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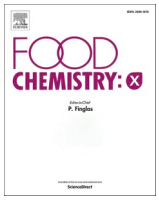

Characterization of volatile metabolites in temperate and tropical sweet corn cultivars under various post-harvest storage conditions

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ABSTRACT

Different post-harvest storage conditions and genetic variability influence the flavor and quality of sweet corn. In this study, the changes in the soluble sugar content and volatile substances were comprehensively analyzed in three temperate and three tropical commercial sweet corn cultivars under various storage conditions. The three tropical cultivars exhibited higher contents of soluble total sugar, moisture, and soluble reducing sugar. Temperate and tropical cultivar groups could be well distinguished under all storage conditions based on the volatile substance profiles. Alkanes were important substances that contributed to the flavor of sweet corn and distinguished different sweet corn accessions and the storage conditions. Moreover, the highest peak area of ethyl acetate and ethanol was 8188.2 and 4833.4, respectively, and these two volatile substances exhibited higher content than others and similar change trend. Collectively, the volatile substances identified in this study can help in the identification and assessment of germplasms and guide future breeding strategies for sweet corn.

1. Introduction

Sweet corn (*Zea mays* L. saccharata Sturt), also called as fruit corn, is widely consumed globally. It normally possesses mutation in maize genes involved in the endosperm starch biosynthesis pathway (Jha et al., 2016). In the 1980s, sweet corn was introduced in China from America (Li et al., 2022). The planting areas of sweet corn in China are increasing, and recently, China has become the top producer of sweet corn in the world (Xiao et al., 2022). Hundreds of valuable compounds including vitamins, minerals, dietary fiber, and phytonutrients are identified in sweet corn (Xiao et al., 2022). Therefore, sweet corn has several potential health benefits and reported to have antioxidant, antidiabetic, anti-inflammatory (Liu et al., 2014), and anticancer (Joshi et al., 2017) properties.

Generally, fresh sweet corn is favored by people and processing industry because of its low starch and high sugar contents. Fresh sweet

Abbreviations: GCMS, Gas chromatography–mass spectrometry; GC–IMS, Gas chromatography–ion mobility spectroscopy; DAP, days after pollination; HS-SPME/GC–MS, headspace solid phase microextraction/gas chromatography–mass spectrometry; sh2, shrunken2; ALF, Aofulan; WC, Wangchao; KPL, Kupula; GLT31, Guangliangtian 31; GLT27, Guangliangtian 27; JBT, Jinbaitian; HPLC, high-performance liquid chromatography; DNS, 3,5-dinitrosalicylic acid; HS-SPME-GC–MS, headspace solid-phase microextraction–gas chromatography–mass spectrometry; DVB, divinylbenzene; CAR, carboxen; PDMS, polydimethylsiloxane; NIST, National Institute of Standards and Technology; PCA, principal component analysis; PLS-DA, Partial Least Squares Discriminant Analysis; VIP, Variable Importance in the Projection; WSN, Wireless Sensor Network; WTMM, widely targeted metabolite modificomics..

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corn is normally harvested at a physiologically immature stage called milk growth stage approximately 21 days after pollination (DAP) (O'Hare et al., 2015). Overall, 57 %–74 % depletion in total sugar content is observed in sweet corn after storage at 18 °C for 10 days (Olsen & Jordan, 1990). Therefore, storage at normal ambient temperature for long time can lead to decreased quality and shortened shelf life, which ultimately reduces the commercial value of sweet corn. Therefore, the post-harvest storage conditions are critical for maintaining the quality of sweet corn, and they significantly influence the shelf life of sweet corn. Accordingly, several studies have reported that sweet corn can be stored at low temperature to maintain its flavor and quality, during which its shelf life is prolonged because of lowered carotenoid metabolic activity and delayed sucrose degradation (Calvo-Brenes, & O'Hare, T., 2020; Hong et al., 2021; Xiao et al., 2024). Generally, sugar and moisture contents of fresh sweet corn are important indicators for evaluating its quality.

The flavor originated from volatile organic compounds is an important criterion for assessing the edible quality of sweet corn. Nevertheless, the breeding programs of maize have focused on yield, leading to slow progress in terms of flavor quality improvement using genetic methods that can help in identifying the key flavor substances and understanding the metabolic pathways. Significantly, almost all-important flavor volatiles are derived from both primary and secondary metabolites and make important contributions to flavor and nutritional diversity of various foods. This suggests that flavor preference should be considered in crop-enhancement strategies (Goff & Klee, 2006). Plant volatiles are generated from both primary and secondary metabolites. More than 7000 flavor volatiles have been identified in foods and are produced at specific development stages (Brown, 2002; Goff & Klee, 2006; Pichersky et al., 2006). Recently, increasing number of studies are focusing on the impact of volatile compounds on the flavor and quality of crops, fruits, and vegetables. The profile of volatile compounds was evaluated using various methods such as headspace fingerprinting, electronic nose, headspace solid phase microextraction/gas chromatography–mass spectrometry (HS-SPME/GC–MS), and gas chromatography–ion mobility spectroscopy (GC–IMS) (Cramer et al., 2005; Li et al., 2023; Yang et al., 2022; Zhang et al., 2022; Zhang, Chen, et al., 2023). Among these techniques, GC–IMS has many significant advantages, enabling quick identification and visualization of the volatile compounds (Ge et al., 2020; Gerhardt et al., 2017; Rodríguez-Maecker et al., 2017; Zhang et al., 2022). However, to the best of our knowledge, the dynamic changes of volatile compound profiles in temperate and tropical sweet corn cultivars are rarely studied during various post-harvest storage conditions using the combination of GC–MS and GC–IMS. Undoubtedly, the technology including artificial intelligence and related packing system have a strong potential to enhance the shelf life and fresh taste of sweet corn (Li et al., 2024; Zhang, Li, et al., 2023). In the future, integrating genomic and metabolomic data to connect artificial intelligence and smart packaging systems will be a trend in improving the value of agricultural products.

It is well known that the kernel characteristics of cooked and fresh sweet corn exhibit genetic variability in various inbred lines of sweet corn (Baseggio et al., 2020; Yactayo-Chang et al., 2022; Zhang, Shen, et al., 2023). It is reported that *shrunken2* (*sh2*) lines have superior filed emergence and eating quality. However, the eating quality of various sweet corn hybrids with the same mutant gene needs to be explored. As sweet corn is consumed in cooked and fresh (as juice) forms, assessing the influence of post-harvest storage conditions on volatile components and sugar content in fresh sweet corn is undoubtedly important. Therefore, it is essential to study the changes in important volatile substances that influence the flavor and quality during different storage conditions after post-harvesting of sweet corn cultivars with varied genetic background. In turn, the flavor and quality of sweet corn can be improved based on the important flavor-related substances using genetic methods. For example, anthocyanin content can be enhanced through genetic engineering focusing on OsTTG1, which is a vital regulator of anthocyanin biosynthesis (Yang et al., 2021).

In this study, we aimed to assess the impact of different post-harvest storage conditions and genetic factors on the quality traits such as volatile metabolites and sugar content in fresh kernels of three temperate and three tropical commercial sweet corn cultivars. First, the moisture and sugar contents of sweet corn kernels were measured. Further, the volatile metabolites were detected by combining GC–MS and GC–IMS. The key volatile metabolites can be used as biomarkers for identifying the freshness level of sweet corn, assessing the nutritional value of different sweet corn germplasms, and guiding future breeding strategies.

