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Characterization of flavor quality deterioration of postharvest Chinese bayberry (*Myrica rubra* cv. Dongkui) at different storage temperatures

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ABSTRACT

The present study characterized the flavor quality deterioration of 'Dongkui' Chinese bayberry (*Myrica rubra*) stored at different temperatures. The fruit were stored at 0 °C, 4 °C, 10 °C and 20 °C for 12 d. The quality deterioration was characterized by combining human sensory evaluation and artificial sensory evaluation, which included e-nose and e-tongue. The sensory quality deterioration was characterized in the aspects of taste strength, bitterness, and odor, in which the odor was found to be most related to the sensory quality deterioration. In the artificial sensory evaluation, 12 e-nose sensors showed differential responses with the elongation of storage time and temperature. Based on the human sensory evaluation and the response values of the 12 e-nose sensors, the stored fruit were classified, with those stored at 20 °C for 2 d or longer and those stored at 10 °C for 7 d or longer classified as "off-flavored" fruit. The artificial neural network (ANN) model verified the classification. A total of 27 volatile compounds were identified by HS-SPME–GC–MS. Eight differential volatiles were screened out with $p < 0.05$ and variable importance in projection (VIP) > 1 by orthogonal partial least squares discriminant analysis (OPLS-DA). Among these volatiles, (*Z*)-3-hexenoic acid methyl ester and (*E*,*E*)-2,4-hexadienal decreased, while octanoic acid, acetaldehyde, ethanol, phenylethyl alcohol, 2-pentylfuran and benzaldehyde increased in the deteriorated fruit.

1. Introduction

Chinese bayberry (*Myrica rubra* Sieb. et Zucc.) is a native fruit in south China with high economic value (Li et al., 2022a), which is popular for its attractive appearance and delicious taste. Chinese bayberry has a unique appearance with juice sacs exposed without protection of pericarp. Thus, the fruit is vulnerable to mechanical damage and pathogens, which leads to decay. According to previous studies, disease incidence of Chinese bayberry reaches more than 40% after 8 days' storage at 1 °C (Chen et al., 2013), and fruit decay rate is about 40% at the 3rd day of storage at 20 °C (Wang et al., 2010). In addition, Chinese bayberry has a vigorous metabolism in the postharvest stage, which accelerates the quality deterioration.

In the postharvest stage of Chinese bayberry, several physiochemical characteristics change significantly with increased storage time. Excessive water loss due to strong fruit respiration and adverse environment leads to collapse and softening of the pulp (Zhang et al., 2010; Yang et al., 2013; Zheng et al., 2021). The cohesiveness, resilience and chewiness also tend to change negatively during storage (Chen, Li, 2009). For the soluble sugars, sucrose content tends to decrease, while fructose and glucose contents remain stable with increased storage time (Zhang et al., 2005; Zhang et al., 2010). The total organic acids content decreases dramatically during storage (Xi et al., 1993).

Correspondingly, sensory quality changes with the deterioration of Chinese bayberry. A sensory panel considered the color, skin wetness, touch, taste, odor and maturation. The panel showed that the preference scores of fruits decreased dramatically after 6 days of storage at 4 °C (Li et al., 2015). Undesirable flavors such as bitterness and unpleasant odors

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Table 1
Human sensory evaluation scheme of Chinese bayberry.

Attributes	Evaluation criteria		
	I	II	III
Taste strength	Flavorful	Medium	Tasteless
Bitterness	Imperceptible	Slightly perceptible	Obviously perceptible
Odor	Typical aroma of fresh fruit	Fermented flavor	Fermented and rotten smell

occur in the stored fruit (Chen et al., 2010). Alcoholic taste is one of the unpleasant odors, which is positively correlated with decay rate (Di et al., 2012) and might be induced under adverse CO_2 concentrations in air (Wang et al., 2011). A linear regression of e-nose response value and physical-chemical indexes (firmness, color index, pH, total soluble solids (TSS), reduced sugar content) has been built (Li et al., 2015), indicating that aroma volatiles might contribute to the fruit quality deterioration.

Several aroma volatiles change in Chinese bayberry with increased storage time, measured using headspace solid-phase microextraction with gas chromatography-mass spectroscopy (HS-SPME–GC–MS) analysis. Hexanal and other associated aldehydes decrease during storage, which might be responsible for the decrease of freshness. Ethanol, ethyl acetate, and acetic acid increase during storage, which might contribute to off-flavor accumulation in stored fruit. Other compounds such as terpinen-4-ol, methyl acetate, and γ-terpinene also increase with increased storage time (Fang et al., 2020). However, the correlation between volatile compounds and postharvest flavor sensory deterioration has not been established yet.

The present study monitored the quality changes of Chinese bayberry 'Dongkui' during storage at 0 °C, 4 °C, 10 °C and 20 °C. The flavor quality of healthy fruit was evaluated by combining sensory evaluation with e-nose and e-tongue artificial sensory evaluation. The volatile compounds were determined by HS-SPME–GC–MS analysis. According to the sensory evaluation results, the stored fruit were divided into "off-flavored" and "consumer acceptable" blocks based on human sensory value and e-nose sensory value scales. This classification was verified by an artificial neural network (ANN) model. Based on the classification, orthogonal partial least squares discriminant analysis (OPLS-DA) was carried out to further identify the characteristic volatile compounds associated with flavor quality deterioration. Differential volatiles potentially contributing to the freshness and off-flavor of postharvest 'Dongkui' fruit were identified with $p < 0.05$ and variable importance in projection (VIP) > 1.

2. Materials and Methods

2.1. Materials

The Chinese bayberry 'Dongkui' fruit were harvested in June 20, 2022 from an orchard in Xianju City, Zhejiang Province. The fully ripe fruit without visible mechanical damage or disease were selected, and stored in refrigerators equipped with temperature and humidity monitoring and controlling systems at 0 °C, 4 °C, 10 °C, and 20 °C. The pulp of fruit was collected, frozen immediately in liquid nitrogen and stored at –80 °C for later use. All the frozen samples were ground into fine powder before analysis.

2.2. Decay rate

Fruit with obvious appearance of decay were recorded on each sampling day during the experimental period. Decay rate was calculated as the ratio of the decayed fruit number to the total fruit number. Two hundred and fifty fruit were used for the decay rate calculation for each treatment.

2.3. Color analysis

The surface color of fruit was measured with a Hunter Lab Mini Scan XE Plus colorimeter (Hunter Associates Laboratory, Inc., Reston, VA). Four measurements were made at four evenly distributed equatorial sites of each fruit. Nine single fruit repetitions were done for each treatment. The CIE 1976 $L^* a^* b^*$ color scale was adopted, and the raw data were obtained as L^*, a^*, and b^*. Color index of red grapes (CIRG) was applied for the calculation of fruit color according to the following formula: $CIRG = (180 - H) / (L^* + C)$, while $C = (a^2 + b^2)^{0.5}$ and $H = \arctan(b^* / a^*)$ (Carreño et al., 1995; Zhang et al., 2005).

