



Effects of pasteurization technologies and storage conditions on the flavor changes in acidified chili pepper

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ABSTRACT

In this study, the effects of thermal processing (TP), high pressure processing (HPP), and preservatives addition, i.e. sodium metabisulfite (SMS), on flavor changes in acidified chili peppers were compared. In addition, their changes during different storage periods (25, 37, and 42 °C for 30 days) were also investigated. The results indicate that TP clearly changed the flavor properties of acidified chili peppers compared to other processing, such as an increase in organic acid contents and titratable acid (TA) values but a decrease in pH value, free amino acid (FAA) concentrations, and some aromatic compound contents (e.g., esters and aldehydes). For SMS groups, more bitter FAAs and higher alcohol concentrations were detected. Some terpenes (e.g., β -ocimene) significantly increased in samples after HPP ($P < 0.05$). In addition, storage conditions also clearly affected their flavor, particularly for high storage temperature. During storage, pH fast decreased but TA values and organic acids increased; FAAs firstly increased but followed decreased; esters and terpenes were the main compounds decreasing. Furthermore, some off-flavor related compounds were produced when samples were stored at high temperature, such as furans, aldehydes, and oxides. The outcome of this study could provide new insights into the effects of processing and storage conditions on flavor changes and guide production for the acidified chili pepper industry.

1. Introduction

Paojiao is a typical fermented Chinese chili pepper and it has been used as an important condiment in Southwest Chinese cuisine (e.g., Yunnan cuisine) for hundreds of years (Li et al., 2022; Shang, Li, et al., 2022a). However, a large amount of high-salt brine is used to produce *Paojiao*. When the used high-salt brines as waste water are discharged, it possibly causes serious pollution to the environment. To solve the problem, some other processing approach without fermentation has been tried and applied to produce low-salt and acidified chili pepper, expecting to have a similar flavor property to *Paojiao*. For this processing procedure, the acidified chili pepper is directly pickled with the unsalted vinegar solution and followed by pasteurization. The type of acidified chili pepper has gradually become popular in the southwest area of China due to its environmental friendly properties and microbiological safety (Tola and Ramaswamy, 2018). As known, flavor properties are

the main driving force for consumers to purchase pickled chili pepper products (Ye et al., 2022b). Although some studies have reported on the flavor properties of fermented chili peppers (Ye et al., 2022a), to the best of our knowledge, flavor changes of acidified chili pepper without fermentation have rarely been analyzed.

At the same time, different pasteurization technologies and storage conditions have been found to be the main indicators affecting the flavor of vegetable products (Bao et al., 2016; Kebede et al., 2015). Among pasteurization technologies, the thermal processing (TP) technique is a common method of food processing but possibly has a negative impact on organoleptic characteristics and chemical composition (Qu et al., 2021). On the other hand, high pressure processing (HPP) as one of the representative non-thermal processing technologies has been widely used in vegetable processing, especially it has been cataloged as a less impact technique on flavors (Marszalek et al., 2018). For example, compared with TP, pickled radish pasteurized by HPP showed a similar flavor profile to the control (Bao et al., 2016). In addition to

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List of abbreviations:

TP	Thermal processing
HPP	High pressure processing
SMS	Sodium metabisulfite
HS-SPME-GC-MS	Headspace-solid phase microextraction-gas chromatography-mass spectrometry
LV	Latent variable
MVDA	Multivariate data analysis
PLS-DA	Partial least squares discriminant analysis
RI	Retention index
VID	Variable identification
TA	Titrateable acid

pasteurization technologies, the rational use of preservatives is another effective way to extend shelf-life. Among the preservatives, sodium metabisulfite (SMS) as a metabisulfite salt is widely used to not only control microorganisms but also inhibit non-enzymatic browning and enzyme-catalyzed reactions (de Araújo Soares et al., 2021). Furthermore, storage conditions can also affect the quality of vegetable products, which is highly related to large amounts of biochemical reactions occurred during storage time (Korkmaz et al., 2020). Storage conditions (e.g., storage temperatures and time) mainly affect flavor changes of vegetable products during prolonged shelf-life and transportation. For example, the content of heptanal and 2-methylbutanal in carrot puree was largely increased at elevated storage temperatures (Kebede et al., 2015). However, researches on the influence of processing and storage conditions on flavor properties of acidified chili pepper were scarcely reported.