2. Materials and methods

2.1. Plant material

Six commercial sweet corn cultivars were used in this study, including three temperate accessions ["Aofulan" (ALF), "Wangchao" (WC), and "Kupula" (KPL)] and three tropical accessions ["Guangliangtian 31" (GLT31), "Guangliangtian 27" (GLT27), and "Jinbaitian" (JBT)]. These six sweet corn cultivars are more popular among consumers in Guangzhou and are representative tropical and temperate sweet corn varieties. All cultivars had *sh2* recessive gene. All plants were grown in 2020 in autumn at Zengcheng Experimental Base of South China Agricultural University (ZC; approximately 113°E and 23°N; Guangzhou City, Guangdong Province, China). The plants were spaced 30-cm apart in rows that were 70-cm apart. Approximately 500 individuals of each cultivar were prepared for the experiments according to the traditional field management practices. The plants were self-fertilized by hand the same day. Approximately 400 ears of each hybrid with consistent growth were harvested at 22 DAP.

2.2. Sample preparation

Harvested ears with husk were immediately transported to the laboratory and stored at normal (23–26 °C; 0, 1, 3, and 5 days; N0, N1, N3, N5) or low (4 °C; 1, 3, 5, and 10 days; L1, L3, L5, L10) temperature with 60 %–70 % humidity. As the edible value of sweet corn decreases after storing at normal temperature for 10 days, the kernels were not sampled at this stage. The subsequent measurements were performed using three biological replicates. Approximately 100 kernels from the central region of one ear were removed when it was sampled, and such kernels from five ears were mixed to form one biological replicate. Unless otherwise noted, all chemical reagents used in this study were of HPLC grade and purchased from Sigma-Aldrich (USA).

2.3. Determination of moisture content

Overall, 50 kernels from one biological replicate were weighed (G_1) and heated at 105 °C and standard atmospheric pressure until constant weight was obtained (G_0). The moisture content was determined as follows:

$$\text{Moisture content} = [(G_1 - G_0/G_0] \times 100\%$$

The measurements were performed using three biological replicates.

2.4. Measurement of the content of soluble reducing and total sugar

The contents of soluble reducing sugar and total sugar were determined using the modified 3,5-dinitrosalicylic acid (DNS) method (Cavaco et al., 2021). The total kernel homogenates (0.002 kg) of each sample were triturated and transferred to a clean centrifugal tube, added to 0.01 L ddH_2O, and centrifuged at 24,200*g* for 10 min. The supernatant was collected in a 0.01-L volumetric flask and further added to 0.01 L ddH_2O. This was added in a centrifugal tube, and centrifugation was repeated. Further, ddH_2O was added to 0.1 L volumetric flask as sample solution. A calibration curve was plotted using standard glucose

solutions with concentrations of 0–0.5 g L^{-1}.

The content of soluble reducing sugar was determined as follows. In boiling water bath, 0.0005 L of sample solution and 0.001 L of DNS reagent were incubated for 5 min. After cooling the mixture to room temperature, 0.005 L ddH_2O was added, and absorbance was measured at 540 nm using UV/Vis spectrophotometer (Hitachi U-2000, Tokyo, Japan).

The content of soluble total sugar was determined as follows. First, 0.0005 L sample solution was mixed with an equal volume of 6 mol L^{-1} HCl and incubated for 5 min at 80 °C in a water bath. The mixture was allowed to cool to room temperature; further, 0.0005 L of 6 mol L^{-1} HCl and 0.002 L ddH_2O were added, followed by the addition of 0.00 2 L DNS reagent and 0.002 L ddH_2O. The absorbance was measured at 540 nm using a UV/Vis spectrophotometer.

2.5. Assessment of volatile compound profile using GC–MS

The headspace solid-phase microextraction–gas chromatography–mass spectrometry (HS-SPME-GC–MS) volatile fingerprinting was performed using the method described by Zhang et al. with slight modifications (Zhang et al., 2022). Kernels (30 g) of each cultivar were placed in a 250-mL brown screw HS-SPME bottle. An HS-SPME fiber (50/30 μm divinylbenzene/carboxen/polydimethylsiloxane; DVB/CAR/PDMS) was applied for extracting volatile compounds. HS-SPME extraction was conducted at 80 °C for 40 min. The volatile compounds were immediately desorbed at 250 °C for 5 min in a splitless injection port for GC analysis with an Agilent 7890B-5977 A model GC–MS system (Agilent, Santa Clara, CA, USA) equipped with a 30 m × 0.25 mm × 0.25 μm DB-5MS capillary column. After each run, the SPME fiber was aged by reheating for 20 min in the injection port. The injector temperature was kept at 250 °C. High purity helium (>99.99 %) was used as the carrier gas at a linear velocity of 0.001 L m^{-1}. The GC oven temperature was kept at 50 °C for 3 m and then increased at a rate of 15 °C m^{-1} to 110 °C, at 3 °C m^{-1} to 170 °C, at 25 °C m^{-1} to 250 °C, and held for 3 m. For MS, the ion source temperature was 280 °C, and electron ionization energy was 70 eV with a mass range of 35–350 m z^{-1} in the full-scan mode for qualitative analysis. After running samples, the total ion chromatograms were analyzed using the NIST spectral library (NIST08) for more precise identification of the peaks. The reverse match factor (similarity >80; the max was 100) was the identified standard for the volatile substances. The relative content of volatile compounds in sweet corn kernels was calculated using peak area normalization method.

2.6. Evaluation of volatile profiles using GC–IMS

A FlavourSpec® analyzer (Hanon Instruments Co., Ltd., Shandong, China) was used to measure the volatile compounds in the kernels of six sweet corn cultivars using the method by Allers et al. (Allers et al., 2016) and Yang et al. (Yang et al., 2022). Sweet corn kernels (0.005 kg; from samples stored at normal temperature for 0 and 5 days and at low temperature for 5 and 10 days) were added to a 0.02-L headspace bottle, incubated at 60 °C, and centrifuged at 2500 *g* for 15 min. The IMS temperature was 45 °C. Subsequently, a heated syringe set at 65 °C was used to automatically inject 500 μL of the detected samples into the injector. Further, the samples were injected into a FS-SE-54-CB-1 column (15 m, ID: 0.53 mm, 1 μm) maintained at 60 °C using a nitrogen (99.999 % purity) flow of 0.150 L m^{-1}, following a predetermined flow protocol of 0.002 L m^{-1} for 2 min and 0.100 L m^{-1} for 18 min. Each sample was measured twice in parallel. For sample fingerprint comparison and difference analysis, the built-in analyzed program Laboratory Analytical Viewer with three plus-ins [Reporter, Gallery Plot, Dynamic PCA, and GC × IMS Library Search program (G.A.S. Gesellschaft für analytische SensorsystememBH. Dortmund)] was used. Two-dimensional qualitative analysis was conducted using the integrated G.A.S IMS migration time database and the NIST 2014 gas-phase retention index database.

2.7. Data analysis

Experimental data were organized and recorded using Microsoft Excel2010. One-way analysis of variance (ANOVA) was used for the statistical analyses. $P < 0.05$ was considered significant. MetaboAnalyst6.0 software (https://new.metaboanalyst.ca/MetaboAnalyst/home.xhtml) (Pang et al., 2021) and the TBtools software (https://github.com/CJ-Chen/TBtools) (Chen et al., 2020) were used for clustering analysis and plotting heatmap. Principal component analysis (PCA) was performed using SIMCA 14.1 (Umetrics, Umeå, Sweden) and MetaboAnalyst6.0. Partial least squares discriminant analysis (PLS-DA) was performed and variable importance in the projection (VIP) score was assessed using MetaboAnalyst6.0 software. The metabolites with VIP >1 were considered as the key differential metabolites. All data were plotted using Graphpad Prism v9.0 (GraphPad Software Inc., La Jolla, CA, USA). Venn diagram was analyzed using jvenn (Bardou et al., 2014) software (https://www.bioinformatics.com.cn/static/others/jvenn/example.html).

