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Insights into the volatile flavor and quality profiles of loquat (*Eriobotrya japonica* Lindl.) during shelf-life via HS-GC-IMS, E-nose, and E-tongue

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ABSTRACT

Loquat fruits are among the most popular Chinese fruits because of their unique taste and aroma. The quality profiles of these fruits during 18 days of shelf-life at 20 °C were elucidated by headspace gas chromatography-ion mobility spectrometry (HS-GC-IMS), E-nose, and E-tongue. During shelf-life period, the properties and variations of 43 (20 aldehydes, 7 esters, 6 ketones, 1 alcohol, and 1 furan) volatile flavored compounds were determined by GC-IMS, which showed that the contents of methyl 3-methyl butanoate, ethyl acetate, and dimethyl ketone gradually decrease with prolonged shelf-life time, while (E)-2-heptenal, heptanal, (E)-2-pentenal, 1-penten-3-one 3-pentanone and 2-pentylfuran increase. The PCA based on the signal intensity of GC-IMS and E-nose, revealed that loquat fruits are well distinguished at different shelf-life times. The taste profile alternates as the storage time increases, along with higher pH, and lower amounts of total soluble solids, vitamin C, and total phenolics. The visual plots of GC-IMS, E-nose, and E-tongue had good consistency, and they characterized the aroma characteristics of loquat fruits well during different shelf-life periods. The findings of this research provide a useful understanding of the flavors of loquat fruits during their prolonged shelf-life, and a potential research basis for advancements in the loquat industry.

Introduction

Loquat (*Eriobotrya japonica* Lindl.) is a subtropical fruit that is harvested in southeastern China (Goulas et al., 2014). Loquat fruits are highly consumed because of their moderate sour and sweet taste, and abundant nutrients (Su et al., 2021; Wang, Shao et al., 2020; Zhang et al., 2022). According to the flesh color, loquat fruits were classified into two types: red-fleshed and white-fleshed cultivars (Zou et al., 2020). These fruits ripe during the rainy season and high temperature, and their qualities deteriorate easily during postharvest shelf-life (Song et al., 2017). Low-temperature storage is a good way to keep fruits nutrients and other values for a prolonged time; however, loquat fruits show chilling damage and lignification during low-temperature storage (Alos et al., 2017; Xu, Li et al., 2019; Xu, Zhang et al., 2019; Zhu, Wu, & Chen, 2018), resulting in decay and economic loss. There is no optimal method to extend shelf-life of loquat fruits. The flavor reflects the sensory properties of food, and affects consumer food choices (Chen et al., 2020; Li et al., 2023). However, the characteristic flavor of loquat fruits during postharvest shelf life is still unclear. Therefore, loquat fruits' safe and reliable aroma profiles need exploration.

Volatile aroma is a critical sensory property that evaluates food quality and affects consumers selection (Lo Bianco et al., 2010). The sensory fruits' aroma perception could be referred by sensory analysis (Kim et al., 2018), and data are commonly subjective and cannot be explored at the molecular level. The volatile compounds, which are produced by the fruit, create aroma, contribute to flavor, and strongly impact the fruit quality and value (Sun et al., 2020). The most frequently used analytical techniques to identify volatile compounds (VOCs) include gas chromatography olfactometry mass spectrometry (GC-O-MS), electronic nose, and gas chromatography-mass spectrometry (GC–MS) (Bai et al., 2021; Huang et al., 2019). In recent years, new techniques have been established to elucidate VOCs, for instance, headspace-gas chromatography-ion mobility spectrometry (HS-GC-IMS), used for accurately analyzing sampled VOCs (Tian et al., 2020;

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Wang, Chen et al., 2020). Compared to GC–MS, GC-IMS can determine more characteristics such as high resolution, rapidness, and visualization of flavor, which has been employed in characterizing VOCs of samples prepared by different processing and storage (Chi et al., 2022; Guo et al., 2022; Liao et al., 2022; Xiao et al., 2022).

This investigation detects volatile components and flavor characteristics of loquat fruits during postharvest (18 days) shelf-life via HS-GC–IMS, E-nose, and E-tongue, to comprehensively identify VOCs and aroma. Multivariate statistical analysis was used to visualize VOCs of loquat fruits during postharvest storage. The results are expected to provide evidence for future research and the development of techniques to keep fruits and vegetables fresh during transportation and storage.

Materials and methods

Regents and chemicals

The potassium chloride (KCl) and sodium chloride (NaCl) were acquired from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All the chemicals utilized in this investigation were analytical or chromatographic grade.

Sample preparation

Loquat fruits of "Baiyu" (*Eriobotrya japonica* Lindl. cv. Baiyu) were collected from Donghan Orchard (coordinate: 31.05448,120.370655) in Wuzhong District, Suzhou, Jiangsu Province, China. The fruits were selected for experiments as described in previous research (Huang, Liu et al., 2022). The criteria included the same size, color, maturity, shape uniformity and no diseases. Twenty loquat fruits were wrapped with a microporous modified atmosphere packaging bag, and then stored for 18 days at a constant 20 °C temperature, and 90% relative humidity. After 6, 12 and 18 days of shelf-life, one bag (total 30 fruits) was sampled to measure their physiological characteristic, quality, and nutritive indexes.

