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Effect of catalase on lipid oxidation and flavor substances of α- instant rice during storage

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Abstract

Lipid oxidation is one of the important factors affecting the quality of α-instant rice. In this study, α- instant rice was detected with hydrogen peroxide value (POV), malondialdehyde value (MDA) and fatty acid value (FAV), colorimeter, electronic nose and HS-SPME GC/MS before and after storage. As well as, the effect of catalase treatment on the quality of α-instant rice during storage was investigated The results showed that the content of MDA in α-instant rice decreased by 41.4% in the late stage of the accelerated storage test (21 days), which was 4.3% lower than that of α-instant rice treated with BHT. At the same time, catalase had more advantages in maintaining the whiteness and aroma of α-instant rice, and inhibiting the aldehydes, ketones, hydrocarbons and various volatile odor substances produced by lipid oxidation of α-instant rice. Therefore, catalase, as a natural substitute for synthetic antioxidants, has great potential in the antioxidation of convenience foods.

Keywords: α- instant rice; catalase; lipid oxidation; storage period; volatile compound.

Practical Application: The application of catalase can reduce the lipid oxidation degree of a- instant rice during storage, and maintain the original flavor of a- instant rice.

1 Introduction

According to the edible method, instant rice could be divided into non-dehydrated and dehydrated rice. Non-dehydrated rice is ready for immediate consumption without rehydration step, but the shelf life is short, usually within 3 months. While α- instant rice, also known as dehydrated rice, is a convenience food prepared by rapidly dehydrating and drying cooked rice (Jiao et al., 2014). With water content less than 10%, dehydrated rice could be stored at normal temperature and pressure for more than two years, which is one of its significant advantages. α- instant rice could be directly eaten after simply soaking for a few minutes in the boiled water. Lipids are generally stable in rice as the intact spheres. However, when the adipocyte membrane is subjected to the action of phospholipase, the lipid begins to hydrolyze under physical damage and high temperature treatment (Djanaguiraman et al., 2018). With the increase of free fatty acid content, lipoxygenase acts on the free fatty acid, producing a variety of lipid oxidation products and flavor substances (Zhang et al. 2021b). The current processing and storage technology generally has the issue of lipid oxidation leading to the rough texture, yellow color, weakened flavor and shortened the shelf life of α-instant rice, which affects the edible quality. Flavor is an important indicator for evaluating the quality of rice, while the odor changes during storage are closely related to the fatty acid content (Ma et al., 2020). The content of lipids in rice is low (Wang, 2022), however the deterioration rate is the fastest during storage, which has a great impact on the flavor and texture of rice. It is due to the fact that fats in foods are decomposed by oxidation to produce free fatty acids, and further oxidation would produce a series of volatile compounds such as small molecular aldehydes, ketones, acids, etc. (Choe & Min, 2006). These volatile compounds lead to the generation of special odors, however the change rules of these volatile compounds during storage is not clear untile now.

Butylated hydroxytoluene (BHT) is commonly used as an antioxidant in the food industry (Carballo et al., 2019) and is also widely used in the pharmaceutical and cosmetic industries (Yehye et al., 2015). Early studies demonstrated the ability of BHT to induce allergic diseases (Yamaki et al., 2007). However, recent studies showed that BHT could induce calcium imbalance and endoplasmic reticulum-mitochondrial dysfunction, causing toxic effect on mouse leydig cells (Ham et al., 2020). Therefore, BHT, as an antioxidant, is gradually replaced in the food field. Natural antioxidants, extracted from natural animals and plants, are considered as relatively safe antioxidants, including polyphenols, polysaccharides, enzymes, phytic acid, vitamins, etc. (Chen et al., 2016). Among them, catalase (CAT) is a good antioxidant that can prevent lipid oxidation (Pérez-Estrada et al., 2019). Catalase (CAT) is an oxidoreductase, which has a good performance in quenching reactive oxygen species (ROS), Kaushal et al. (2018) and it is described the application of catalase in the food industry, which acts as an antioxidant protecting cells from oxidative stress. In addition, CAT is capable of decomposing more than one million substrate molecules per second and has the highest rate of reaction with substrates (Loncar & Fraaije, 2015). CAT is widely used in the food industry because it can reduce the formation of free radicals and lipid peroxidation.