2.4. Total soluble solids (TSS) and titratable acidity (TA) analysis

TSS contents were determined with a PR101-a refractometer (Atago, Japan) following the manufacturers' protocols, with nine fruits for each treatment, and two measurements per fruit. For TA determination, 1 g of the grounded fresh pulp was added with 25 mL ddH_2O, and titrated with 0.1 mol/L NaOH using a T890 automatic titrator (Hanon Instruments Co. Ltd., China) with pH 8.2 as the titration end-point (Cao et al., 2019). Three repetitions were done for each treatment, with three fruits in each repetition.

2.5. Human sensory evaluation

The human sensory evaluation was carried out at 0 d, 2 d, 3 d, 4 d and 7 d of storage. The fruit without visible mechanical damage or disease were selected for the human sensory evaluation. Thirty volunteers (ranging in age from 22 to 38 years old, half male and half female) were invited to participate in the human sensory assessment at room temperature. Before evaluation, necessary explanations of the evaluation scheme and criteria were made to all the volunteers. Each volunteer was asked to evaluate three fruits of each group at each time. The sensory quality of fruit was evaluated based on the following aspects: (a) visual observation of the appearance; (b) odor estimation of the fruit by human nose; (b) taste estimation of the fruit. The evaluation criteria for sensory attributes are shown in Table 1. After the evaluation, the volunteers were asked to give an overall rating on consumer acceptance of the stored fruit according to the following criteria: (I) with good edible and commodity quality and scored as 1; (II) with medium edible and commodity quality and scored as 2; (III) still edible but the commodity quality was not good and scored as 3; (IV) lost edible quality and scored as 4.

2.6. E-tongue analysis

E-tongue analysis was carried out using an Astree II V5 electronic-tongue (Alpha MOS Company, France). In brief, 1 g of the grounded fresh pulp was put into a sample cup and suspended with 20 mL distilled water. The probe, consisting of seven sensors, AHS (sensitive to sour flavor, abbreviating as "sour"), CTS (salt), NMS (umami), CPS (complex), ANS (sweet), SCS (bitter) and PKS (complex), was dipped into the sample suspension, and the response values were collected for 120 s with time intervals of 1 s. The sum of the response values at 120 s of each sample was used for further analysis. Three biological repetitions were done with the mixture of three fruit in each repetition, and three analyses were done for each repetition.

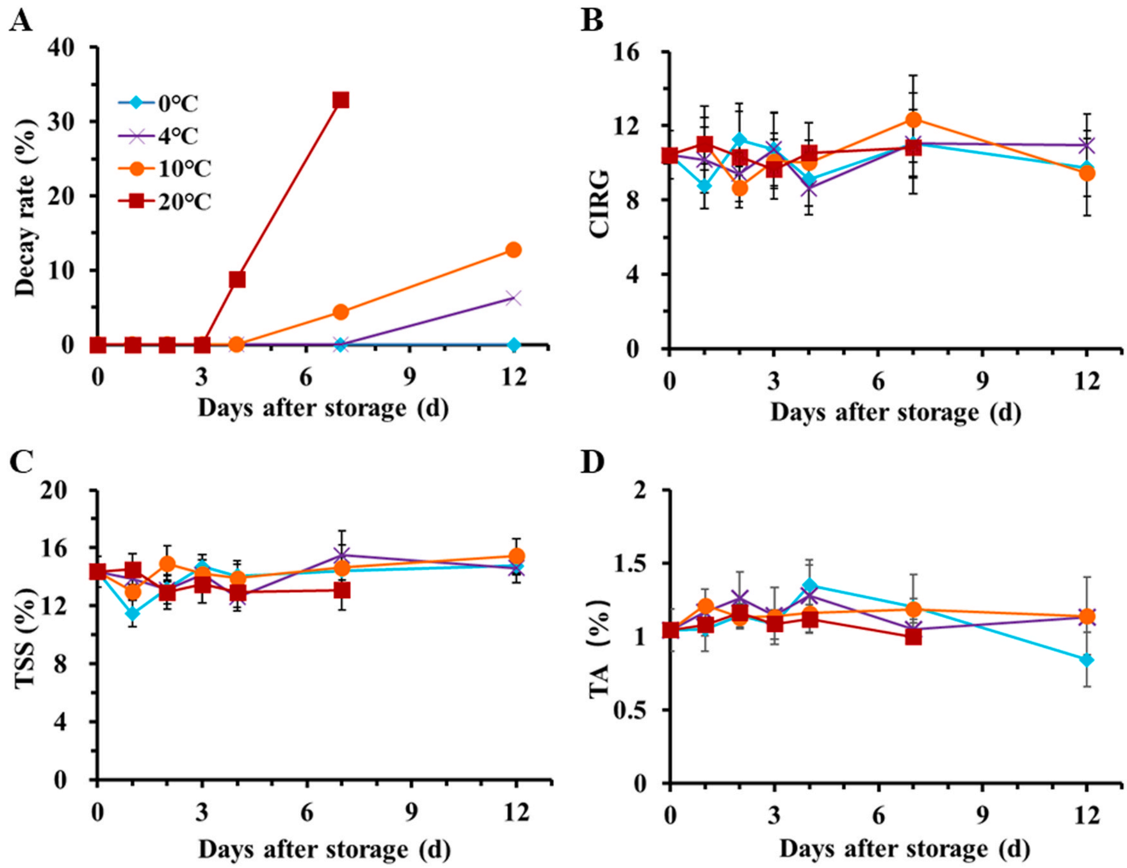

Fig. 1. Dynamic changes of decay rate (A), CIRG value (B), TSS (C) and TA contents (D) of Chinese bayberry 'Dongkui' fruit stored at different temperatures.

2.7. E-nose analysis

E-nose analysis was carried out using a FOX4000 electronic-nose (Alpha MOS Company, France) equipped with 18 sensors (Table S1). In brief, 3 g of ground fresh pulp were mixed with 5 mL of saturated NaCl solution. Then, 2 mL of the mixture were obtained, put into a 10-mL glass vial and sealed. The samples were heated at 40 °C for 30 min. Then, 2 mL of the headspace above the sample were injected into the injection chamber of e-nose. The analysis parameters were as follows: acquisition duration of 120 s, equilibration time of 300 s, air flow speed of 150 mL/min. The maximum response value of each sample was used for further analysis. Three biological repetitions were done with the mixture of three fruit in each repetition, and three analyses were done for each repetition.