HS-GC-IMS analysis

The VOCs were assessed via the FlavourSpec® (the G.A.S. Department of Shandong Hai Neng Science Instrument Co., Ltd., Shandong, China). Briefly, loquat (3.0 g) was sampled in a 20 mL headspace vial, and then incubated for 20 min at 40 °C. Subsequently, 500 μL of headspace gas was administered using an 85 °C injection needle. Then the separation of volatile components was performed using GC-IMS at 45 °C. The capillary column was an MXT5 (15 m × 0.53 mm ID). The capillary column was an MXT5 (15 m × 0.53 mm ID). Nitrogen (purity ≥ 99.999%) was selected as a carrier gas (Yuan et al., 2020), and the programmed flow used was: 2 mL/min holding for 2 min, flow ramped up to10 mL/min within 8 min, ramped up to 100 mL/min within 10 min, and lastly, flow ramped up to 150 mL/min within 10 min. The analytical ions were directed to the drift tube at 45 °C with drift gas set at 150 mL/min.

Electronic nose analysis

The Electronic nose (E-nose) assessment was conducted with an E-nose (WinMuster Airsense Analytics Inc., Schwerin, Germany), comprising a sampling apparatus, gas detection system, and pattern recognizing software (Li, Yang, Zhu, Ben, & Qi, 2022). The gas detection system has 10 metal oxide gas sensors, where different sensors recognize different odors (Supplementary Table 1).

According to previously reported methods (Baietto & Wilson, 2015; Guarrasi, Farina, Germana, San Biagio, & Mazzaglia, 2011), briefly, loquat (5 g) small pieces of loquat flesh of the same size was incubated in a 20 mL headspace vial at ambient temperature for 1 h to achieve emission of the volatile compounds. After balanced, a syringe needle connected to the E-nose was inserted to adsorb the balanced headspace gas and quickly flowed in the E-nose system. The instrument parameters were set as: 60 s sensor self-cleaning time, 5 s sensor zeroing time, 5 s sample preparation time, and 60 s acquisition analysis. The loquat gas flow rate was 400 mL/min. Each assay was repeated 6 times. And then principal component analysis (PCA) was performed on these data. PCA is a multivariate statistical method used for reducing the dimension of multiple indicators' data into eigenvectors and classifying them linearly.

Electronic tongue analysis

The loquat fruits' taste properties were assessed by SA-402B Electronic Tongue (E-tongue) (Insent Inc., Japan), comprising a sensor array, and data acquisition system and analysis system. The sensor array has six taste sensors including C00, AAE, CA0, AE1, CT0, and GL1 for bitter, umami, sour, astringent, salty, and sweet, respectively and two reference electrodes (Yu et al., 2022).

Loquat fruit (30.0 g) was diluted with 50 mL distilled water, homogenized, and then filtered through 100 mesh gauze. The supernatant was collected. 70 mL of each sample was used for analysis. The test procedure is as follows: cleaning solution, 90 s, 120 s, 120 s; Conditioning solution, 30 s; and the detection time was set to 30 s.

Measurement of contents of TSS, TA, and Vc in loquat fruits

The contents of total soluble solids (TSS), and vitamin C (Vc) were measured according to the previous methods (Lin et al., 2020). TSS contents were presented in unit of %, while content of titratable acidity (TA) was expressed as pH, which measured by portable pH meter (Mettler Toledo Co. Ltd.). Vc content was presented as the unit of mg/g.

Measurement of total phenolics content in loquat fruits

The content of phenolics was determined according to the method of Huang et al. (2020). One grams of fresh loquat tissue were applied to measure the contents of total phenolics. The content of total phenolics was expressed as g gallic acid (GA) kg^{-1} of fresh loquat fruits, respectively.

Statistical analysis

The HS-GC-IMS and E-nose data were standardized via Origin 2021 (OriginLab, China) for heatmap clustering and principal component analysis (PCA). The qualitative analysis was performed by GC-IMS Library Search using a two-dimensional cross-qualitative method.

Results

GC–IMS topographic plots of loquat fruits during shelf-life

The VOCs of loquat fruits during prolonged shelf-life were elucidated via HS-GC-IMS. The 3D topography (Y = drift time, X = retention time, and Z = peak intensity) revealed that different groups' volatilities had different peak intensities (Fig. 1). Topography displayed the total loquat fruit aroma at different shelf-life time. Similar images of loquat fruit during different shelf-life periods and different signal intensities representing differences were acquired. Some VOCs increased while others decreased over the shelf-life.