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Isaksen & Adler-Nissen (1997) it was proved in early studies that the addition of catalase could effectively inhibit the lipid oxidation of mayonnaise during storage. CAT has been widely used in the dairy industry to remove hydrogen peroxide from milk and inhibit the effect of oxidation on dairy flavor (Mir-Khan & Selamoglu, 2020). At present, research mainly focuses on controlling the oxygen content in the finished product packaging to control lipid oxidation (Mobina et al., 2022) but there is a lack of research on the inhibition of antioxidants on the oxidation of lipid- containing starchy foods.

In this study, pre-made α- instant rice was treated with CAT and antioxidant BHT and α-instant rice without antioxidant were used as the control group. The headspace- solid phase microextraction- gas chromatography- mass spectrometry (HS-SPME GC/MS) was used to analyze the changes of flavor components of α- instant rice during storage. By analyzing the indexes of α- instant rice such as hydrogen peroxide value (POV), malondialdehyde value (MDA), fatty acid value (FAV), electronic nose and colordifference, the influence of CAT on the lipid oxidation process of α- instant rice was discussed, which would provide reference for improving the quality of dehydrated and dried rice and prolonging its shelf life.

2 Materials and methods

2.1 Experimental materials

Rice, Daohuaxiang No.2 was purchased from wuchang city, Heilongjiang, China. Food grade catalase (CAT) and butylated hydroxytoluene (BHT) were purchased from Qilu Biotechnology Co., Ltd., Shandong, China. All chemicals used in this work were analytical grade.

2.2 Experimental methods

Preparation of α- instant rice

Under normal pressure, rice was steamed with water at the ratio of 1: 1.5 (w/ w) for 30 minutes. The standard for cooked rice was heating until there was no hard core in the inner center of the rice. The cooked rice was cooled to 25 ± 0.5 °C, soaked in the 0.012% CAT (w/w) solution for 15 min, taken out and drained, and then dried in an electric- heating blast drying oven at 40 ± 1 °C to prevent enzyme from being inactivated. Accelerated storage test was conducted according to the method of Huai- xiang et al. (2019). Through accelerated storage test, the shelf life of instant rice at 25 ± 0.5 °C was predicted. The α-instant rice was put into a sealed bag and stored in a thermostat at 50 ± 0.5 °C (1 day at 50 ± 0.5 °C is equivalent to 8 days at 25 ± 0.5 °C), and the quality changes of α- instant rice were observed during storage.

Determination of peroxide value of α- instant rice

The peroxide value could effectively reflect the degree of lipid oxidation (Zhang et al., 2021a). According to the method of Jensen et al. (2011), the following three solutions were prepared. 0.4g $BaCl_2 \bullet 2H_2O$ and 0.5 g $FeSO_4 \bullet 7H_2O$ were mixed in 50 ml of water, 100 μm filtered, and 2 ml of 10 mol/L HCl solution was added to obtain solution A. Solution B was 30% (w/w) of NH_4SCN. Solution C was CH_3OH: CHCl (1:1, v/v). Theferrous $Fe(SCN)_2$ solution was prepared, mixing A, B, and C solutions at the 1: 1: 98 (v/v/v) ratio. 1 g of the crushed sample was mixed with 5 mL of water and 10 mL of solution C to prepare the sample extract. The extracts was vortexed for 30 s and centrifuged (5000 g, 10 min). We transferred the lower extraction phase to a test tube, mixed with 1 ml of ferrous $Fe(SCN)_2$ solution, and let stand for 5 min. And used multipurpose spectrophotometer (Shimadzu, UV 2550, JPN) to measure absorbance at 500 nm with pure chloroform as blank.