2.8. HS-SPME–GC–MS analysis

The identification and quantitation of volatile compounds was carried out according to Cheng et al. (2016) with slight modification. In brief, 3 g of the ground fresh pulp were mixed with 5 mL of saturated NaCl solution. Then, 2 mL of the mixture were put into a 10-mL glass vial and sealed. Cyclohexanone (471.6 μg/mL, 10 μL in each sample) was used as an internal standard. Solid-phase microextraction (SPME) of volatiles was carried out using a fiber coated with 50/30 μm divinylbenzene/Carboxen™/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco, Bellefonte, PA) at 45 °C for 20 min. Chromatographic analysis was carried out with an Agilent 7890A–5975 C GC–MS system (Agilent, Santa Clara, CA) equipped with a DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm; Agilent J & W, Folsom, CA), using high-purity helium (99.999%) as carrier gas at constant flow rate of 1 mL/min. The inlet temperature was set to 240 °C and the samples were desorbed in splitless mode. The column heating procedure was as follows: the column was programmed to start at 40 °C for 5 min, heated to 145 °C at 3 °C/min, and then to 240 °C at 5 °C/min and then kept at 240 °C for 4 min.

The mass spectrometry parameters were as follows: full scan mode acquisition, EI, electron bombardment energy of 70 eV, interface temperature of 280 °C, ion source temperature of 230 °C, quadrupole temperature of 150 °C, scan mass range of *m/z* 45–350, scan frequency of 4.58 scans/s. Volatiles were identified by computer matching with the reference mass spectra of the MS library of NIST 11/Wiley 7.0. The identities of most of the volatiles were then confirmed by comparison with authentic standards. The abundance of the compounds was semi-quantitatively determined by comparing the peak area of each compound with that of the internal standard cyclohexanone (Zhu et al., 2020). Three biological repetitions were done with a mixture of three fruit in each repetition.

2.9. Data analysis

The correlation analysis was conducted with Pearson correlation using corrplot package in R 4.1.2. The heatmap was generated using TBtools software (Chen et al., 2020). Principal component analysis (PCA) and OPLS-DA were completed on SIMCA software (V14.1, Umetrics, Umeå, Sweden). The differences between groups were compared by Student's *t*-test. ANN was conducted by Matlab online (https://matlab.mathworks.com), by applying back-propagation neural network (BPNN), and the model was trained by BFGS method.

3. Results

3.1. Changes of decay rate, fruit color, TSS and TA of fruit stored at different temperatures

In the 12 days of storage, no fruit decay happened in the 0 °C group.

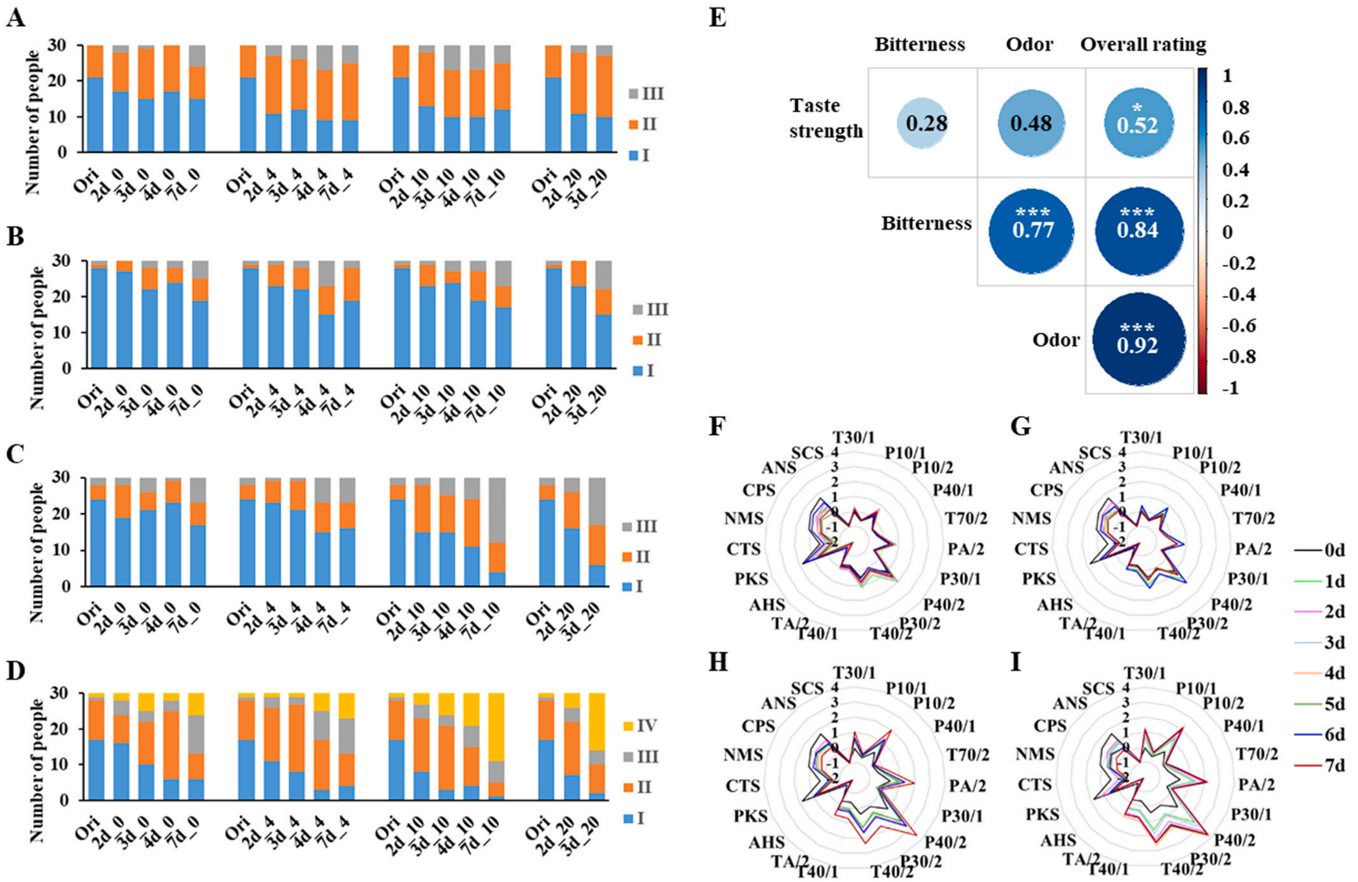

Fig. 2. Sensory evaluation of Chinese bayberry 'Dongkui' fruit stored at different temperatures. Ori, fresh-picked fruit (0 d). A, taste strength; B, bitterness; C, odor; D, overall rating of consumer acceptance; E, heatmap of correlations between different human sensory attributes, * and *** indicated significant difference with $p < 0.05$ and $p < 0.001$ respectively; F-I, radar chart on e-nose and e-tongue responding characteristics of 0 °C, 4 °C, 10 °C and 20 °C group. The representations of I, II, III, and IV of different attributes were as referred to in Table 1.