To clarify the VOCs alterations, the top GC-IMS 3D topographic map view of the loquat fruits was obtained (Fig. 2). The ordinate and abscissa indicated gas chromatography retention time and ion migration time which were normalized, respectively. Each RIP point on the right depicted a VOC, and most signals appeared in 100 s and 400 s with a drift time of 1.0 s and 1.75 s. The loquat fruit at 0 d was considered standard, and other days' fruit values were subtracted from the standard values (Fig. 2B). White = similar values, red = greater values, and blue

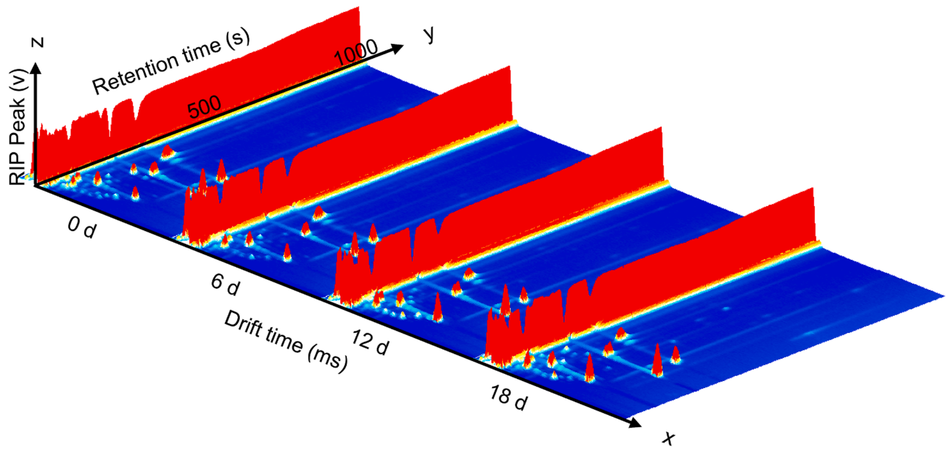

Fig. 1. GC-IMS 3D topographic plot of loquat fruits during different shelf-life time.

= lower values than standard, respectively. The results indicated that loquat fruits' VOCs changed with shelf-life time.

Gallery plots of loquat fruits during shelf-life

To define the volatile changes during shelf-life, fingerprint profiles were generated automatically by Gallery plot using all the analysis signals (Fig. 3A). Each row represents a sample, and each column depicts a VOC. The color degree depicts the compounds' content level, where brighter color represents higher content. The fingerprint spectrum is very useful for determining VOCs during different shelf-life time. The flavor of loquat fruits changed significantly during 18 days of shelf-life. Compared to the fresh loquat fruits sample, the content of 9 flavor compounds, including (E)-2-Heptenal (PubChem CID: 5283316), (E)-2-Pentenal(D), 1-Penten-3-one (PubChem CID: 15394), 3-Pentanone(M) (PubChem CID: 7288), 3-Pentanone(D), 2-Pentylfuran (PubChem CID: 19602), (E)-2-Pentonal(D), (E)-2-Octenal (PubChem CID: 5283324), (E)-2-Pentonal(M), and Heptanal (PubChem CID: 8130), increased substantially at shelf-life 18 days. However, the flavors including 4 aldehydes [Nonanal (PubChem CID: 31289) ((E)-2-Heptenal (PubChem CID: 15204988), (E)-2-Pentenal(M) (PubChem CID: 5364752), Heptanal, and (E)-2-Pentenal(D) (PubChem CID: 166643339)], 3 ketones [3-Pentanone(M) (PubChem CID: 7288), 1-Penten-3-one (PubChem CID: 15394), and 3-Pentanone(D)], and a furan (2-Pentylfuran (PubChem CID: 19602)) decreased with prolonging of shelf-life time.

For further differences in VOCs of loquat during shelf-life, PCA (Fig. 3B) and Fingerprint Similarity Analysis (FSA) (Fig. 3C) were performed according to the area signal strength of the VOCs. PCA is a multidimensional data analysis with quantitative variables. Sample similarity represents small difference, and distance represents an obvious component difference. In the PCA plot (Fig. 3B), the contribution of PC1 is 86.4% and that of PC2 is 10.0%, with a total cumulative contribution of 96.4%, indicating that it was sufficient to explain the similarity between different shelf-life. FSA was applied to analyze the similarity of fingerprints of VOCs during different shelf-life (Fig. 3C). As shown, PCA and FSA can discriminate loquat fruits during different shelf-life. As the shelf-life increases, the distance from the fresh sample also increases, and the flavor of the sample also differs significantly.

Differences in volatile compounds in loquat fruits during shelf-life

The qualitative analysis of flavors in loquat fruits during shelf-life was determined (Fig. 4A). The VOCs were characterized based on their retention index and drifted time by comparing them with the fresh samples (Supplementary Table 2). A total of 43 classes were elucidated by GC-IMS, aldehydes (20), esters (6), ketones (7), alcohol (1), furan (1), and unidentified compounds (8). The most VOCs in loquat fruits were aldehydes. To furtherly display flavor cluster characteristics, the heatmap of these 43 VOCs was generated (Fig. 4B). Altogether, aldehydes were essential for the synthesis of volatile components of fruits during shelf-life, which was in line with a previous report (Huang, Li et al., 2022).