The resulting standard curve was Y = 0.03346 x- 0.00147, $R^2 = 0.9998$.

$$POV(meq/Kg)=\frac{A\times K\times 0.5}{55.46\times m\times 2}\times 1000 \quad (1)$$

In Formula 1, POV is the peroxide value of the sample, meq/kg. A is the absorbance of the tested sample solution. K is the slope of the standard curve of Fe^{3+} . 55.46 is the atomic weight of iron. M is the weight of the sample, g. 0.5, is the molar ratio of O/Fe. 2 is the coefficient (meq/kg) of converting oxygen to POV. The result is accurate to 0.01.

Determination of fatty acid value of α- instant rice

Referencing Farhoosh et al. (2012), the research methods was slightly modified.5.00 g of the sample accurately was weighed into a conical flask, 50 mL of hexane was added and shaked them at 25 °C for 30 min. Left it for 10 minutes, then filtered. Added 2.5 mL phenolphthalein solution to 25 mL filtrate, and titrated with potassium hydroxide to pink, which could remain unchanged for 30 s. Recorded the number of milligrams of potassium hydroxide consumed to neutralize the fatty acid.

Determination of malondialdehyde value of α- instant rice

Refer to Grintzalis et al. (2013) with minor modifications. We accurately weighed 2.00 g of samples in a mortar, add 5 ml of 10% trichloroacetic acid solution, groung it into homogenate, then added 25 mL of 10% trichloroacetic acid solution, shaked well and centrifuged (9184 g, 10 min) them. After centrifugation, 4 mL of the supernatant was mixed with 4 mL of 0.6% (w/ w) thiobarbituric acid, and boiled in boiling water for 15 min. The sample solution was cool to 25 °C. The absorbance was measured at 450 nm, 532 nm, and 600 nm, respectively.

$$MDA/\mu mol=\frac{6.45\times(A_{450}-A_{600})-0.56\times A_{532}}{100\times m} \quad (2)$$

In Formula 2: A_{450} is the absorbance value at 450 nm. A_{532} is the absorbance value at 532 nm. A_{600} is the absorbance value at 600 nm. m is the mass, g.

Determination colordifference of α- instant rice

The colorimeter (Konica Minolta, CR-400, JPN) was used for the determination of samples. Where a* positive value indicates

the degree of red, and negative value indicates the degree of green. The value of b* positive value indicates the degree of yellow, and negative value indicates blue. The value of L* reflects the brightness, the larger value gives higher brightness. Whiteness (W) calculates according to the Formula 3:

$$W=100-[(100-L)^{*2}+a^{*2}+b^{*2}]^{1/2} \quad (3)$$

Determination electronic nose of α- instant rice

The 10.0 g sample was weighed, pulverized and put into a sample bottle and then sealed, standing for 30 min. And inserted into the electronic nose probe to determine the volatile flavor components. The data were processed by WinMuster software, and the principal component analysis (PCA) chart was obtained.

Determination HS-SPME GC/MS of α- instant rice

Headspace conditions: crushed the samples into a pulverizer, sieved them with a 0.15 mm sieve, 5 g of the screened samples was weighed, and put into a 20 mL headspace vials. 10 μL cyclohexanone- ethanol solution (0.789 μg/ mL) was added as the internal standard. Then sealed the bottle cap, and inserted the sampler equipped with a probe. When the sample was balanced in a magnetic stirrer with a water bath at 80 °C for 10 min, the probe was pushed out of the vial and extracted for 30 min. The extraction probe was quickly taken out and immediately inserted into the injection port of a GC instrument, followed by thermal desorption. GC Condition Referencing (Peng et al., 2020): the chromatography column was HP- 5MS 5%Phenyl Methyl Siloxane elastic time-sensitive capillary column (30 m× 0.25 mm × 0.25 μm).Temperature programmed: the column temperature was kept at 40 °C for 3 min, and then the temperature was raised to 250 °C at the rate of 5 °C/ min . The injection and detector temperature was maintained at 250 °C for 3 min. The carrier was high purity helium (99.999%).

2.3 Statistical analysis

All measurements were repeated three times of the same sample. The data were expressed as mean with standard deviation. Duncan's multiple range tests were used to identify significant statistical separation among the means at $p = 0.05$. Software SPSS 22.0 was applied in analyzing data and Origin 9.0 for plotting charts and integrating graphs.