3. Results and discussion

3.1. The moisture and sugar contents in the kernels of temperate and tropical sweet corn cultivars

To compare the quality of temperate and tropical sweet corn accessions under different storage conditions (varying temperature and time), the cobs of three temperate accessions (ALF, WC, and KPL) and three tropical accessions (GLT31, GLT27, and JBT) were stored at normal temperature for 0, 1, 3, and 5 days or at low temperature for 1, 3, 5, and 10 days. The contents of soluble total sugar (Figs. 1A and D), moisture (Figs. 1B and E), and soluble reducing sugar (Figs. 1C and F) in the kernels of six sweet corn cultivars decreased under low and normal temperatures; this was consistent with a previous study (Xiao et al., 2024). The soluble total sugar content was higher in the kernels of three tropical accessions (GLT31: 69.43 g kg^{-1}, GLT27: 68.22 g kg^{-1}, and JBT: 68.02 g kg^{-1}) than in those of three temperate accessions (ALF: 66.44 g kg^{-1}, WC: 67.34 g kg^{-1}, and KPL: 63.41 g kg^{-1}). Although those sweet corn cultivars may carry the same super sweet mutant gene, differences in sugar content (a quantitative trait) still existed. The highest and lowest total sugar content was 69.43 and 43.23 g kg^{-1} in freshly harvested GLT31 and KPL cobs stored at normal temperature for 5 days, respectively. Moreover, the rate of decrease of soluble total sugar content was slower in three tropical accessions (GLT31: 4.90 g kg^{-1}, GLT27: 4.93 g kg^{-1}, and JBT: 4.51 g kg^{-1}) than in three temperate accessions (ALF: 5.23 g kg^{-1}, WC: 4.96 g kg^{-1}, and KPL: 5.05 g kg^{-1}) under normal temperature from 0 to 5 days. For the same sweet corn variety, low temperature storage condition could slow down the rate of decrease of soluble total sugar content (GLT31: 3.14 g kg^{-1}, GLT27: 2.14 g kg^{-1}, and JBT: 1.39 g kg^{-1}, ALF: 3.06 g kg^{-1}, WC: 3.60 g kg^{-1}, and KPL: 3.19 g kg^{-1}).

Consistent with the above results, the soluble reducing sugar content exhibited similar trends (Figs. 1C and F). During the storage period of 0 to 5 days, the soluble reducing sugar content of six sweet corn cultivars ranged from 20.95 g kg^{-1} (in ALF stored at normal temperature for 5 days) to 37.08 g kg^{-1} (in freshly harvested GLT27) under normal temperature and from 27.68 g kg^{-1} (in KPL stored at low temperature for 5 days) to 37.08 g kg^{-1} (in freshly harvested GLT27) under low temperature. A previous study reported that the sugar content significantly decreased during storage particularly under the storage at 23 °C (Hong et al., 2021). Furthermore, the soluble reducing sugar content of sweet corn kernels after 10 days of storage under low temperature was still higher than that after 5 days of storage under normal temperature, indicating that low-temperature storage condition is more conducive to maintaining sugar content of sweet corn kernels. This is consistent with

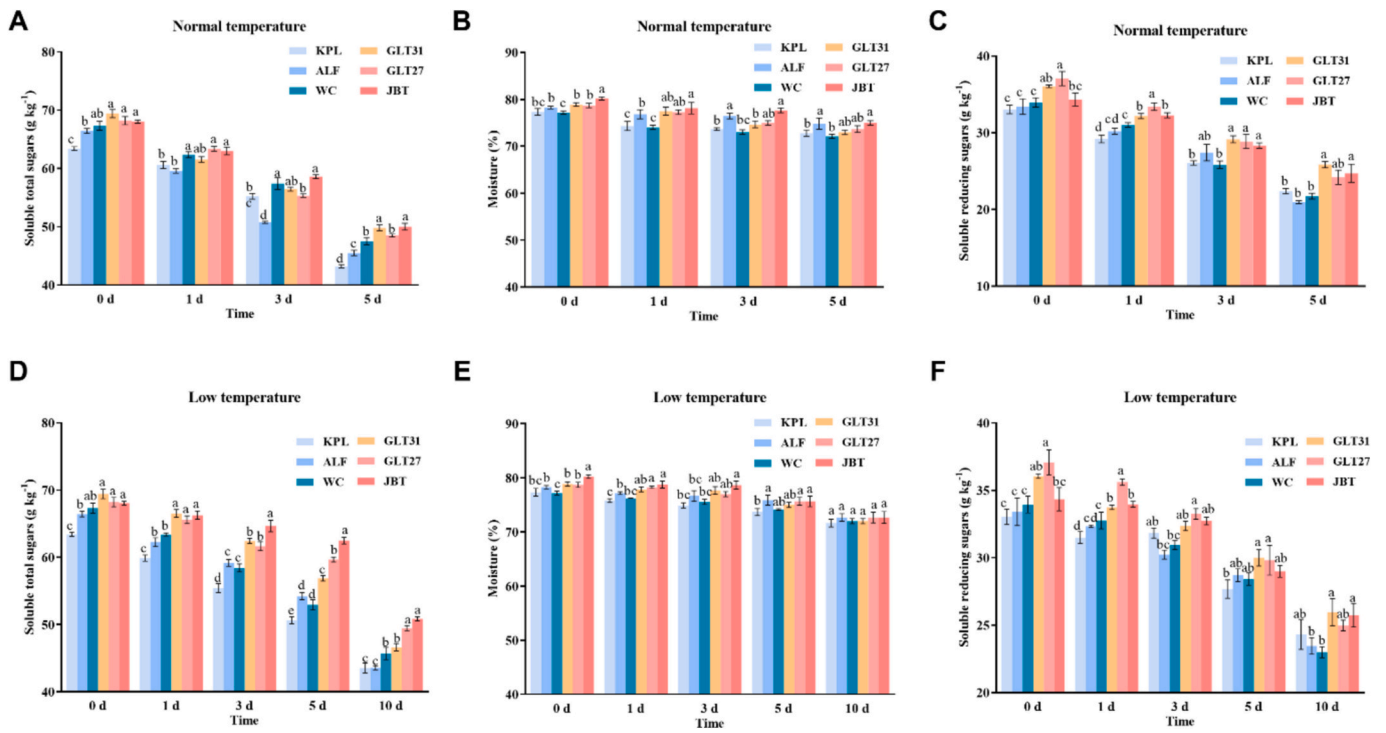

Fig. 1. The moisture and soluble sugar contents in the kernels of six sweet corn cultivars under different post-harvest storage conditions.

a previous study reporting that low temperature slows down the sugar metabolism in fresh sweet corn (Yactayo-Chang et al., 2022).

The moisture content was higher in the tropical accessions than in the temperate accessions. The moisture content of JBT was always the highest compared with other accessions under the same storage conditions during the storage period of 0 to 5 days. When stored for 10 days under low temperature, the moisture content of all accessions (72 % or 73 %) was not significantly different. Probably, this was the threshold for reducing the moisture content in these cultivars at low temperatures. Although the moisture content in all samples exhibited decreasing trend, the rate of decrease in moisture content was different from that of decrease in sugar content. The rate of decrease in moisture content was slower in the temperate accessions than in the tropical accessions under normal and low temperatures. This may be one of the reasons underlying the change in the sugar content of these samples.

In conclusion, tropical sweet corn accessions exhibited higher total sugar, reducing sugar, and moisture contents. Low-temperature storage condition was more conducive to maintaining these important quality indexes in all six sweet corn accessions. Therefore, sweet corn cultivars carrying the same super sweet mutant gene may still exhibit differences in terms of sugar content, which is a quantitative trait (Chen et al., 2022; Yactayo-Chang et al., 2022). This indicated that genetic background has a significant impact on the sugar content and flavor of the accessions. In the future, multiomics combined with genetic methods should be conducted to identify the genes that affect sugar and moisture contents, which will help in sweet corn breeding. Furthermore, there are many different packaging forms for fresh sweet corn in the market, and the impact of these packaging forms on the sweetness and humidity of sweet corn should be studied. Importantly, aflatoxin is a risk factor to be monitored in fresh sweet corn during storage. This is because corn is one main host for *Aspergillus section Flavi*, which produces aflatoxins. Moreover, children are at a high risk for aflatoxin M1 exposure (Xiong et al., 2022). Therefore, aflatoxin contamination of fresh sweet corn should be included in the critical quality parameters for fresh sweet corn. However, this field has not yet attracted the attention of researchers.