Analysis of volatile compounds in loquat fruits by E-nose during shelf-life

The E-nose system is sensitive to odors within the measurable range (Chen et al., 2020). The relative aroma intensity of loquat fruits during shelf-life was displayed by radar plot (Fig. 5A). The response values of sensors W1S, W1W, W5S, W2S, and W1W in loquat fruits increased markedly during the shelf-life time, indicating that aldehydes and ketones are present in the highest concentrations. PCA of VOCs in loquat fruits during shelf-life was shown in Fig. 5B. The main PCA information was based on the first two principal components. The first central component's variance contribution rate was 92.4%, while that of the second was 4.9%, with a cumulative contribution rate of 97.3%, which included the whole flavor information elementarily. The first principal component's variance contribution rate exceeded 85% with storage time, suggesting that it contained principal information. The greater the distance between different varieties of loquat fruits on the X-axis, the greater the difference. The flavor components of loquats in different storage periods are located in regions with an obvious boundary. Therefore, PCA could significantly distinguish loquat fruits during different storage time. PCA analysis further confirmed the conclusion from Radar graph. The data from this analysis was consistent with that of GC-IMS analysis.

Taste analysis of loquat fruits by E-tongue during shelf-life

The difference in taste attributes of loquat fruits during different shelf-life was evaluated by E-tongue. The basic sensory taste indices are presented in the form of radar image (Fig. 6A). The taste profiles of loquat fruits stored at 18 d were notably different from the fresh samples. The sensory taste indices (sourness, saltiness, richness, and umami) were changed markedly, where typical loquat tastes could be distinguished significantly. Among all sensory indices, that of sourness and saltiness showed a substantial decrease, whereas, with the passage of time, richness and umami increased substantially, while bitter and sweet taste only raised slightly. The astringency, after taste-A and after taste-B, remained unchanged. The content of TSS, VC, and total phenolics decreased quickly on the day 6 of shelf-life, and then went down slowly on the coming shelf-life, while pH increased evidently (Fig. 6B, 6C, 6D, and 6E). These data suggested that the taste properties of loquat fruits faded with time, consistent with the data acquired from E-nose and GC-IMS analyses.