3 Results and discussion

3.1 Analysis of peroxide value of α-instant rice during storage

The peroxide value, reflecting the degree of lipid oxidation of the fat-containing food, is used to determine whether a sample deteriorates because of oxidization (Pinzón-Martinez et al., 2022). As shown in Figure 1. Lipid oxidation in α- instant rice accumulates hydroperoxides during storage, producing a conjugated dienes which combines with oxygen to form a cyclic peroxide (Claudia & Giovanni, 2003), resulting in an increase of peroxide. The peroxide value in the control group reached 0.547 meq/kg at 21 days. The peroxide values in treatment groups were lower than that in the control group, which indicates that the selected antioxidants has the ability to inhibit lipid oxidation. The increase rates of the three groups of samples were in the order: blank control group > CAT group > BHT group. The reason for the analysis is that CAT reduces the enzyme activity and thus the ability to inhibit lipid oxidation under the condition of maintaining 50 ± 0.5 °C for a long time in the accelerated oxidation experiment. The results showed that although CAT was affected by temperature in the α- instant rice system, there was no significant difference ($p > 0.05$) between CAT and BHT, an antioxidant commonly used in food industry, in the overall anti-fatty acid oxidation effect.

3.2 Analysis of fatty acid value of α-instant rice during storage

The fatty acid value reflects the content of free fatty acids in α-instant rice during storage, and the change of free fatty acid reflects the deterioration degree of rice quality. The change of fatty acid value in pure storage is shown in Figure 2. Fatty

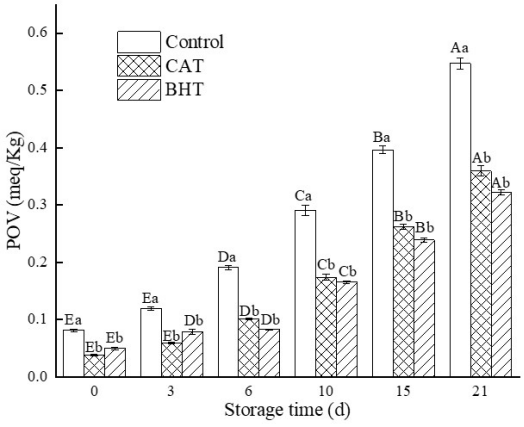

Figure 1. Hydrogen peroxide value of α- instant rice. Note: Different capital letters indicated significant differences of the same substance at different days ($p < 0.05$). Different small letters indicated significant differences among different substances on same days ($p < 0.05$).

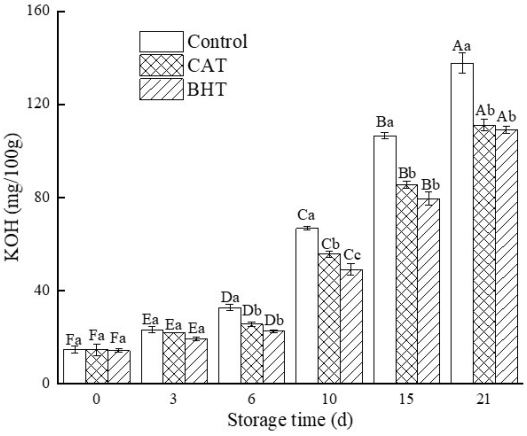

Figure 2. Changes of fatty acid value of α-instant rice. Note: Different capital letters indicated significant differences of the same substance at different days ($p < 0.05$). Different small letters indicated significant differences among different substances on same days ($p < 0.05$).

acids produced by decomposition of triglycerides contained in the α- instant rice were easily oxidized during storage. At the same time, fat was hydrolyzed by endogenous lipase or free fatty acid was produced under thermodynamic action (Nazeri et al., 2018), which leads to the increase of fatty acid value during the storage. In addition, the molecular structure of unsaturated fatty acid contains one or more unstable double bonds, which makes unsaturated fatty acids more easily oxidized (Rendón et al., 2014). On the 21st day of the accelerated test during storage, it was found that there was still no significant difference between the CAT and BHT treatment groups ($p > 0.05$), which was much lower than the control treatment group. Indicating that CAT might replace BHT in inhibiting the fatty acid oxidation of α-instant rice.