The decay rate increased with the increase of storage temperature. In the 4 °C group, no fruit decay happened in the 7 day's storage, and the decay rate increased to 6.21% in 12 d. In the 10 °C group, no fruit decay happened in the 4 day's storage, but the decay rate increased to 12.79% in 12 d. In the 20 °C group, fruit decay began to happen on the 4th day of storage and increased to 32.95% in 7 d, and then the fruit deteriorated quickly (Fig. 1 A). Thus, low storage temperature of 0 °C was most effective in preventing fruit decay of 'Dongkui' bayberry, which was consistent with previous studies (Xi et al., 1993; Chen et al., 2013).

The dynamic changes of CIRG value, TSS and TA contents of fruit showed irregular fluctuation, and no significant difference can be seen among different groups (Figs. 1B, 1 C and 1D), indicating that these quality indicators were stable and rarely affected by storage temperature in the short-term postharvest storage. The postharvest changes of color, TSS and TA of Chinese bayberry were related to the harvest maturity. The fruit with high maturity tend to keep stable on these quality indexes, while those with lower maturity tend to have greater color change and TA decrease in the postharvest period (Yang et al., 2013).

3.2. *Sensory characteristics of fruit stored at different temperatures*

3.2.1. *Human sensory evaluation*

The decayed fruit were discarded, and those without visible mechanical damage or disease were selected for the human sensory evaluation. The proportion of sensory evaluators in each sensory attribute degree is shown in Fig. 2A. The flavor quality deterioration of Chinese bayberry 'Dongkui' was characterized by changes of taste strength, bitterness and odor.

The strength of taste flavor was lower in 4 °C, 10 °C and 20 °C groups than that in 0 °C group during the storage (Fig. 2A), indicating that low temperature was effective in maintaining the flavor of 'Dongkui' bayberry. This is inconsistent with stability of TSS and TA contents, which indicated that the postharvest taste strength reduction of 'Dongkui' Chinese bayberry in the short storage period were not attributed to the TSS and TA reduction. According to a previous study, the taste perception such as sweetness could be affected considerably by volatiles (Fan et al., 2021). Thus, it was deduced that the changes of some quality attributes other than TSS and TA might lead to the taste strength reduction of short-term stored 'Dongkui' Chinese bayberry.

With the extension of storage time, the flesh bitterness became perceptible, but there was no significant difference among different temperatures (Fig. 2B). The bitterness of Chinese bayberry is deduced to be related to the total polyphenols' content (Yu et al., 2018), which increases with increased storage time (Zhang et al., 2008). However, further study is required for exploring the metabolites contributing to the bitterness of Chinese bayberry fruit.

The odor changed significantly during the storage. Unpleasant odors including fermented flavor and rotten smells developed with the extension of storage time, especially in those fruit stored at 10 °C and 20 °C (Fig. 2C). The Chinese bayberry develops alcoholic odor easily at high temperature due to the production of ethanol by exuberant respiratory metabolism. The bayberry juice produced at room temperature has a strong "fermented" flavor compared to that produced at 4 °C (Cheng et al., 2021).

Ultimately, the sensory evaluators were asked to give an overall rating on marketability and edibility qualities of the fruit. More than

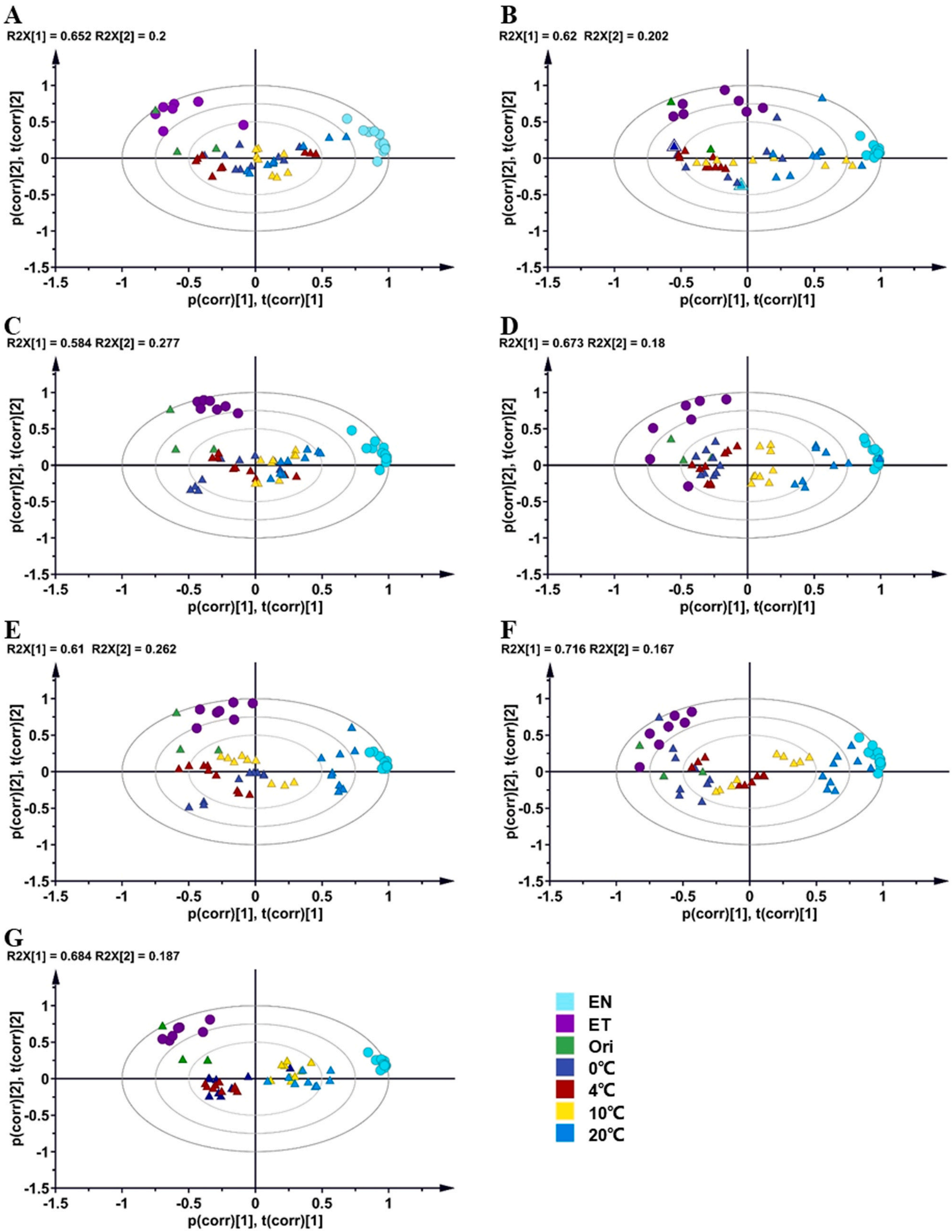

Fig. 3. Bi-plot of PCA analyses on e-tongue and e-nose sensory evaluation. EN, e-nose sensors; ET, e-tongue sensors; Ori, fresh-picked fruit (0 d). A, 1 d; B, 2 d; C, 3 d; D, 4 d; E, 5 d; F, 6 d; G, 7 d.

