling Qu: Conceptualization, Writing – review & editing, Project administration. Wenhao Li: Formal analysis, Writing – original draft. Qiankui Yang: Formal analysis. Yunze Xia: Formal analysis. Peng Lu: . Mei Hu: .

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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