3.3 Analysis of malondialdehyde value during storage of α-instant rice

The results of the malondialdehyde values during storage are shown in Figure 3. Malondialdehyde is the product of lipid peroxidation caused by free radicals in plants, and its content could be used to measure the degree of lipid oxidation (Wu et al., 2013). With the increase of storage time, the content of malondialdehyde in the sample increased to varying degrees. After accelerated storage for 21 days, the malondialdehyde (MDA) value of CAT treatment group was the lowest, which is 0.00288 μmol/L, which is 4.3% lower than that of the BHT treatment group and 41.4% lower than that of the control group. This phenomenon might be due to the reduction of hydrogen peroxide by catalase, resulting in the reduction of secondary oxidation products produced by oxidative decomposition, and slow growth of malondialdehyde. Although temperature affected CAT, the CAT treatment group still showed the best inhibition effect on MDA. Therefore, CAT had great potential in the inhibition of lipid oxidation in α-instant rice.

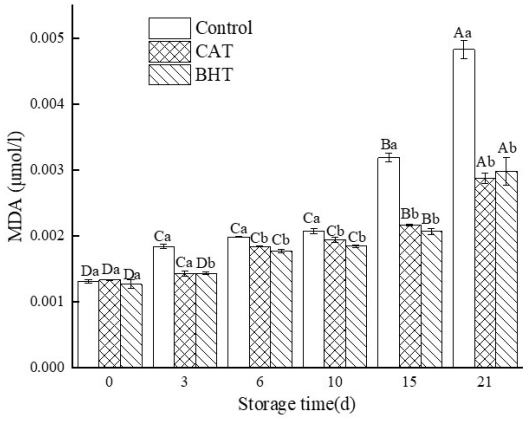

Figure 3. Changes of malondialdehyde value of α- instant rice. Note: Different capital letters indicated significant differences of the same substance at different days ($p < 0.05$). Different small letters indicated significant differences among different substances on same days ($p < 0.05$).

3.4 Colordifference- whiteness analysis of α-instant rice

The color of fresh α- instant rice was almost the same as that of normal fresh japonica rice. But the whiteness of α- instant rice decreases with storage time (Table 1). Rancidity occurred in the later stage of storage, leading to the yellow appearance of α-instant rice accompanied by a bad flavor. At the same time, the fat itself contained natural dyes, and the phospholipids are easily oxidized to produce dark substances during storage (van Nieuwenhuyzen, 2015). In addition, Liu et al. (2021) also found that carbonyl compounds generated by automatic oxidation of carbohydrates and lipids in rice during storage reacted with amino compounds provided by protein in rice, which leads to browning of rice. Therefore, with the increase of storage time, the whiteness of α- instant rice decreased gradually.

During storage, the whiteness of treatment group was significantly lower than that of the control group ($p < 0.05$). Yehye et al. (2015) found that the main factor for BHT delaying the oxidation reaction during storage was that H^+ provided by BHT inhibited the production of free radicals, thus inhibiting the lipid oxidation. On the other hand, CAT catalyzesd the decomposition of hydrogen peroxide in the matrix and inhibits the production of free radicals. Besides, Gligorijević et al. (2021) found that CAT contain peptides with antioxidant activity. Therefore, CAT could catalyze the decomposition of hydrogen peroxide into water and molecular oxygen in the α- instant rice system to inhibit the generation of free radicals, and at the same time provide peptides with antioxidant activity to slow down free radical induced oxidation. Therefore, CAT treatment was more beneficial to alleviating the decrease of α- instant rice whiteness caused by lipid oxidation.

3.5 Analysis of electronic nose of α- instant rice during storage

The electronic nose has many advantages in simple operation, high speed, less sample pretreatment, low cost, etc., and is widely

Table 1. Changes of whiteness of α- instant rice during storage.