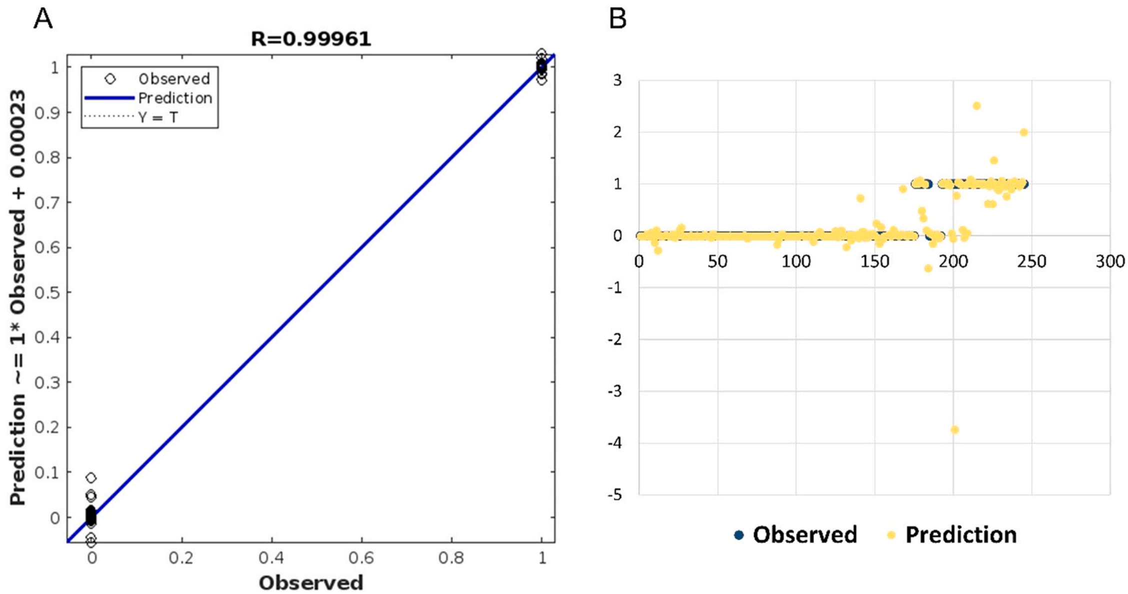

Fig. 4. The artificial sensory evaluation (ANN) model with two hidden layers for prediction of sensory quality of 'Dongkui' fruit. A, scatter plots of prediction and observed data; B, representative details of prediction and observed data.

Table 2
Identification of volatile compounds by HS-SPME–GC–MS analysis.

Code	Compounds	Flavor Description[a,b]	Formula
	Aldehydes		
A1	Acetaldehyde[2]	floral, green apple	C_2H_4O
A2	Hexanal[1−7]	grass, tallow, fat	$C_6H_{12}O$
A3	(*E*)-2-Hexenal[1−5,7]	apple, green	$C_6H_{10}O$
A4	(*E*,*E*)-2,4-Hexadienal[1−3]	green	C_6H_8O
A5	Benzaldehyde[2,4,6]	almond, burnt sugar	C_7H_6O
A6	Octanal[1−5,7]	fat, soap, lemon, green	$C_8H_{16}O$
A7	Nonanal[1−7]	fat, citrus, green	$C_9H_{18}O$
A8	3,5-Dimethylbenzaldehyde	almond	$C_9H_{10}O$
	Alcohols/ Terpenols		
B1	Ethanol[2−3,5]	sweet	C_2H_6O
B2	Linalool[2−4,6−7]	flower, lavender	$C_{10}H_{18}O$
B3	Phenylethyl alcohol[2,4−6]	honey, spice, rose, lilac	$C_8H_{10}O$
	Esters		
C1	(*Z*)-3-Hexenoic acid methyl ester[1−7]	earthy sweet, slightly fruity	$C_7H_{12}O_2$
C2	Methyl benzoate[1−3,5,7]	almond, burnt sugar	$C_8H_8O_2$
C3	3-Nonenoic acid methyl ester[1,3,5]	oil, fat	$C_{10}H_{18}O_2$
	Terpenes		
D1	Limonene[1−3,6]	citrus, mint	$C_{10}H_{16}$
D2	α-Pinene[1−3,6−7]	pine, turpentine	$C_{10}H_{16}$
D3	(*E*)-β-Ocimene[2−7]	sweet, herb	$C_{10}H_{16}$
D4	β-Elemene[1−4,6]	herb, wax, fresh	$C_{15}H_{24}$
D5	Isocaryophyllene[1,3,5,7]	Wood	$C_{15}H_{24}$
D6	Alloaromadendrene[1,3,5−6]	Wood	$C_{15}H_{24}$
D7	β-Caryophyllene[1−7]	wood, spice	$C_{15}H_{24}$
D8	Humulene[1−6]	Wood	$C_{15}H_{24}$
D9	γ-Muurolene[3,6]	herb, wood, spice	$C_{15}H_{24}$
D10	Isoaromadendrene epoxide[1,3]	hawthorn like smell	$C_{15}H_{24}O$
	Others		
E1	2-Pentylfuran[2−3,5,7]	butter, floral, fruit, green bean	$C_8H_{14}O$
E2	Octanoic acid	sweat, cheese	$C_{10}H_8$
E3	Caryophyllene oxide[1−7]	herb, sweet, spice	$C_{15}H_{24}O$

a, http://www.flavornet.org/flavornet.html; b, https://www.femaflavor.org/flavor-library. [1], Cheng et al., 2015a; [2], Cheng et al., 2015b; [3], Cheng et al., 2016; [4], Cheng et al., 2021; [5], Fang et al., 2020; [6], Kang et al., 2012; [7], Wu et al., 2019

50% of volunteers regarded that the fruit lost their edibility in 3 d at 20 °C, and this could be extended to 7 d when stored at 10 °C. For those stored at 0 °C and 4 °C, the edibility quality of the fruit after 7 d storage remained acceptable to more than 70% of volunteers, but more than 50% of volunteers thought that the fruit had lost the commodity value (Fig. 2D).

The correlation among sensory attributes was calculated by Pearson correlation analysis and the results are shown in Fig. 2E. The sensory attributes of bitterness ($|r|$=0.78) and odor ($|r|$=0.92) were highly correlated with the consumer's overall rating scores ($p < 0.05$), while the correlation between the taste attribute and consumer's overall rating score was relatively low ($|r|$=0.52, $p < 0.05$). Among the sensory attributes, the odor showed the highest correlation with the consumer's overall rating score, and thus might play an important role in the general sensory quality of 'Dongkui' fruit in postharvest. The finding that odor has a close relationship with customers' purchase decision has also been verified in other fruits, such as grape (Cefola et al., 2018), watermelon (Mendoza-Enano et al., 2019), and rambutan (González González et al., 2016).