3.2. Differences in flavor substances between temperate and tropical sweet corn cultivars under different post-harvest storage conditions determined using GC–MS

3.2.1. Variation in volatile metabolites in sweet corn depending on the genetic factors and storage environments

To assess the effect of genetic background and different post-harvest storage conditions on the maintenance and production of volatile compounds in sweet corn, GC–MS was performed using the samples stored at normal temperature (0, 3, and 5 days; named N0, N3, and N5) and low temperature (3, 5, and 10 days; named L3, L5, and L10). A total of 119 volatile metabolites were detected in all samples (Table S1). All metabolites were classified into 12 categories, including alkanes (53), esters (17), olefins (23), phenols (4), arenes (7), ketones (4), aldehydes (3), amides (2), salts (1), ethers (1), heterocyclic compounds (3), and acids (1) (Fig. 2, Table S1). Among these, the types and quantities of alkanes, esters, and olefins were high in the six accessions, indicating that these volatile compounds significantly contribute to the flavor of sweet corn. However, salts (6-beta-naltrexol), ethers (benzene,1-nitro-2-(octyloxy)), and acids (4-(anisylideneamino)-cinnamic acid) were only detected in the samples of temperate accessions (WC-N0, WC-L5, and KPL—N5, respectively). The types of volatile substances detected in temperate accessions were more diverse (Fig. 2, Table S1), indicating that the flavor of temperate accessions of sweet corn may be superior to that of tropical accessions. Moreover, freshly harvested temperate accessions of sweet corn exhibited the highest variety of volatile substances (ALF has 23, WC has 20, and KPL has 23), and after storing for 10 days at low temperature, the samples ALF (5), WC (5), and KPL (11) exhibited the least variety of volatile substances. This suggested that stored temperate accessions of sweet corn would lose some flavor. In contrast, the types of volatile substances increased during storage in three tropical accessions (GLT31-L10 has 19, GLT27-N5 and -L5 have 18, JBT-L10 has 23), indicating that several volatile compounds were produced and released during the storage. Additionally, it may be because the harvest time of tropical sweet corn cultivars is later than that of temperate cultivars. In summary, different sweet corn cultivars can produce different types and quantities of volatile compounds, which

is consistent with a previous study (Yactayo-Chang et al., 2022). Therefore, genetic factors have a significant impact on the flavor of sweet corn.

The trend of change in volatile compound content was studied under various storage conditions to understand how temperature and duration of storage affect the volatile compounds in sweet corn (Fig. 3, Table S1). When stored under low temperature from 3 to 10 days, the relative content of alkanes gradually increased in the tropical accessions but gradually decreased in the temperate accessions. Moreover, the relative content of alkanes gradually increased in ALF, GLT27, and JBT under normal temperature but first increased and then decreased in WC and GLT31. The venn diagram (Fig. S1) revealed only 1–6 common volatile substances in the samples of the same variety under different storage conditions or samples of different accessions with the same storage condition. In conclusion, the volatile compounds in different sweet corn cultivars under the same or different conditions were all different. In other words, the flavor of sweet corn is influenced by genetic factors and storage conditions. Therefore, the flavor and shelf life of sweet corn would be improved using genetic methods, which is consistent with a previous study (Yactayo-Chang et al., 2022). Further, revealing the key genes and their mechanisms via identifying the metabolic pathways of these volatile substances is the foundation for precise improvement of sweet corn flavor. Moreover, consumption of fresh sweet corn involves cold chain transportation. Easy and direct tracing and controlling the critical quality parameters in actual cold-chain logistics helps to provide consumers with the best quality sweet corn. In a previous study, the critical quality parameters of table grapes were monitored in cold chain logistics by integrating Wireless Sensor Network (WSN) and correlation analysis (Xiao et al., 2017). Therefore, this method has high application value for future research on quality assurance for fresh sweet corn in cold-chain logistics.

3.2.2. *Determination of characteristic volatile substances via clustering analysis, principal component analysis (PCA), and partial least squares discriminant analysis (PLS-DA)*

To differentiate the volatile profiles of the six cultivars of sweet corn identified using GC–MS, the online analytical software MetaboAnalyst was used. The PCA score plot is displayed in Fig. 3B. During 0–10-day storage, the cumulative variance contribution rate of PC1 and PC2 ranged from 31.64 % to 71.64 %. Regardless of the temperature, the two groups (three tropical accessions vs three temperate accessions) were independently dispersed on the visualization map on the same storage day (Fig. 3B). Based on these data, it was suggested that the volatile profile of the three tropical accessions differed from that of the three temperate accessions. This finding confirmed that the genetic factors significantly influenced the volatile profile and flavor of sweet corn kernels. Moreover, the heatmap revealed that the six sweet corn cultivars were well clustered into two major categories (three tropical accessions vs three temperate accessions) under six different storage conditions (Fig. 3A).

Based on these results, the key volatile substances were identified according to a PLS-DA model (Fig. S2). Volatile substances with a VIP > 1 were considered to have the greatest impact on distinguishing sweet corn cultivars and their storage time. In this model, 12–25 variables (KPL has 15, ALF has 15, WC has 12, JBT has 25, GLT27 has 20, and GLT31 has 14; Figs. S2 A–F) under the six storage conditions were calculated with VIP > 1. The implication is that these volatile substances were essential and could be used to differentiate sweet corn cultivars with respect to storage temperature and duration, each of which had a unique profile under various storage conditions. It is beneficial for assessing the freshness and flavor of sweet corn. Among these, dodecane (alkanes, VIP = 2.77; ALF), phenol,2,4-bis(1,1-dimethylethyl) (phenols, VIP = 2.34; KPL), 9-methylheptadecane (alkanes, VIP = 2.31; WC), methyleugenol (phenols, VIP = 2.30; JBT), decane, 2-methyl-(alkanes, VIP = 2.28; GLT27), and tricyclo [2.2.1.0(2,6)] heptane,1,7,7-trimethyl- (alkanes, VIP = 2.62; GLT31) were identified as having the highest VIP values in six cultivars under the same storage condition. Moreover, they were considered to be the key volatile substances with VIP > 1 under the same storage condition to distinguish the diverse sweet corn cultivars (Figs. S2 G–L). These volatile substances play crucial roles in identifying the origin of sweet corn material. Ethyl oleate (esters, VIP = 2.60; N0), 9-methylheptadecane (alkanes, VIP = 2.24; N3), 2-bromododecane (alkanes, VIP = 2.28; N5), kaur-15-ene (amides, VIP = 2.16; L3), undecane,3-methyl- (alkanes, VIP = 2.56; L5), and benzene, 1-(1,1-dimethylethyl)-3,5-dimethyl- (arenes, VIP = 3.73; L10) had the top VIP value in six cultivars under the same storage condition. In summary, alkanes were important substances that not only contributed to the flavor of sweet corn but also distinguished different sweet corn accessions and the storage conditions. Therefore, a comprehensive analysis of the metabolism of alkanes and genetic analysis of sweet corn quality may be good methods for distinguishing sweet corn germplasms.