	Storage time/d	Control	BHT	CAT
whiteness	0	48.70 ± 0.69^{Ba}	51.09 ± 0.80^{Aa}	52.09 ± 0.94^{Aa}
	3	44.85 ± 0.47^{Bb}	49.27 ± 0.12^{Aa}	48.45 ± 0.38^{Ab}
	6	36.14 ± 0.82^{Bc}	45.11 ± 0.35^{Ab}	44.85 ± 0.39^{Ac}
	10	33.48 ± 0.64^{Bd}	39.61 ± 0.32^{Ac}	39.50 ± 0.44^{Ad}
	15	29.01 ± 0.20^{Be}	36.89 ± 0.05^{Ad}	37.02 ± 0.63^{Ae}
	21	20.75 ± 0.63^{Cf}	33.31 ± 0.39^{Be}	35.40 ± 0.38^{Ae}

Note: Different capital letters in the same line indicated significant differences among different substances ($p < 0.05$). Different lowercase letters in the same column indicated significant differences among different days ($p < 0.05$).

used in the rapid detection and food quality control (Ghasemi-Varnamkhasti et al., 2018). Miao et al. (2017) used electronic nose to distinguish the storage time of canned foods. The longer time it is stored, the greater the change of volatile flavor compounds remains in canned food, the larger the distance exists between the principal components in PCA. Therefore, the electronic nose could intuitively compare the quality differences of samples stored in different times.

Figure 4 shows the influence of different treatment groups on electronic nose PCA of α-instant rice during storage. In the three groups of samples, the contribution rates of the first principal component were 89.18%, 71.68% and 70.26% respectively, and the contribution rates of the second principal component were 8.53%, 14.35% and 20.53% respectively. The cumulative contribution rates of the three groups of samples were 97.71%, 90.79% and 86.03%, respectively, which were greater than 85%. This means these two principal components could basically represent the main information characteristics between these samples. Therefore, the electronic nose could effectively distinguish the main volatile components of α- instant rice in different treatment groups during storage. As shown in Figure 4A, there is a distance between the main ingredients, which indicates that lipid oxidation has a great influence on the flavors of α-instant rice during storage. The dense distribution of main ingredients shows CAT has a good effect on inhibiting rancidity of α- instant rice and keeping the original flavors (Figure 4B). Shown as Figure. 4 C, there is an obvious change in the main flavors component on 21 days, It could be considered that BHT treatment group could only inhibit the lipid oxidation of α- instant rice in short time. The comprehensive results showed that compared with BHT treatment group, the inhibition effect of CAT on the rancidity of α- instant rice was greatly improved, which was beneficial to keep the aroma of α- instant rice itself.

3.6 HS-SPME GC/MS results analysis of α-instant rice during storage

Based on the PCA analysis results of electronic nose, the volatile flavor substances of α-instant rice during storage were further analyzed (Figure 5). The results of HS-SPME GC/MS analysis showed that the volatile flavor compounds were mainly aldehydes, ketones, esters, alcohols and various hydrocarbons during storage. Among them, aldehydes were on the rise as time, which indicates that aldehydes were the main factor caused the flavors changes of α- instant rice during storage. Biao et al. (2019) confirmed this point. A small amount of aldehydes could make rice produce fruit flavors, but with the increase of the content, it would produce unpleasant smell. The main aldehydes, including hexanal, nonanal and sunflower aldehyde, showed a significant increase in the blank control group. Zeng et al. (2008) suggested that aldehyde products were produced by oxidation of oleic acid. Hidalgo & Zamora (2019) found that the oxidation product of linoleic acid, trans-2-octenal, and the degradation product of phenylalanine, benzaldehyde, were the main factors that caused the bitter taste of rice. Therefore, the aldehydes produced by rancidity had a bad influence on the flavor of α- instant rice, while the CAT treatment group had a good inhibitory effect on aldehydes.

Volatile products of ketones mainly come from fat oxidation and amino acid degradation (Narváez-Rivas et al., 2014). Ketones contribute to the formation of food flavors, but fatty ketones produced by the automatic oxidation of unsaturated fatty acids will produce bad flavors. In this study, the volatile metabolites of ketones increased first and then decreased with the passage of time, while the changes of treatment group tended to be stable. CAT treatment group had the greatest influence on ketones, and the overall trend of fitone was the most significant, which suggests that fitone might be the main volatile product of ketones caused flavor changes.