3.2.2. *Artificial sensory evaluation*

In the 18 sensors of e-nose, 12 sensors (T30/1, P10/1, P10/2, P40/1, T70/2, PA/2, P30/1, P40/2, P30/2, T40/2, T40/1, TA/2) were responsive to the headspace volatiles of 'Dongkui' fruit. All the 7 e-tongue sensors (SCS, ANS, CPS, NMS, CTS, PKS, AHS) were responsive to the suspension of 'Dongkui' bayberry pulp. Thus, the response values of these 19 sensors were applied for the artificial sensory characterization of 'Dongkui' bayberry (Table S2). The radar chart of normalized response values showed the general response characteristics of the 19 sensors. In 0 °C and 4 °C groups, the response value of the 12 e-nose sensors remained stable in the whole storage period. In 10 °C and 20 °C groups, the response value of all the 12 e-nose sensors showed trends of increase with increased storage time, with the increase higher in the 20 °C group than in the 10 °C group. On the contrary, the response values of e-tongue sensors remained stable and fluctuated irregularly with the elongation of storage time, and no significant difference was found among different storage temperatures (Fig. 2F–I). These results were in line with the result that TSS and TA remained stable during storage at 0 °C, 4 °C, 10 °C and 20 °C, and in line with the result of human sensory evaluation that the odor changed more significantly than the taste with increased storage time and increased temperature.

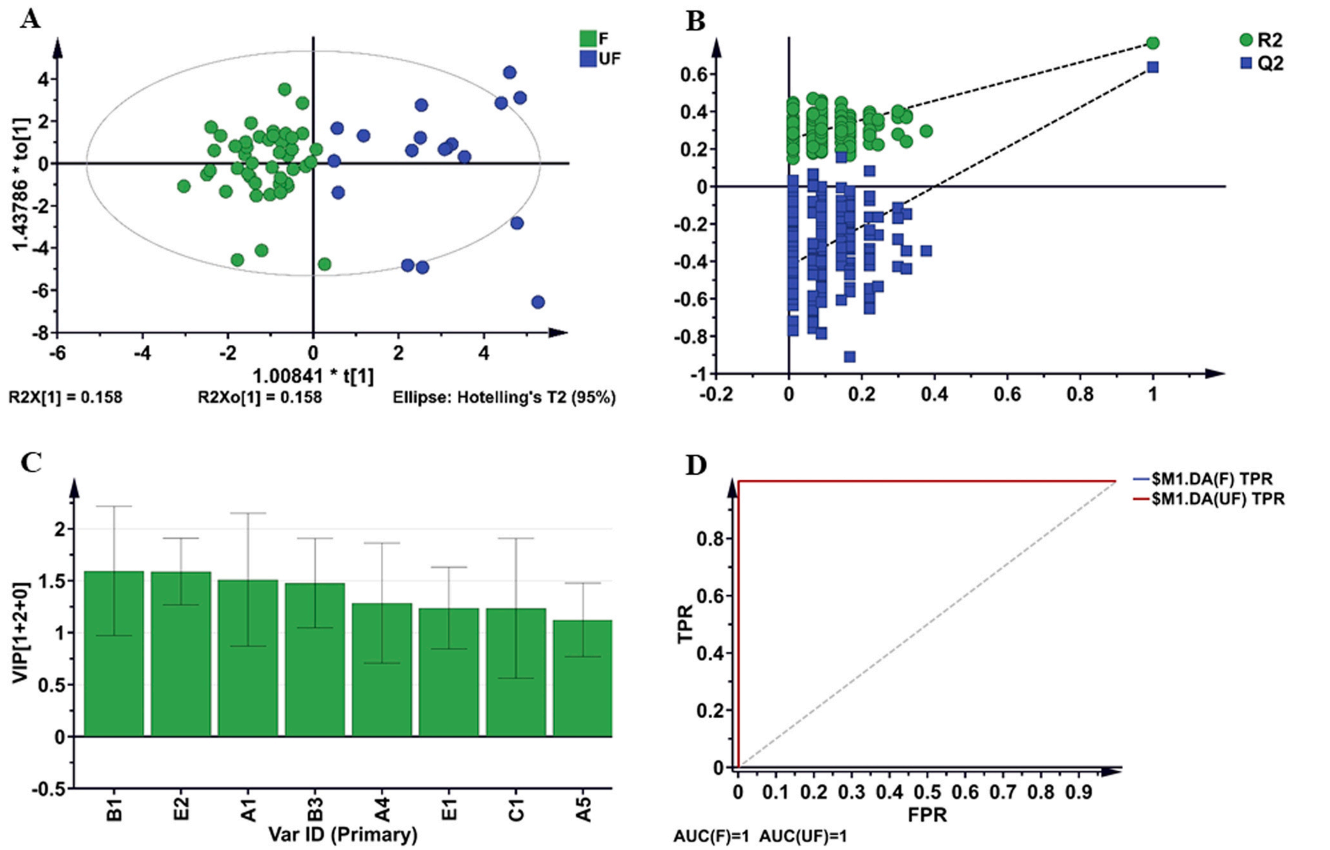

Fig. 5. Orthogonal partial least squares discriminant analysis (OPLS-DA) of 'Dongkui' fruit in different sensory quality groups based on the concentration of volatiles. A, the scores plot of samples; B, variable importance in projection (VIP) scores of volatiles; C, 200 permutation tests; D, receiver operating characteristic curve (ROC). F, acceptable block; UF, off-flavored block.

PCA was applied to evaluate the general effects of storage time and temperature on the e-nose and e-tongue sensory characteristics of 'Dongkui' bayberry. The response values of the 19 sensors were applied for PCA. The score bi-plot and loading of the first two principal components are shown in Fig. 3. The results confirmed that the sensory characteristics of 10 °C and 20 °C stored fruit were gradually separated from that of CK (0 d), 0 °C and 4 °C stored fruit with increasing storage time, and the 12 e-nose sensors were located on the same positive side as these two groups from the 3rd day of storage. These results indicated that the headspace volatiles released from fruit were applicable in distinguishing the flavor quality deterioration of stored 'Dongkui' bayberry, which was consistent with the results of the correlation analysis shown in Fig. 2.

In the study of Yu et al. (2018), e-tongue was found to be applicable in distinguishing different Chinese bayberry cultivars. However, we found that it was not able to distinguish 'Dongkui' fruit at different postharvest stages. This might be due to the variation of soluble compounds such as sugars, acids and phenolics being more obvious among different cultivars than among fruit of the same cultivar at different storage stages. In comparison, e-nose seems to be more applicable in distinguishing fruit of different storage stages. For example, linear discriminant analysis (LDA) of e-nose data distinguished Chinese bayberry of different packaging and delivery modes (Jiang et al., 2021). Li et al. (2015) also found that the PCA analysis of e-nose response values could separate fruits of different storage times at 4 °C. The results of the present study indicated that twelve of the e-nose sensors were potentially applicable in differentiating the sensory quality of postharvest Chinese bayberry based on the headspace volatiles.