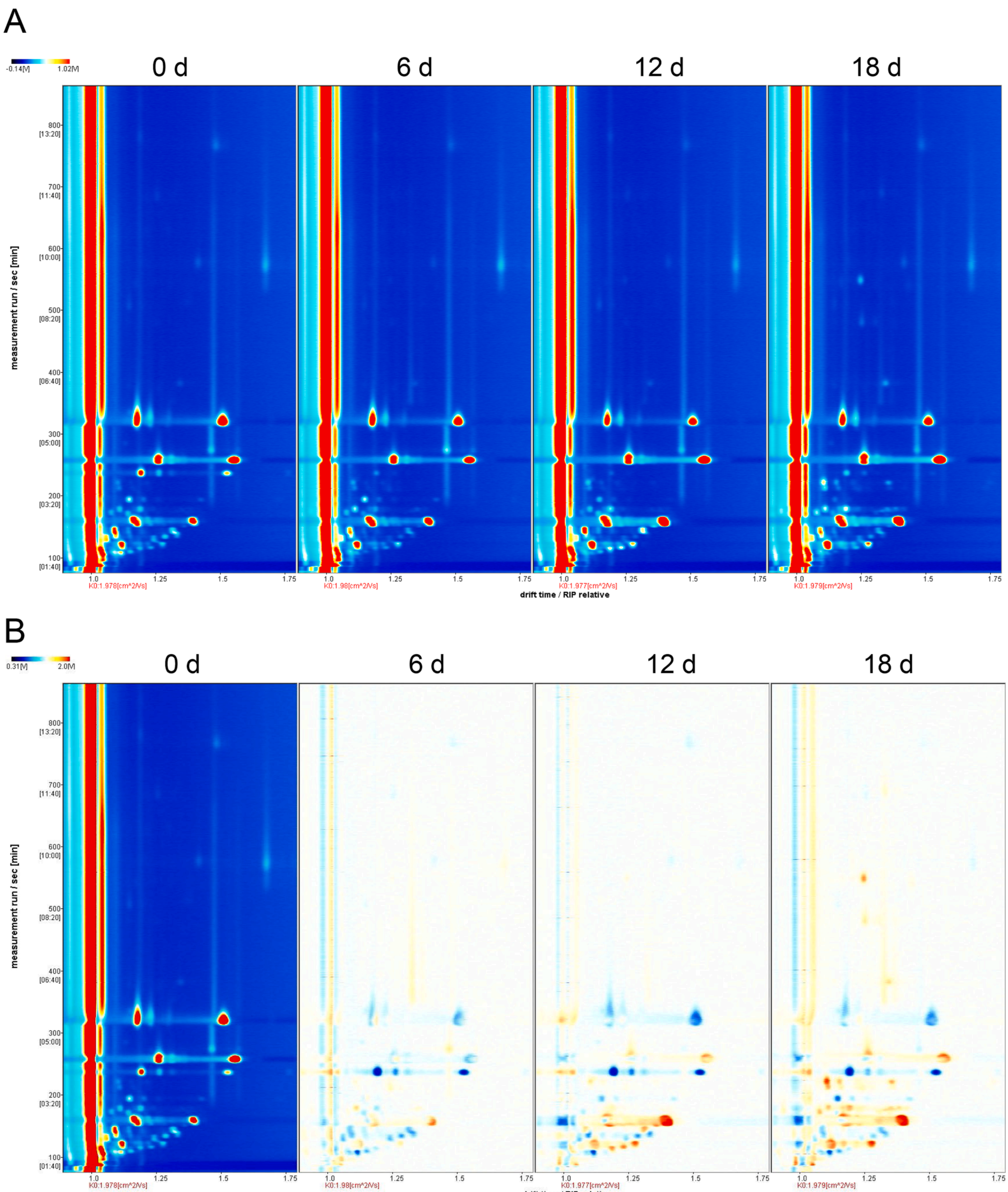

Fig. 2. Two-dimensional chromatography (A) and sub-traction plot (B) of loquat fruits during different shelf-life times.

Discussion

One of the essential qualities of fruits, vegetables, or any other food is freshness. Consumers' choices are greatly affected by this property since fruits and vegetables can easily deteriorate, age, and, therefore, lose their economic value. VOCs play a vital role in evaluating the nutritional and flavor quality of fruits and vegetables. In recent years, rapid detection technology of VOCs in fruits and vegetables has progressed