Among the volatile products of esters, diisobutyl phthalate, ethyl palmitate and methyl palmitate, which are the main components, showed a downward trend, while the decline of esters in the CAT treatment group was slower. Alcohols is another volatile flavor compound. Most of the Special aroma of rice come from alcohols, but alcohol produced by lipid oxidation, such as 1- octene -3- ol, has musty smell. According to Refsgaard et al. (1999), alcohols with 3-8 carbon atoms might be produced by lipid peroxidation and hydroperoxide decomposition of fatty acids. The data (Figure 5) shows that heptanol and 1- octene -3- ol are the main inhibitory alcohols, which indicates that they might be the main alcohol in the lipid oxidation products of α- instant rice. Most hydrocarbons, especially branched- chain alkanes from lipids are the aromatic source of faint scent and sweet flavors. The quality of α- instant rice begins to decline

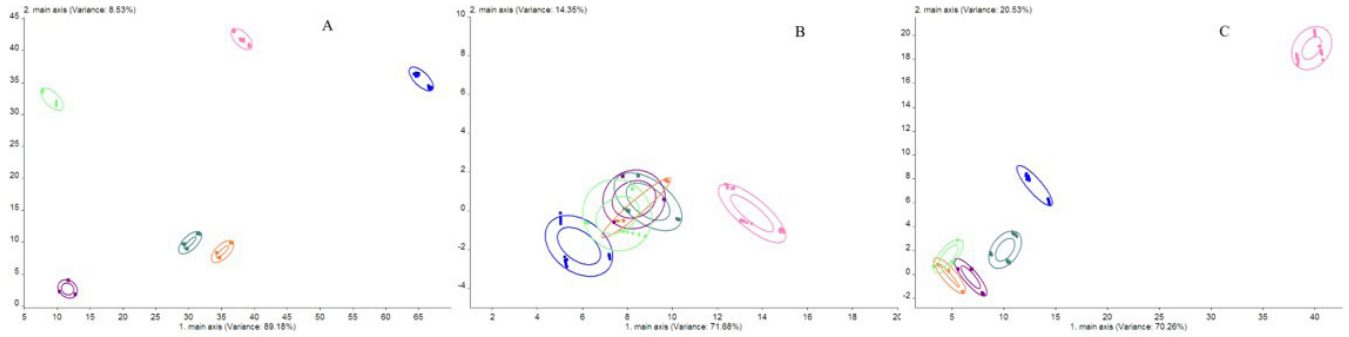

Figure 4. Volatile PCA of α- instant rice during storage. Figures 4A, 4B and 4C were PCA diagrams of volatile components of blank control group, CAT treatment group and BHT treatment group, respectively.