3.2.3. Sample classification based on human and artificial sensory evaluation

To identify the characteristic volatiles related to the off-flavor of 'Dongkui' Chinese bayberry, we classified the samples combining human sensory scores and e-nose evaluation. In a previous study, Li et al. (2015) used SNR-max values of e-nose from SR method to represent the physical and chemical indexes of bayberry fruit. However, the relationship involving the consumer's preference had not been built yet. The subjectivity and diversity of human sensory responses increase the difficulty in building an accurate regression relationship between human sensory scores with the physiochemical indexes and e-nose values. Tenenhaus et al. (2005) established a method to predict the consumer's preference by several attributes of physical-chemical and sensory by partial least square (PLS) analysis, through blocking the consumers according to the preference scale, with R^2 higher than 0.9. We used a similar strategy and first classified the samples according to the consumer's overall rating scale, with the groups of average score $>$ 3.0 being defined as "off-flavored" (UF) and the other groups being defined as "acceptable" (F). By this standard, the fruit stored at 20 °C for 3 days or longer as well as those stored at 10 °C for 7 days or longer fell into the UF block. Using k-means to make unsupervised binary classification of the examination of the 12 e-nose sensors, we found that the differentiation in the division of sensory scores was only 13.47%. According to the result of k-means, the samples stored 2 days at 20 °C were classified as UF to get a better differentiation value of 11.43%. The classification was shown as the sample score plots of PLS-DA analysis (Fig. S1).

To verify the rationality of the new classification, we made an ANN model to predict the classification result basing on the response values of the 12 sensors (Fig. 4). BPNN was applied and BFGS method was used to train the model. The activation function of hidden layer was a sigmoid

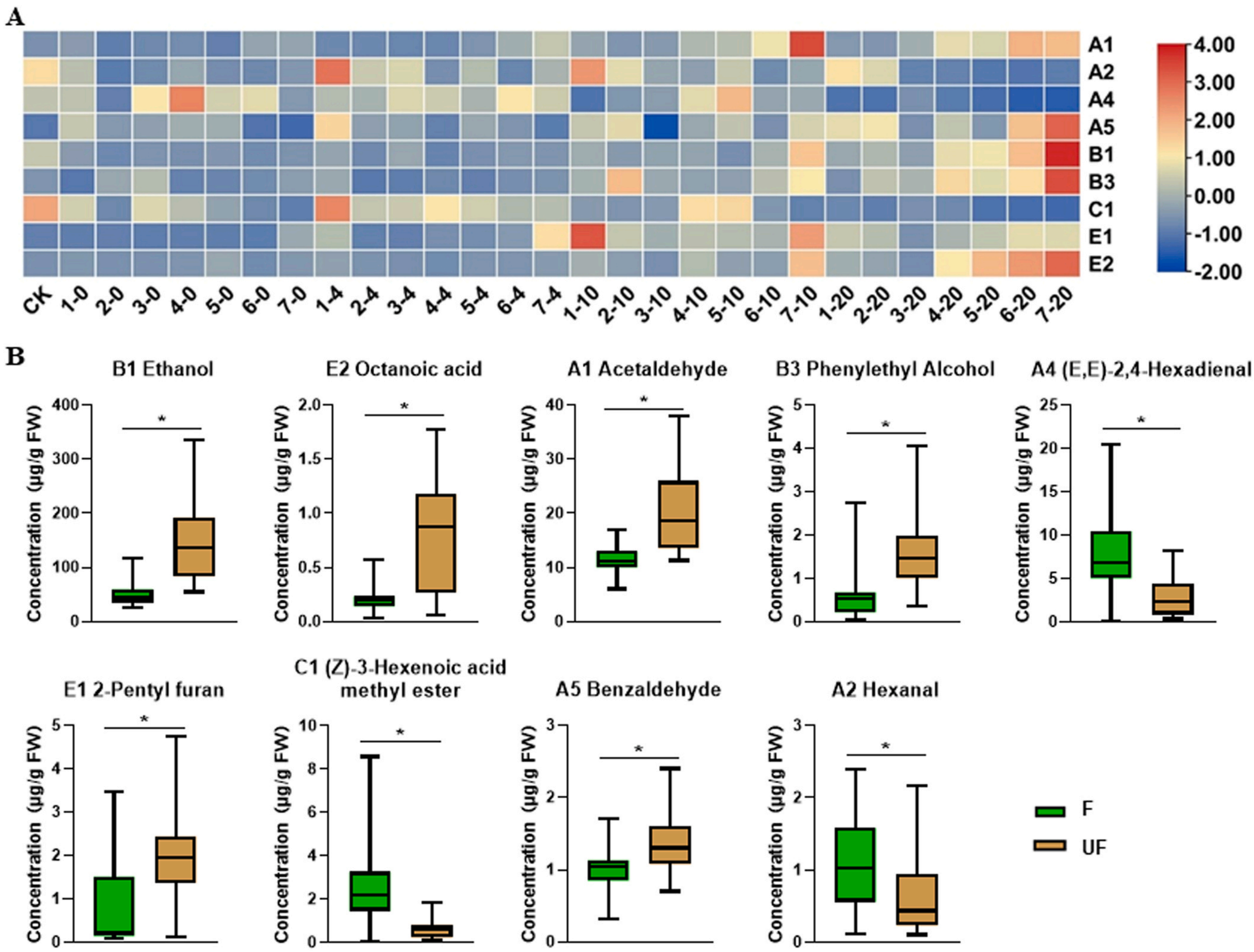

Fig. 6. Relative abundances of differential volatiles. A, heatmap on the dynamic changes of differential volatiles in the storage period of different temperature and the data represented the normalized mean value of three repetitions; B, concentrations of differential volatiles in fruit of different consumer acceptability; F, acceptable block; UF, off-flavored block; *, significant difference by Student's *t*-test ($p < 0.05$).

function and that of the input layer was a linear function. The model had 2 hidden layers and 20 nodes in one layer. The data was cut into training data and test data randomly in a proportion of 7:3 and the original division of F samples and UF samples remained. The output of more than 0.7 was classified as UF and the rest was F. The generated ANN model had 97.27% and 91.94% prediction accuracy for F and UF fruit respectively, with an overall accuracy of 95.92%. As the training datasets and test datasets were divided randomly, the prediction accuracy changed every time but it reached more than 95% in 5 repeats. The highest prediction accuracy of F samples was 99.48% and the highest overall prediction accuracy was 96.73%. Thus, the classification is reasonable and applicable in defining the consumer's acceptability of the stored 'Dongkui' bayberry fruit.