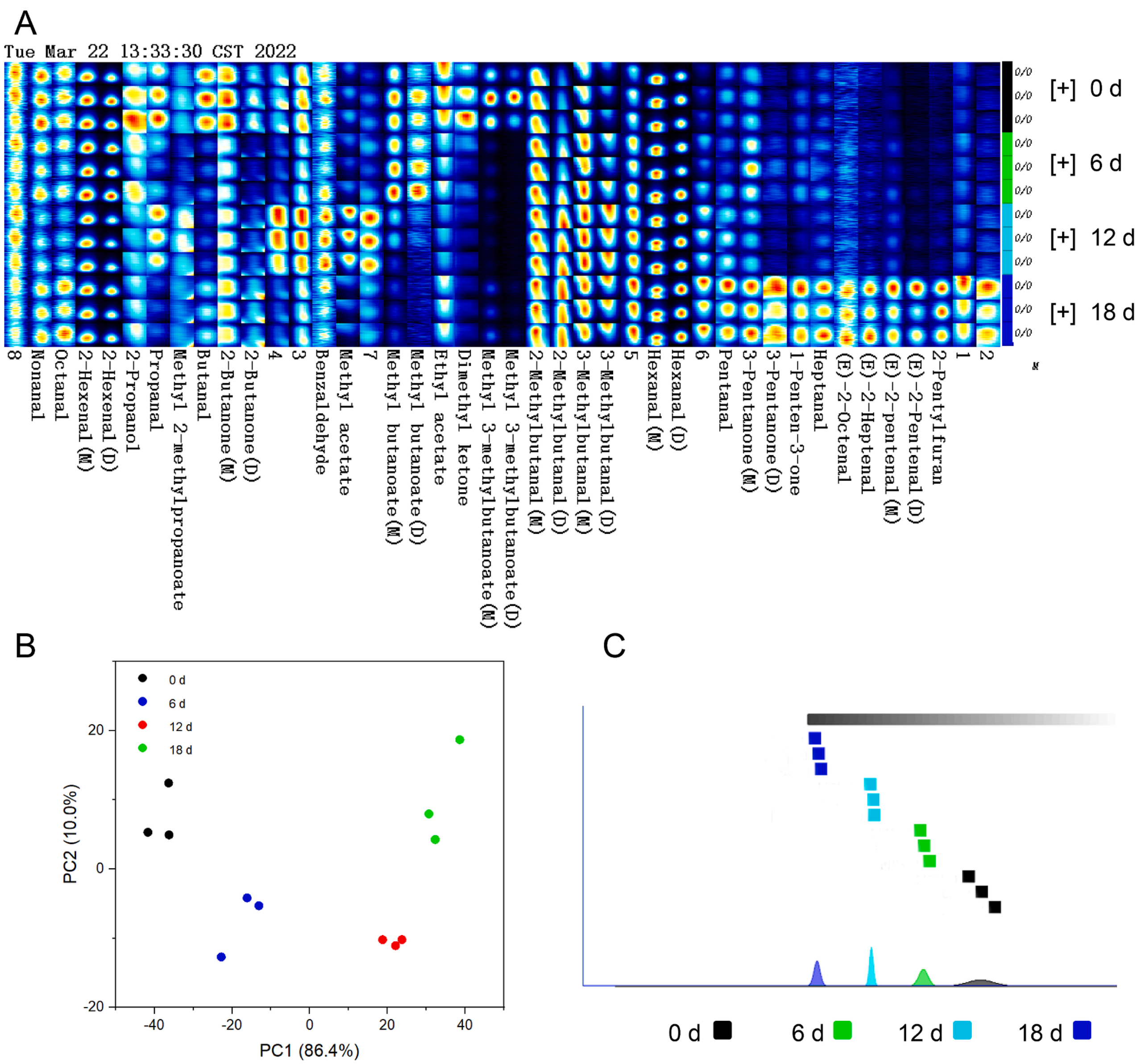

Fig. 3. Gallery plot of the selected peak (A), PCA plot (B) and FSA (C) of VOCs from loquat fruits with different shelf-life times in NFC Chinese bayberry juice during different storage periods.

greatly, such as GC-IMS, E-nose, and E-tongue which are fast, sensitive, and efficient novel systems (Li et al., 2023). These could be used in the supply and production chains of fruits and vegetables to elucidate how to increase freshness time and for predicting prolonged shelf-life (Zhu et al., 2021). The results presented in this study outline the potential advantages of E-nose, GC-IMS, and E-tongue for flavor detection during different shelf-life periods.

Flavor determines the overall sensory properties of fruits (Montero-Prado et al., 2013). In this research, the contents of most detected esters reduced gradually as the shelf-life duration increased. The qualitative and quantitative results of the VOCs are presented in Fig. 4A and Supplementary Table 2. Methyl 3-methylbutanoate, methyl butanoate, and ethyl acetate were responsible for the fruity apple, grape, and cherry, respectively (Liu et al., 2023), which greatly contributed to the aroma of customer preference. PCA can generate two-dimensional principal components from multiple data sets (Yin & Zhao, 2019). The sensory characteristics of loquat fruits during different storage times were separated, as assessed by PCA of E-nose. PCA is often used to reveal the relationships among multiple variables through a few principal components or to extract a few principal components from the original variable while retaining as much information about the original variable as possible (Xuan et al., 2022). The PCA showed that loquat fruits were clearly distinguished at different shelf-life, especially at 18 days. This result indicates that loquat fruits are more likely to form off-flavor compounds during long-time storage. The whole flavor of loquat fruits was very different from the original fresh samples. To further elucidate the variabilities, the taste of loquat fruits at different sampling stages was identified by E-tongue to digitize comprehensive sample information. The response profiles obtained by six working membrane sensors of the E-tongue system revealed that the overall loquat fruits' taste during different storage periods was significantly varied, suggesting that the volatile compositions of loquat fruits changed significantly during storage. The results of the different techniques E-nose, GC-IMS, and E-tongue for flavor detection during different shelf-life periods showed that the longer the storage time, the greater the difference of the flavors.