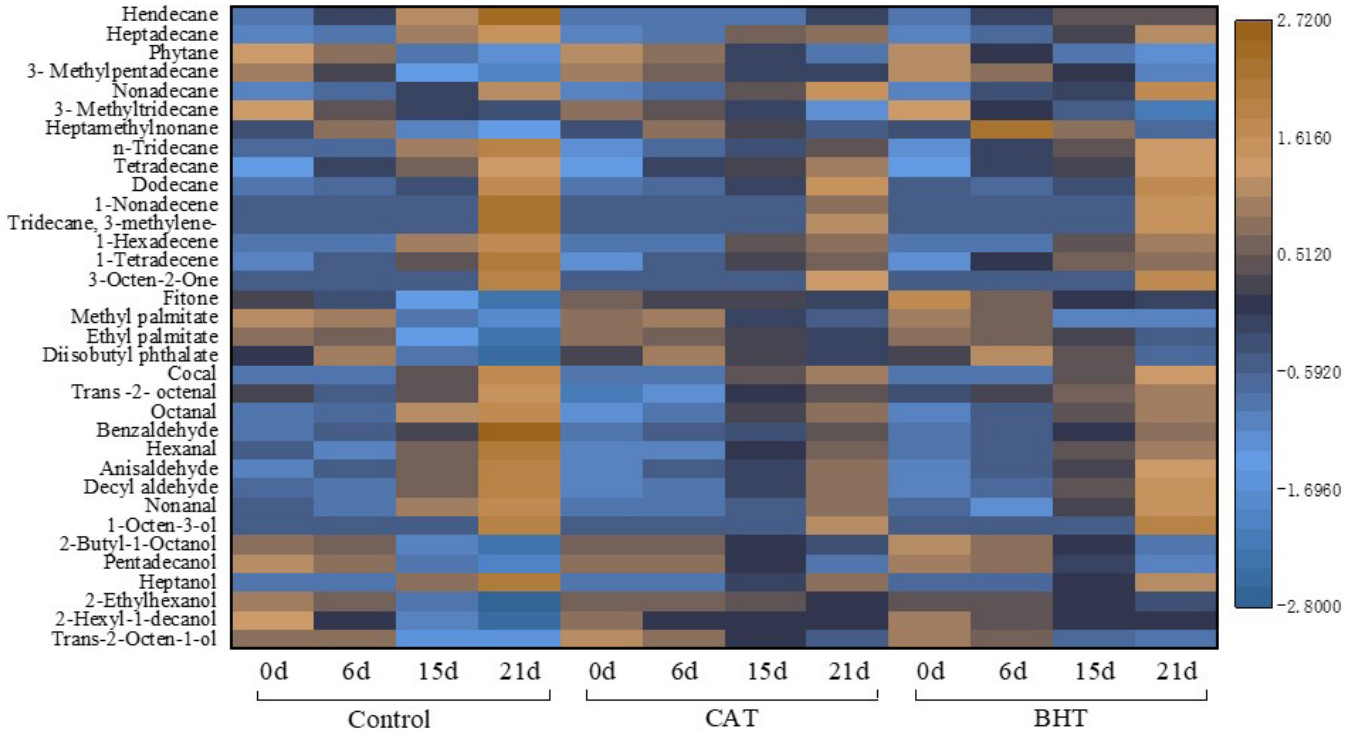

Figure 5. Heatmap: changes of flavor substances of α-instant rice during storage heat picture.

when the hydrocarbon substances drops. The result in Figure 5 shows that that CAT treatment group has a better effect on the maintenance of hydrocarbon in the α-instant rice than the BHT treatment group.

To sum up, according to the analysis in Figure 5. Compared with BHT treatment group, CAT further reduced the free radical induced lipid oxidation degree of α- instant rice during storage, and weakened the formation of free fatty acids during storage. Therefore, CAT more could effectively inhibit the lipid oxidation rate in α-instant rice, and reduce the proportion of lipid oxidation products such as benzaldehyde, nonanal, 1- octene -3- ol, heptanol and trans -2- octenal during storage. And the content of fitone, hydrocarbon and ester flavor compounds during the storage period of the α- instant rice was effectively maintained. The results showed that the α-instant rice treated by CAT was more beneficial to reducing the rancidity of rice during storage and keeping the original flavor of α- instant rice.

4 Conclusion

In this study, catalase was used to inhibit the degree of lipid oxidation in α -instant rice. During storage, α- instant rice treated with catalase was not only more effectively suppressed the lipid oxidation of rice, but also had more advantages in maintaining the whiteness during storage. At the same time, catalase could better maintain the flavor of the cooked rice during storage and reduce the bad flavors substances produced by rancidity in α- instant rice. It had the best effect of inhibition on the main odor sources such as benzaldehyde, nonanal, 1- octene -3- ol, heptanol and trans -2- octenal, and had a positive maintenance effect on fitone, hydrocarbons and esters which were aromatic to the α- instant rice. To sum up, the application of catalase in α-instant rice could reduce the degree of lipid oxidation during storage, maintain the original whiteness and the flavor of the rice, and effectively improve the quality of the rice. This study provided a new theoretical basis for the development and quality improvement of α- instant foods.

Conflict of interest statement

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Acknowledgments

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