3.3. Changes of volatile compounds of fruit stored at different temperatures

3.3.1. Identification and quantitative analysis of volatile compounds

By HS-SPME–GC–MS, a total of 27 volatiles were identified, including 8 aldehydes (acetaldehyde, hexanal, (*E*)-2-hexenal, (*E*,*E*)-2,4-hexadienal, benzaldehyde, octanal, nonanal, and 3,5-dimethylbenzaldehyde), 2 alcohols (ethanol and phenylethyl alcohol), 1 terpenol (linalool), 3 esters ((*Z*)-3-hexenoic acid methyl ester, methyl benzoate, 3-nonenoic acid methyl ester), 10 terpenes (limonene, α-pinene, (*E*)-β-ocimene, β-elemene, isocaryophyllene, alloaromadendrene, β-caryophyllene, humulene, γ-muurolene, and isoaromadendrene epoxide), and 3 other compounds (2-pentylfuran, octanoic acid, and caryophyllene oxide) (Table 2).

The concentration of volatile compounds is shown in Supplementary Table S3. Terpenes was the main volatiles species of Chinese bayberry 'Dongkui', and β-caryophyllene was the main terpene. (*E*,*E*)-2,4-Hexadienal, (*E*)-2-hexenal, and hexanal were the most abundant aldehydes. (*Z*)-3-hexenoic acid methyl ester and 3-nonenoic acid methyl ester were the main esters of fresh Chinese bayberry 'Dongkui'.

3.3.2. Differential volatile compounds in characterizing postharvest flavor deterioration

The correlation of different volatiles with the odor or overall rating of consumer acceptance was calculated by Pearson correlation analysis. Only acetaldehyde, 2-pentylfuran and octanoic acid showed relatively high correlation ($|r|>0.50$, $p < 0.001$) with the odor or overall rating of consumer acceptance (Table S5). This indicated the difficulty in building an accurate regression relationship between human sensory scores with particular volatile compounds, which might be due to the subjectivity and diversity of human sensory scores.

Thus, we adopted the strategy of Tenenhaus et al. (2005) again, and carried out OPLS-DA analysis on the two sample groups as classified in *Section 3.2.3* (Fig. 4), to explore the characteristic volatiles contributing to flavor deterioration (Fig. 5A). The results of permutation tests indicated a good interpretability and an acceptable predictability of the model (Fig. 5B). The receiver operating characteristic (ROC) curve with the area under curve (AUC) of 1.0 also showed a good performance of the model (Fig. 5D). Eight volatiles were with variable importance in projection (VIP) > 1, which were octanoic acid, acetaldehyde, ethanol, phenylethyl alcohol, 2-pentylfuran, (*E*,*E*)-2,4-hexadienal, benzaldehyde, and (*Z*)-3-hexenoic acid methyl ester (Fig. 5C, Table S4). These results indicated that these compounds could be potentially applied in distinguishing the sensory flavor of 'Dongkui' bayberry.

Among the 8 volatiles with VIP > 1, (*Z*)-3-hexenoic acid methyl ester and (*E*,*E*)-2,4-hexadienal were significantly lower in the UF than the F group ($p < 0.05$), and thus might be potentially contributed to the fresh flavor of Chinese bayberry. The heatmap also showed that these compounds showed a trend of decrease with the extension of storage time (Fig. 6A). In addition to these two compounds, hexanal also decreased in the UF compared to the F group ($p < 0.05$) (Fig. 6B), although the VIP value was 0.99. (*E*,*E*)-2,4-Hexadienal, and hexanal are aldehydes, which are products from lipoxygenase enzymatic pathway and are clarified as green leaf volatiles (GLVs) (Gigot et al., 2010). These two compounds have green and grass-like smell and contribute to the fresh flavor of many fruits. In other fruit species, hexanal, (*E*)-2-hexenal and (*E*,*E*)-2,4-hexadienal are considered as contributors or markers to the fresh flavor of fruits like sapodilla (Lasekan and Yap, 2018), apple (Kebede et al., 2020), and grape (Cefola et al., 2018). The significant decrease of hexanal and other aldehydes during the storage of Chinese bayberry was also reported in the study of Fang et al. (2020).

Ethanol, acetaldehyde, octanoic acid, phenylethyl alcohol, 2-pentylfuran and benzaldehyde were significantly higher in the UF group than the F group ($p < 0.05$). The heatmap also showed that these compounds increased with the extension of storage time and temperature (Fig. 6B). Ethanol has been widely considered as an off-odor volatile of postharvest fruit and vegetables including Chinese bayberry. It is produced from anaerobic respiration and is correlated with the alcoholic and fermented smell (Karakurt et al., 2000; Tietel et al., 2011; Imahori et al., 2005; Fang et al., 2020). Acetaldehyde is the precursor of ethanol production, which is found to increase simultaneously with ethanol in fruit under an anaerobic environment (Saberi et al., 2018; Li et al., 2022b). Octanoic acid, a product of lipid oxidation with unpleasant flavor, has been found to be elevated in the stored dried ripe *Capparis spinosa* fruit and *Corylus avellana* (Zhang et al., 2023; Shakiba et al., 2023). Phenylethyl alcohol, 2-pentylfuran and benzaldehyde are found to be elevated in 'Dongkui' fruit at certain time points of storage at 4 °C, and even more remarkably elevated in the early days of storage at room temperature (Cheng et al., 2015b). Thus, it can be deduced that these elevated compounds might potentially contribute to the off-flavor of 'Dongkui' Chinese bayberry in the postharvest period.

4. Conclusion

The present study provided a comprehensive understanding on the flavor quality deterioration of Chinese bayberry 'Dongkui' during postharvest storage at 0 °C, 4 °C, 10 °C and 20 °C. The increase of storage temperature accelerated fruit quality deterioration, which could be characterized by the decreased flavor strength, increased bitterness and unfavorable odors. The dramatic change of odor was most correlated with the consumer acceptability of stored 'Dongkui' bayberry, which could also be detected by the e-nose sensors. Combining human sensory evaluation and the response values of the selected 12 e-nose sensors, the stored fruit were divided into off-flavored and consumer acceptable blocks. The consumer unacceptable sample block included the fruit stored at 20 °C for more than 2 days, and the fruit stored at 10 °C for more than 7 days. The ANN model verified the classification, with an accuracy higher than 95%. A total of 27 volatiles were identified in the 'Dongkui' bayberry by HS-SPME–GC–MS analysis. Eight differential volatiles were screened out with $p < 0.05$ and VIP > 1 in distinguishing the consumer acceptable and inacceptable fruit. Among these volatiles, (*Z*)-3-hexenoic acid methyl ester, and (*E*,*E*)-2,4-hexadienal were decreased in the deteriorated fruit with the extension of storage time, and thus might potentially contribute to the fresh flavor of 'Dongkui' baberry. Octanoic acid, acetaldehyde, ethanol, phenylethyl alcohol, 2-pentylfuran and benzaldehyde increased in the deteriorated fruit, and thus might potentially contribute to the off-flavor of 'Dongkui' bayberry in the postharvest period.

Declaration of Competing Interest

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

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2024.106146.

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