3.3. *Comparison of fingerprints of volatile substances in six sweet corn cultivars under four post-harvest storage conditions using GC–IMS*

3.3.1. *Topographic plots of various samples of sweet corn using GC–IMS*

The volatile substances of six sweet corn cultivars were obtained under four post-harvest storage conditions, and their topographic plots are shown in Figs. 4 and S3. The majority of the red signals from the three temperate accessions at freshly harvested time (N0; 0 day at normal temperature) appeared in the retention times of 100–150 s and drift times of 1.0–1.25 s, whereas the three tropical accessions exhibited

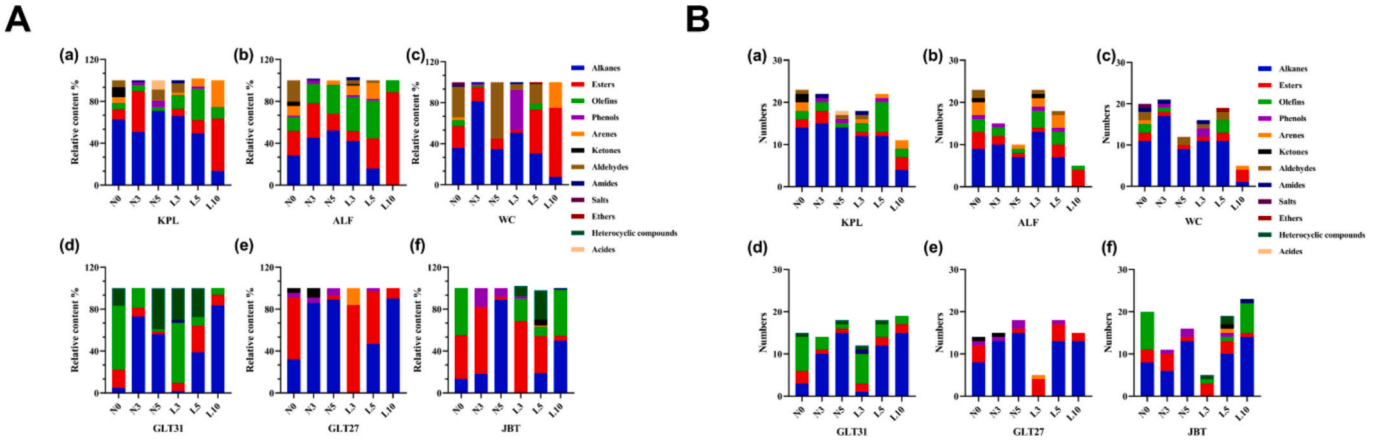

Fig. 2. The contents and number of various volatile metabolites of six sweet corn cultivars under six types of different post-harvest storage conditions as revealed by GC–MS.

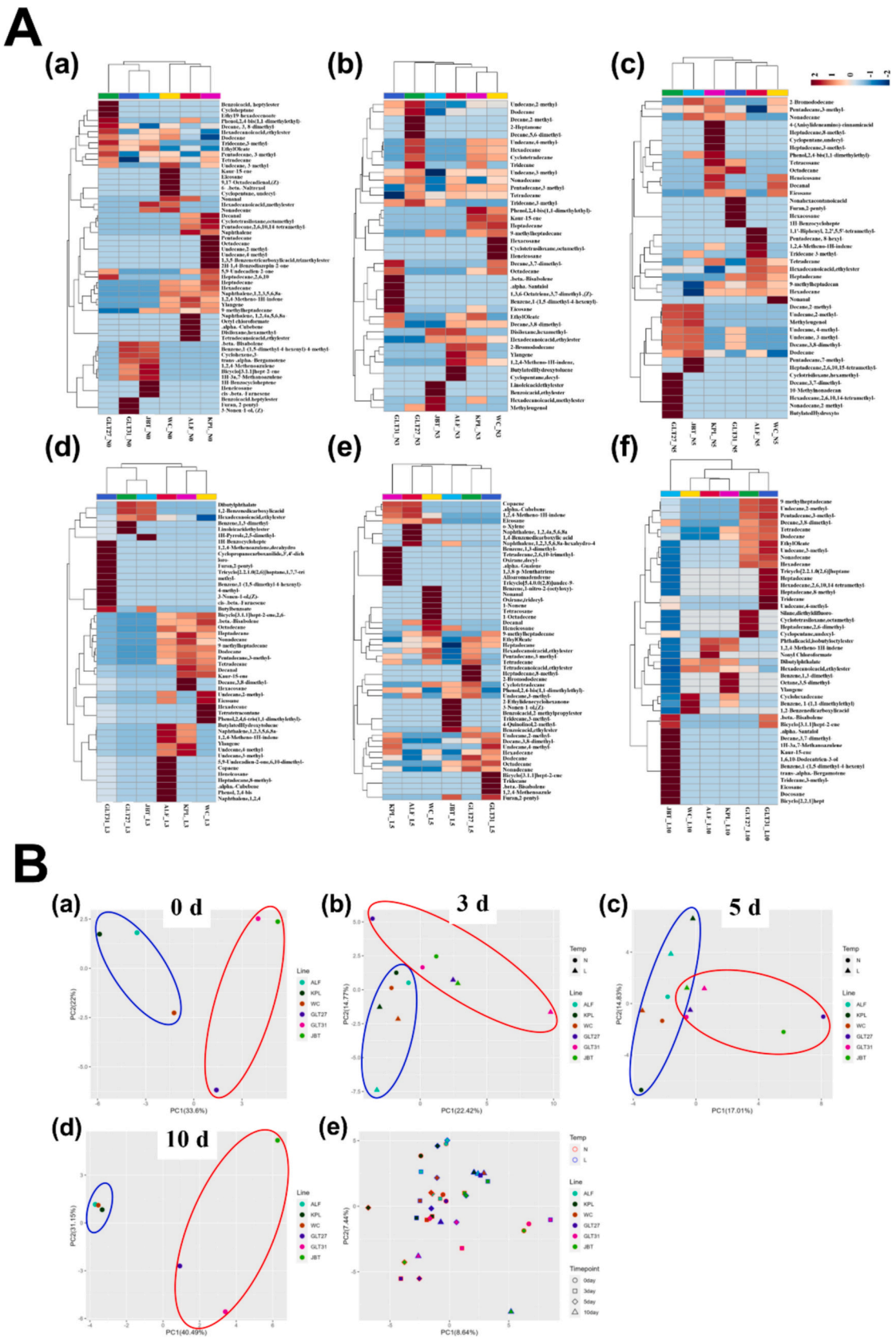

Fig. 3. Heatmap and principal component analysis (PCA) of various sweet corn samples to determine the characteristic volatile substances according to GC–MS.