In the present study, the effects of processing treatments (HPP, TP, and SMS) and storage conditions (stored at 25, 37, and 42 °C for 30 d, respectively) on the taste and aroma of acidified chili pepper were investigated. Considering large and complex datasets obtained in the study, a multivariate data analysis (MVDA) was used to extract the main changed key aroma/taste compounds in acidified chili peppers and follow their change trends during processing and storage. The findings of this work may provide new insights into the flavor profile and changes of acidified chili pepper and guide production for the acidified chili pepper industry.

2. Materials and methods

2.1. Materials and reagents

Green peppers (*Capsicum frutescens* L.) are provided by the Yunnan Hongbin Green Food Group Co. LTD (Yunnan, China). Peppers were selected with the same size, color, and weight without visible blemishes, disease, and/or physical damages.

Food additives including acetic acid, citric acid, calcium chloride, sodium D-isoascorbate, monosodium glutamate, and sodium metabisulfite were purchased from Shandong haihua company limited (Shandong, China). The reference standard of isoamyl acetate and organic acids (oxalic acid, lactic acid, malic acid, acetic acid, citric acid, tartaric acid, quinic acid, fumaric acid, and succinic acid) were purchased from Aladdin (Shanghai, China), n-alkanes (C3–C9, C10–C25) were supplied by O2si (North Charleston, SC), and amino acid solution (type H) was acquired from Wako (Wako-shi, Japan). High-performance liquid chromatography (HPLC) grade acetonitrile and methanol were obtained from Sigma-Aldrich (Milan, Italy).

2.2. Sample preparation

The acidified liquid (90 mL) consists of 0.75% acetic acid, 0.75%

citric acid, 0.1% calcium chloride, 0.18% sodium D-isoascorbate, and 0.18% monosodium glutamate. The fresh samples (30 g) were immersed in 90 mL of the acidified liquid, and they then were packed into a vacuum food bag (90 mm × 130 mm × 0.16 mm) by DZD-500/2SC vacuum packing machine (Tengtong Co., Ltd., Jiangsu, China).

2.3. Processing and storage conditions

Acidified chili pepper was treated with HPP, TP, and SMS for pasteurization, respectively. TP was carried out using a thermostatic water bath (Shanghai Boxun Industry & Commerce Co., Ltd., Shanghai, China) and held at 80 °C for 20 min (Bao et al., 2016). Then samples were cooled to room temperature for storing. HPP was conducted in a 10-L high-pressure vessel (HPP-10 L/600 MPa, Baotou KEFA High Pressure Technology Co., Ltd., Baotou, China) at ambient temperature (20 °C). The pressure increased at the rate about of 600 MPa/min and held for 5 min at 600 MPa (Li et al., 2021). Then the pressure was released immediately. SMS treatment was carried out by adding 0.1 g/kg sodium metabisulfite into the acidified liquid, which is under the limit of that the sodium metabisulfite concentration in pickled vegetables is 0.1 g/kg according to the Chinese national standard for food safety (GB 2760-2014). The resulting solution was mixed with peppers and put into vacuum food bags immediately and packed.

Finally, samples of each treatment were stored at 25 °C, 37 °C, and 42 °C for 30 days, respectively according to a previous study (Kebede et al., 2015). The taste properties of acidified chili pepper, including pH, TA, organic acids, and free amino acids, were measured at 0, 1, 3, 5, 7, 14, 21, and 30 d, respectively. The volatiles of acidified chili pepper were measured at 0 and 30 d.