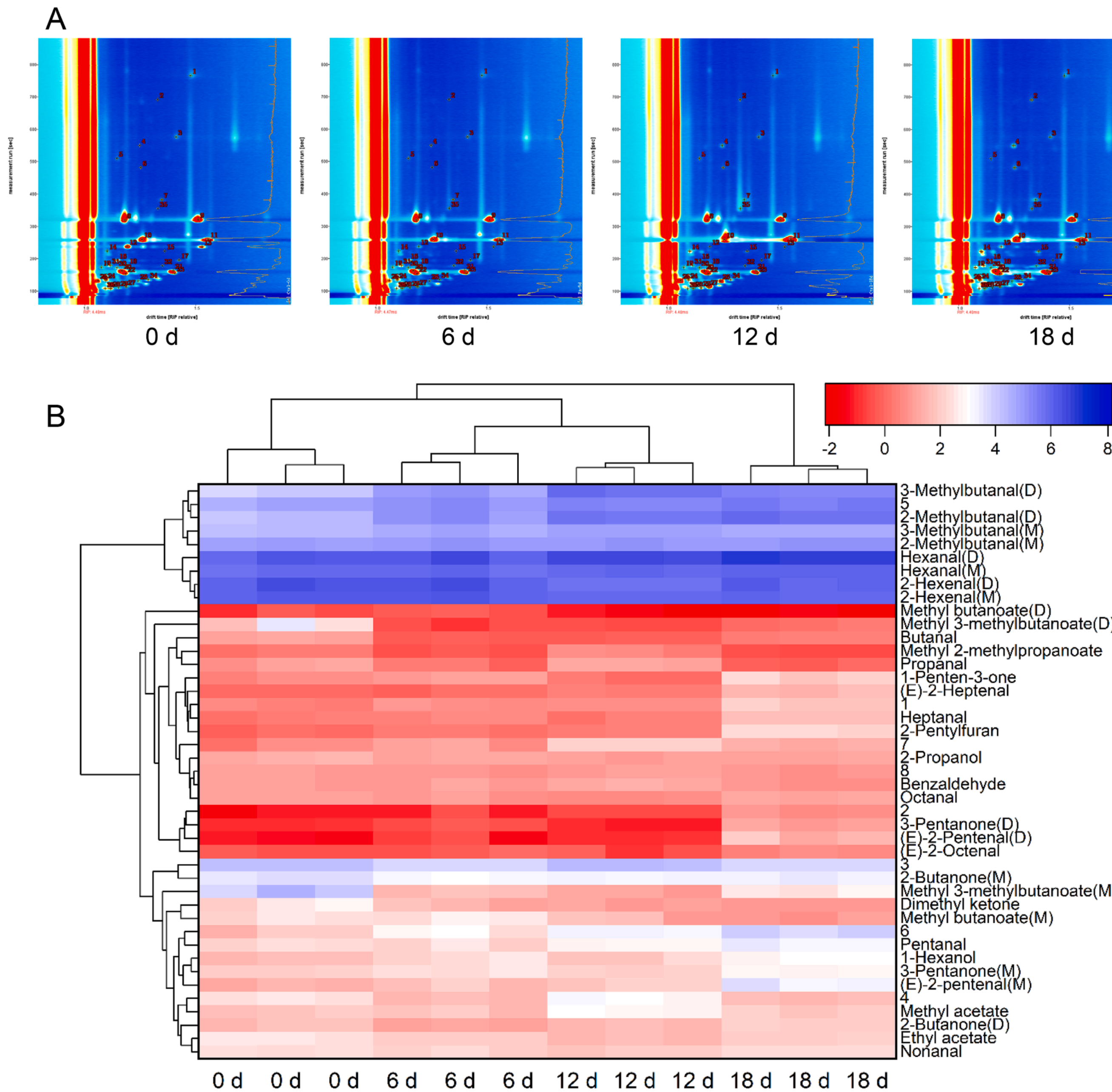

Fig. 4. (A) Topographic plots of GC-IMS spectra with individual volatile compound identification marked 1–43; (B) Heatmap visualization and colors of loquat fruits during different shelf-life time.

Conclusions

In summary, GC-IMS, in combination with E-nose and E-tongue, were applied to analyze qualities of loquat fruits during prolonged storage. This research tracked the variations of volatile flavor components by GC-IMS at varied storage times, and 43 characteristic VOCs were identified, with aldehydes being the most abundant. The content of 2-hexenal and hexanal was the highest and showed no significant alterations during the entire study. The flavor components of fruity and floral aromas gradually reduced with increasing storage time, such as methyl 3-methylbutanoate, methyl butanoate, and ethyl acetate. The loquats from different storage durations were distinguished efficiently. The PCA generated by GC-IMS and E-nose data showed that the loquat fruits were distinguishable at different storage periods. As one of the important factors of quality evaluation, the sourness faded gradually. Storage time had a significant effect on the VOCs of loquat fruits. The advantage of this study is that, for the first time, VOCs and their variations in loquat fruits were analyzed at different storage times. In addition, the combination of GC-IMS, E-nose and E-tongue techniques could compensate for their respective shortcomings and reflect the changes of VOSs more comprehensively. This study could provide a simple, specific, and reliable method to analyze the effect of storage time on the VOCs change of loquat fruits and provide useful knowledge for understanding the characteristics of VOCs in loquat fruits.

CRediT authorship contribution statement

Gui-Li Huang: Conceptualization, Data curation, Funding acquisition, Writing – original draft. **Tian-Tian Liu:** Conceptualization, Data curation, Funding acquisition, Writing – original draft. **Xiao-Mei Mao:** Conceptualization, Data curation, Funding acquisition, Writing – original draft. **Xin-Yao Quan:** Methodology, Validation. **Si-Yao Sui:**

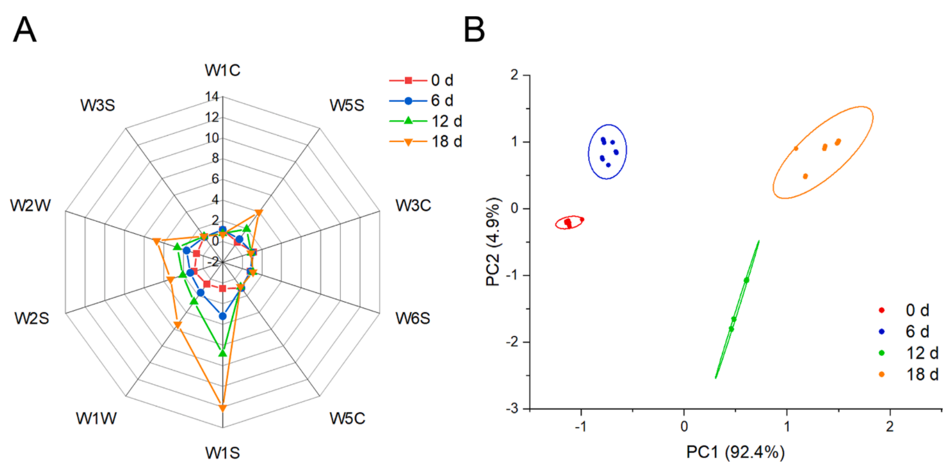

Fig. 5. Principal component analysis (A) and radar plot (B) of aroma in loquat fruits at different storage time.