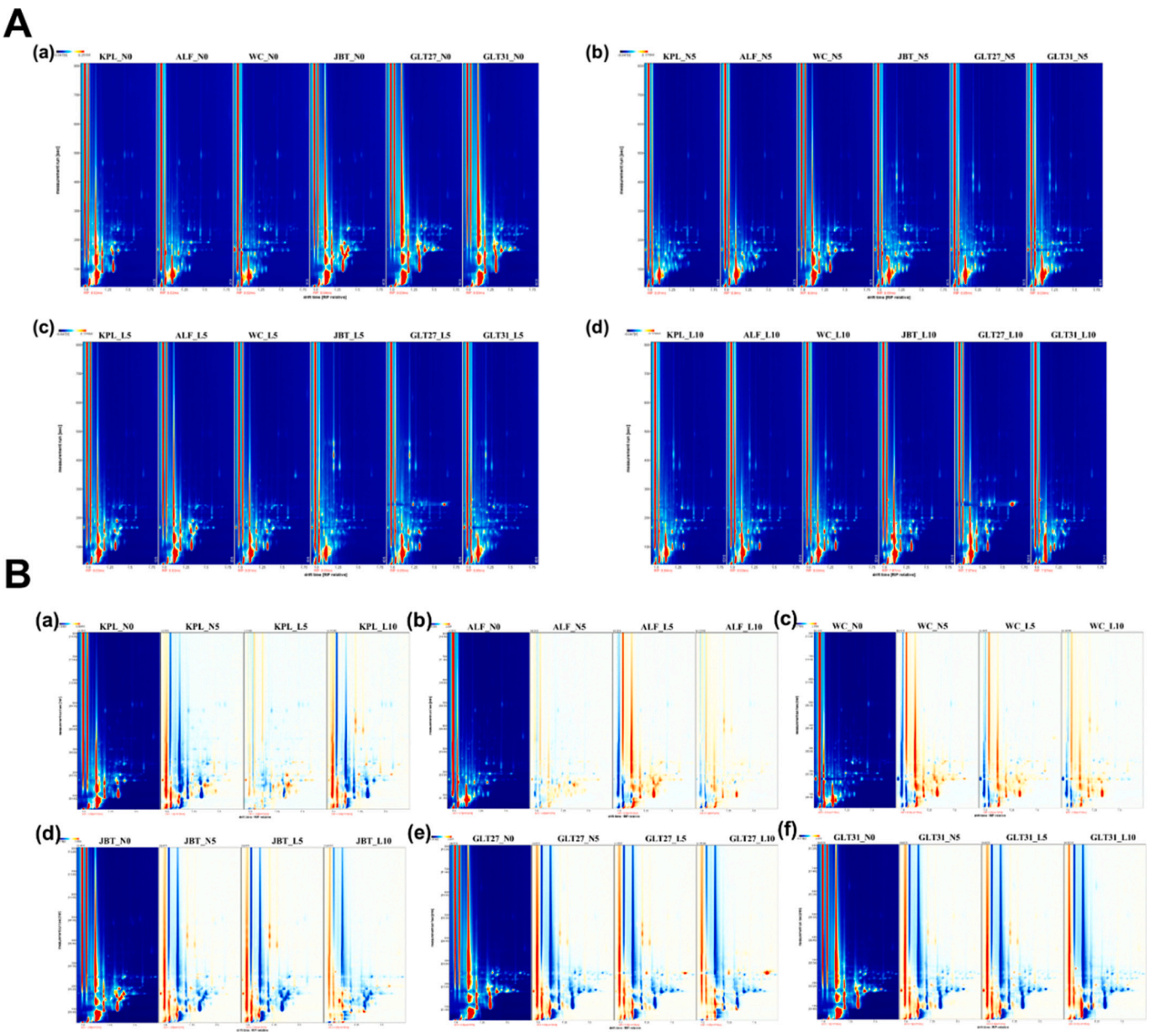

Fig. 4. The two-dimensional spectrum of volatile compounds in six sweet corn cultivars under four different post-harvest storage conditions (top view).

the retention and drift times of 100–200 s and 1.0–1.3 s, respectively (Fig. 4A-(a)). Consistent with the above results, the retention and drift times were different among six accessions under N5 (5 days at normal temperature), L5 (5 days at low temperature), and L10 (10 days at low temperature). These results indicated that a large number of volatile components were different between temperate and tropical accessions, suggesting that six sweet corn cultivars had unique flavors and the cultivars with similar ancestry had similar flavors.

To compare the characteristics of volatile components in sweet corn accessions under different storage conditions, the difference comparison model of the internal software was used. The topographic plot of each freshly harvested variety was chosen as a reference and deducted from the other sample plots (Fig. 4B). The results revealed that as storage time increased, the volatile components of the temperate accessions significantly changed but those of the tropical accessions slightly changed. This indicated that storage time has a significant impact on the flavor of the three temperate accessions.

3.3.2. Variations in the volatile components and flavor among six cultivars of sweet corn under various post-harvest storage conditions according to GC-IMS

To assess whether the volatile components influenced the flavor of sweet corn, the RI and drift time were used to identify each volatile compound (Fig. S4 and Table S2). In total, 50 organic volatiles were assessed, including 5 alcohols, 11 aldehydes, 1 arenes, 6 esters, 2 heterocyclic compounds, 6 ketones, 1 olefin, 1 phenol, and 17 unidentified compounds (Tables S2 and S3). The average peak area represents the amount of substance (Table S3 and Fig. 5A). High levels of alcohols (885.98) and esters (1019.26) were found in the fresh JBT kernels. However, the levels of alcohols and esters in other samples ranged from 36.06 (WC; L5) to 46.06 (ALF; N0) and 29.55 (JBT; L10) to 258.75 (WC; N0), respectively. Among all components, aldehydes exhibited the highest content, indicating that GC–IMS was more sensitive to aldehydes. This is consistent with a previous study (Chen et al., 2021). Interestingly, aldehyde contents in the temperate accessions first increased at 5-day storage and further decreased at 10-day storage. In contrast, the aldehydes in the tropical accessions initially decreased at 5-

day storage and further slightly increased at 10-day storage.

To visualize the differences in volatile components in six sweet corn cultivars under four post-harvest storage conditions, a gallery plot was created. Fig. 6 shows the fingerprints of all the samples, with each row denoting a sample and each column a volatile compound. Each sample included two replicates. The substances in the green and red boxes indicate generally higher content in the temperate and tropical accessions, respectively. For the freshly harvested six sweet corn cultivars, the gallery plot (Fig. 6), heatmap (Fig. 5B), and PCA analysis (Fig. 5C) consistently distinguished the three temperate accessions from the three tropical accessions. GLT27 and GLT31 exhibited similar characteristics of volatile components. Moreover, the contents of the volatile components in the green and red boxes were different among the temperate and tropical accessions, respectively. For instance, maltol and methional contents were higher in KPL; acetophenone and E_2_octenal contents were higher in ALF; nonanal, octanal, E_2_heptenal, heptanal, hexanal, acetal, 1_hexanol, 1_pentanol, 2_hexanone, and 2_pentylfuran contents were higher in WC; 2_pentanone, p_xylene, and styrene contents were higher in JBT; acetoin, 3_methylbutanol, and ethyl acetate contents were higher in GLT27; 3_sec_butyl_2_methoxypyrazine, ethyl 3-methylbutanoate and ethyl 2-methylpropanoate contents were higher in GLT31. Notably, irrespective of how volatile components change in all samples, both the clustering analysis (Fig. 5B) and PCA analysis (Fig. 5C) could divide the six sweet corn cultivars into two groups, which were the temperate and tropical accessions. After storage for 5 days under normal temperature, the content of volatile compounds in sweet corn significantly decreased (Fig. 6B), indicating that low temperature is beneficial for maintaining the aroma quality of sweet corn kernels. Methional, ethanol, ethyl acetate, and 3_methylbutanol contents were higher in WC. Nonanal, E_2_octenal, gamma_octalactone, E_2_heptenal, acetoin, 1_hexanol, hexanal, octanal, 2_pentylfuran, heptanal, 1_pentanol, acetophenone, and Z_3_hexenyl acetate contents were higher in JBT. Similarly, each of the six sweet corn cultivars exhibited specific and different volatile components after storage for 5 and 10 days under low temperature (Figs. 6C and D). In summary, the volatile component content significantly differed among the six sweet corn accessions. Moreover, the impact of storage conditions on the flavor of six cultivars varied. According to the volatile components, the six sweet corn cultivars could be divided into two groups, which were the temperate and tropical accessions. Therefore, this demonstrated that the genetic factors have significant effects on the flavor of sweet corn.