2.4. pH and titrateable acid (TA) determination

Samples were homogenized and weighed for pH value and TA determination. The pH value of acidified chili pepper was detected by a pH meter (Mettler Toledo, Shanghai, China). The TA, which was expressed in percentage acetic acid, was determined by an automatic potentiometric titrator (907 GPD titrino, Metrohm, Switzerland) with 0.05 mol/L NaOH until the pH value was reached 8.1 (Ye et al., 2022b). The TA of acidified chili peppers was calculated by Equation (1).

$$TA \text{ (as \% acetic acid)} = \frac{C \times V \times K}{m} \times 100 \quad (1)$$

where C is the concentration of NaOH (0.1 mol/L); m is the weight of acidified chili peppers; V (mL) is the volume of used NaOH; and K is the conversion factor of citric acid.

2.5. Organic acid determination

The extraction procedure of organic acids was in accordance with a previously described method (Shang et al., 2022b). The extract was determined by a HPLC system (Agilent 1260, Agilent Technologies, USA) with a Prevail Organic Acid column (250 mm × 4.6 mm, 5 μm particle size, Avantor, New Jersey, USA). The injection volume was 30 μL. The 25 mmol/L potassium dihydrogen phosphate buffer (pH 2.5) was used as the mobile phase and the flow rate was 0.8 mL/min. A UV-DAD detector was set at 210 nm. The analyses for each extract were conducted in triplicate. External standards were conducted for the quantification of organic acids including oxalic, lactic, malic, acetic, citric, tartaric, quinic, fumaric, and succinic acids.

2.6. Free amino acid determination

Extraction and analysis of free amino acid (FAA) followed the method of our previously reported procedures with minor modifications (Ye et al., 2020). After being vortexed with trichloroacetic acid (10 g/L),

the homogenate of acidified chili peppers was left at room temperature for 1 h. Then, centrifuging the mixture at 4000 g for 20 min and filtering supernatant by 0.22 µm syringe filters. The injection volume was 20 µL. An amino acid analyzer (L-8900, Hitachi, Tokyo, Japan) equipped with an ion-exchange resin 2622 column (4.6 mm × 60 mm, 3 µm) and a UV detector was used at 570 and 440 nm. Each sample underwent a triple analysis.

2.7. Volatile compound analysis

The volatile compounds were analyzed using the method of our previous work with some modifications (Yi et al., 2018). Briefly, a screw-capped amber glass vial with a PTFE/silicone septum seal was filled with 3 g sample homogenates, 3 mL of saturated NaCl solution were added and vortexed for 1 min. An isoamyl acetate (0.1 µg/mL) as internal standard (IS) was spiked (200 µL) with a gastight syringe before the sample analysis. To equilibrate the solution and headspace, the vials were incubated at 40 °C for 15 min under agitation at 500 rpm. Volatile compounds in the headspace were extracted using SPME fiber coated with 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Zhenzheng, Qingdao, China) with the same condition for 40 min. The volatile compounds were thermally (250 °C) desorbed from the fiber into the injector port of the gas chromatography (GC) for 5 min.

A GC–MS system (QP2010, Shimadzu, Japan) equipped with a HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness, Agilent Technologies, USA) was used for the separation and detection of volatiles. Helium (purity >99.99%) was used as a carrier gas at a constant flow of 2.0 mL/min. The temperature of the column oven was maintained at 45 °C for 5 min at first and then was elevated to 250 °C at a rate of 5 °C/min, held at 250 °C for 2 min. The mass spectra were captured using the scanning range of m/z 35–500 and an electron ionization mode at 70 eV. Ion source and transfer line temperatures for the mass spectra were 230 °C and 280 °C, respectively. The six replications were used to determine the volatile content of each sample.

Tentative identification of volatile compounds was performed by comparison of the database from the NIST 2014 library and by comparison of the experimentally determined retention index (RI), which was calculated using n-alkanes (C3–C25, Shanghai, China) as the external reference under the same operating conditions, with literature data.