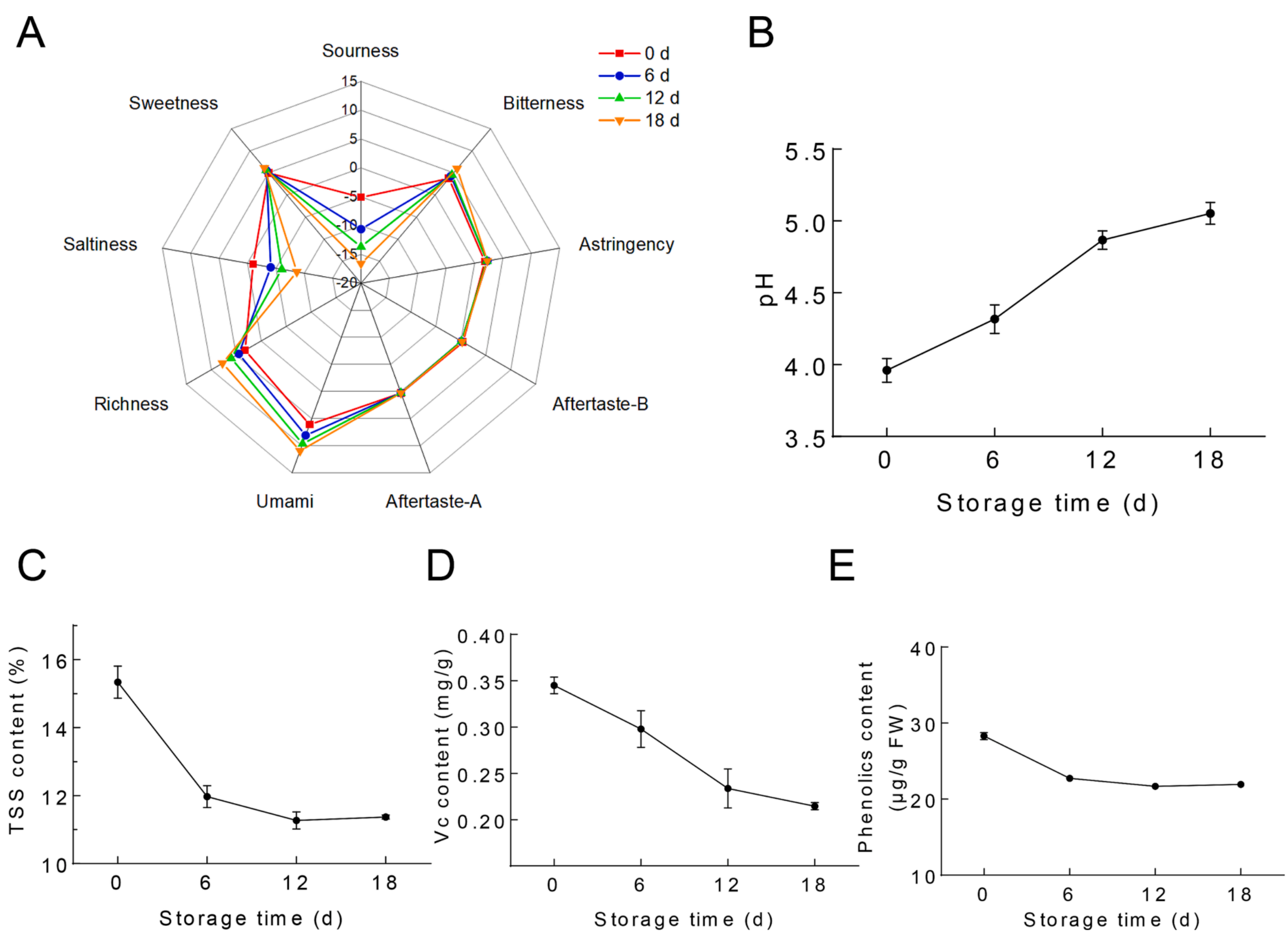

Fig. 6. Radar plot of relative taste intensity (A), the change of pH (B), the content of TSS (C), Vc (D), and total phenolics (E) of loquat fruits during different storage time. Notes: Aftertaste-A, astringent Aftertaste; Aftertaste-B, bitter Aftertaste.

Methodology, Validation. **Jia-Jia Ma:** Methodology, Validation. **Ling-Xiang Sun:** Methodology, Validation. **Hao-Cong Li:** Methodology, Validation. **Qian-Shuo Shao:** Data curation, Writing – review & editing. **Yu-Ning Wang:** Data curation, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100886.

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