According to the peak area of volatile compounds detected by GC-IMS (Table S3), the amount of ethyl acetate (esters; fruity) was the highest compared with that of other volatile compounds, followed by ethanol (alcohols; sweet flower) and acetone (ketones; bitter almond), indicating that fruity and sweet flower might the main order of the fresh sweet corn. In addition, fatty fragrance was the second main order followed by sweet flower according to the radar chart of the samples (Fig. S5). However, the volatile compounds with high contents in fresh sweet corn were different from the detected volatile compounds in cooked sweet corn (Yactayo-Chang et al., 2022) and sweet corn juice (Feng et al., 2020), indicating that different sweet corn materials and their treatments result in the different volatile profiles. Nevertheless, ethyl acetate was a dominate volatile compound in artisan bakeries and mung bean flour (Sanmartín et al., 2024; Xue et al., 2024), indicating that anaerobic respiration pathway plays an important role in the production of the main volatile compound. The trend of change of ethyl acetate and ethanol was similar in all samples (Fig. 7A). Fresh sweet

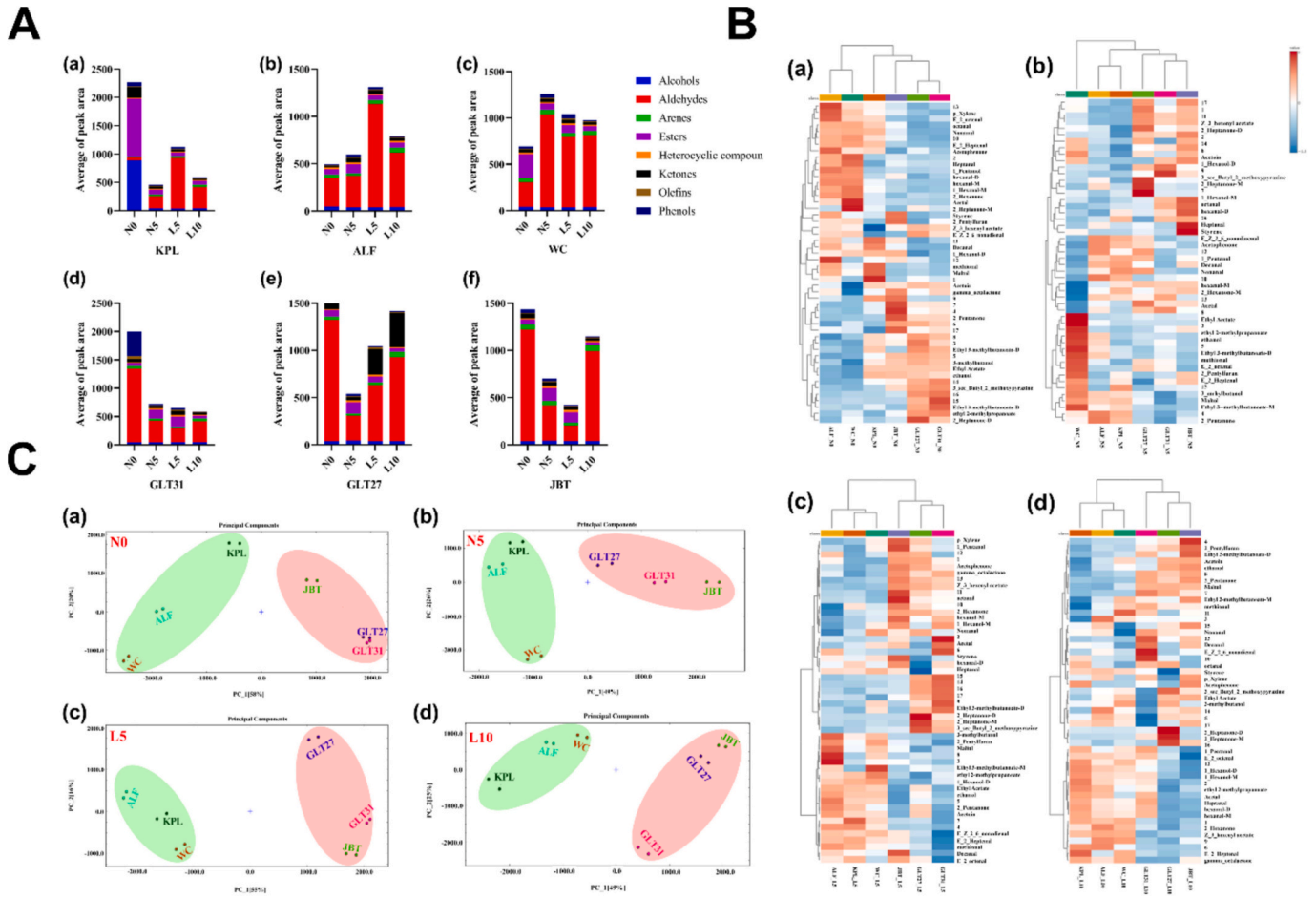

Fig. 5. Analysis of volatile components of various sweet corn samples as detected by GC–IMS under four different post-harvest storage conditions.

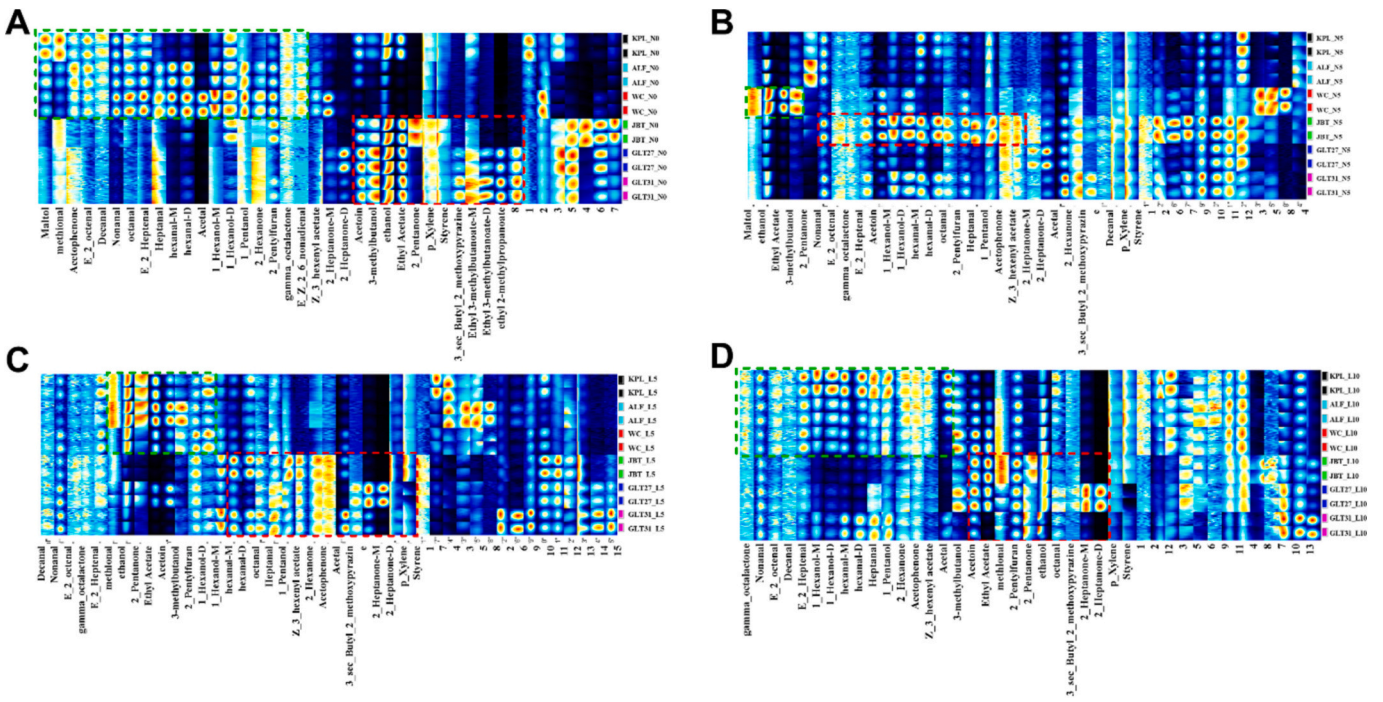

Fig. 6. Gallery plot fingerprint of sweet corn samples under different post-harvest storage conditions.

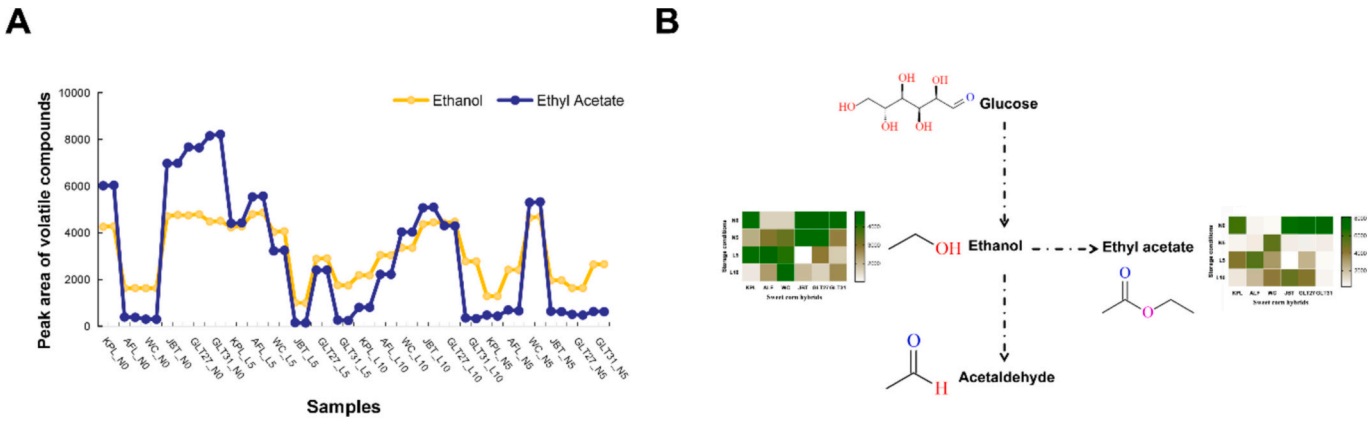

Fig. 7. The change trend (A) and metabolic pathway (B) of ethyl acetate and ethanol under four post-harvest storage conditions of six sweet corn cultivars using GC-IMS data.

corn with husks exhibits anaerobic respiration during growth and storage, where glucose metabolism produces ethanol, followed by the production of ethyl acetate (Fig. 7B). For the temperate and tropical accessions, the amount of ethyl acetate and ethanol in the freshly harvested sweet corn of tropical accessions was higher than that of temperate accessions. This was consistent with the trend of differences in solute sugar content. Therefore, the fruity flavor may not only come from sugar but also from ethyl acetate. Moreover, at the fresh harvest (N0), the highest amount of ethyl acetate was observed in JBT, GLT27, GLT31, and KPL, and the lowest amount of ethyl acetate was observed in AFL and WC. This suggested the influence of genetic factors on the flavor differences of sweet corn. Furthermore, various storage conditions exhibited diverse effects on different sweet corn cultivars and volatile compounds. Low temperature storage (L5 was better than L10) was beneficial for the preservation of ethyl acetate. Furthermore, low temperature storage was better than normal temperature storage for the retention of flavor in the majority of sweet corn accessions in this study. Interestingly, WC was a special cultivar because the sweet flowery and fatty flavors were the highest under normal temperature storage for 5 days (Fig. S5), indicating that WC has a special genetic background compared with other sweet corn cultivars.

Based on the VIP values calculated by PLS—DA model according to the data from GC–IMS (Fig. S6), the marker substances were identified to distinguish the samples. Under the same storage conditions, acetal and 3-s-butyl-2-methoxypyrazine were the key substances in freshly harvested samples; ethyl acetate and acetal were the key substances in samples stored for 5 days under normal temperature; 2_hexanone-D and 2_hexanone-M were the key substances in samples stored for 5 and 10 days under low temperature. For the same accessions under four different storage conditions, 2_pentanone in ALF and KPL, acetal in WC and JBT, 2_hexanone-D in GLT27, and ethyl 2-methylpropanoate in GLT31 were the marker volatile components. Moreover, 2_hexanone-D and acetal were relatively important marker volatile metabolites (Fig. S6K). Further research on the effects of these compounds on human health and the metabolic pathways of these volatile compounds is needed, which will guide the molecular breeding process for sweet corn

hybrids. The emission of volatile compounds from plants can be altered by plant viruses transmitted by aphids (Zhang et al., 2024). Therefore, these compounds may be the indicators of pest attacks and may be useful for generating pest-resistant crop varieties through genetic methods.

3.4. Comparison of volatile metabolites in six sweet corn cultivars under different post-harvest storage conditions using GC–MS and GC–IMS methods

The volatile metabolites of six sweet corn cultivars under different storage conditions were analyzed using GC–MS and GC–IMS. These two methods exhibit different sensitivities for the identification of different volatile metabolites (Chen et al., 2021; Guo et al., 2018). Some studies (Zhang et al., 2022) reported that GC–IMS identifies acids and furan compounds more precisely than GC–MS; however, GC–MS was more sensitive to aldehydes and benzene ring compounds in green wheat samples. This is slightly inconsistent with our study. A previous study reported that GC–MS was more sensitive to pyrazines, whereas GC–IMS measured aldehydes and ketones more accurately (Chen et al., 2021). Therefore, the samples from different organisms have a significant impact on the detection ability of GC–MS and GC–IMS. In all samples in this study, seven categories including phenols, olefins, ketones, heterocyclic compounds, esters, arenes, and aldehydes were detected by both methods. However, alcohols were only detected by GC–IMS, and alkanes, amides, salts, ethers, and acids were only detected by GC–MS. Furthermore, only three common volatile metabolites (nonanal, decanal, and 2-heptanone) were detected by GC–MS and GC–IMS in all samples, indicating these three volatile metabolites are stable and important for the flavor of sweet corns. Moreover, GC–IMS could distinguish the monomers and dimers of the same compounds but GC–MS could not.

Thus, for the comprehensive analysis of the volatile compounds in food samples, a combination of GC–MS and GC–IMS is currently required (Chen et al., 2021). However, the six sweet corn cultivars under all storage conditions in our study could be divided into two groups, which were the temperate and tropical accessions, according to the volatile components detected by GC–MS or GC–IMS. Moreover, Yang et al. proposed that plant-modified metabolites detected by a widely targeted metabolite modificomics (WTMM) strategy can be used for plant biomarker development (Yang et al., 2024). Therefore, the key volatile compounds identified by GC–MS or GC–IMS could be considered as biomarkers of different germplasms.

4. Conclusion

To the best of our knowledge, this is the first study to comparatively analyze sugar and moisture contents and volatile metabolites of temperate and tropical sweet corn cultivars under different post-harvest storage conditions. The results revealed that tropical sweet corn accessions exhibited higher contents of soluble total sugar, moisture, and soluble reducing sugar. Furthermore, 169 volatile substances in the six cultivars were identified: 119 using GC–MS and 50 using GC–IMS. Additionally, alkanes were important substances that not only contributed to the flavor of sweet corn but also distinguished different sweet corn accessions according to GC–MS results. Moreover, the highest peak area of ethyl acetate and ethanol was 8188.2 and 4833.4 respectively. These two volatile substances exhibited higher content than others, and a similar trend of change was observed in GC–IMS. In addition, storage conditions had a greater impact on the flavor of temperate sweet corn cultivars. In addition, clustering and PCA from GC–MS and GC–IMS clearly indicated that the six sweet corn cultivars could be divided into two groups, which were the temperate and tropical accessions, according to the volatile components. In summary, this study provided excellent genetic resources for enhancing sweetness using tropical sweet corn accessions and improving flavor using temperate sweet corn accessions.

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CRediT authorship contribution statement

Lihong Zhai: Writing – review & editing, Writing – original draft, Project administration, Investigation, Funding acquisition, Conceptualization. **Yunqi Tang:** Software, Methodology, Data curation. **Mingfei Dong:** Software, Methodology, Formal analysis, Data curation. **Gengshen Chen:** Software, Methodology, Data curation. **Yang Wang:** Writing – review & editing, Software, Methodology, Investigation. **Feng Teng:** Writing – review & editing, Writing – original draft, Resources. **Jun Huang:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that none of their known financial conflicts or interpersonal connections could have influenced the work that was published in this paper.

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Data availability

Data will be made available on request.

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