2.8. Data analysis

2.8.1. Kinetic modeling

The first-order fractional conversion model (Equation (2)), one of the empirical kinetic models, was used to evaluate the dynamic change of pH and TA in acidified chili peppers during storage (Shang et al., 2022b).

$$C = C_{\infty} + (C_0 - C_{\infty}) \exp(kt) \quad (2)$$

In the equation, C is the parameter value at a specific storage time (days), C_0 is the initial value of storage time (day 0), C_{∞} is the value of the stable fraction, and k is the reaction rate constant (days^{-1}), and t is the number of storage time. The first-order fractional conversion model was carried out by OriginPro 2021 software (Origin Lab Corporation, USA).

2.8.2. Multivariate data analysis

The clustered heatmap was plotted by TBtools (version 1.098) (Ni et al., 2022). All untreated volatile data were analyzed by MVDA on Solo (Version 9.0, 2020; Eigenvektor Research, Manson, WA) (Yi et al., 2018). Variable identification coefficients (VID) were used to quantitatively select the discriminant volatile compounds per group.

Table 1

The pH, titratable acid, organic acids, and free amino acids in acidified chili peppers after different processing.

	Untreated	TP	HPP	SMS
pH	4.43 ± 0.01 ^a	4.21 ± 0.05 ^c	4.31 ± 0.03 ^b	4.42 ± 0.05 ^a
TA (as % Acetic acid)	0.50 ± 0.01 ^b	0.64 ± 0.01 ^a	0.51 ± 0.01 ^b	0.51 ± 0.01 ^b
Organic acids (mg/g)				
Oxalic acid	0.51 ± 0.04 ^c	0.90 ± 0.02 ^a	0.62 ± 0.00 ^b	0.53 ± 0.01 ^c
Tartaric acid	0.09 ± 0.01 ^c	0.20 ± 0.00 ^a	0.13 ± 0.01 ^b	0.13 ± 0.00 ^b
Quinic acid	0.95 ± 0.02 ^c	1.66 ± 0.01 ^a	1.09 ± 0.02 ^b	1.09 ± 0.01 ^b
Malic acid	2.13 ± 0.09 ^c	2.63 ± 0.02 ^a	2.31 ± 0.09 ^b	2.72 ± 0.03 ^a
Lactic acid	0.62 ± 0.03 ^c	1.39 ± 0.01 ^a	0.67 ± 0.01 ^c	1.08 ± 0.02 ^b
Acetic acid	1.06 ± 0.05 ^c	2.29 ± 0.01 ^a	1.23 ± 0.02 ^b	1.29 ± 0.02 ^b
Citric acid	2.30 ± 0.11 ^c	3.81 ± 0.02 ^a	2.71 ± 0.08 ^b	2.45 ± 0.05 ^c
Succinic acid	0.40 ± 0.01 ^{ab}	0.44 ± 0.02 ^a	0.37 ± 0.06 ^{ab}	0.30 ± 0.05 ^b
Fumaric acid	0.01 ± 0.00 ^a			
Total organic acids	8.07 ± 0.17 ^d	13.33 ± 0.06 ^a	9.13 ± 0.22 ^c	9.60 ± 0.12 ^b
FAAs (mg/100g)				
Asp	13.99 ± 0.34 ^b	13.65 ± 0.31 ^b	12.04 ± 0.55 ^c	22.79 ± 0.90 ^a
Thr	77.12 ± 1.49 ^a	37.83 ± 0.96 ^c	63.78 ± 1.99 ^b	79.00 ± 1.60 ^a
Ser	40.22 ± 0.92 ^b	25.35 ± 0.69 ^d	32.26 ± 0.90 ^c	46.10 ± 1.27 ^a
Glu	59.04 ± 2.20 ^c	69.69 ± 0.71 ^a	63.11 ± 2.18 ^{bc}	64.23 ± 0.75 ^b
Gly	2.35 ± 0.08 ^a	0.94 ± 0.03 ^c	1.64 ± 0.04 ^b	2.26 ± 0.04 ^a
Ala	11.21 ± 0.41 ^a	6.21 ± 0.07 ^c	8.31 ± 0.28 ^b	11.46 ± 0.20 ^a
Cys	1.82 ± 0.11 ^{ab}	0.78 ± 0.10 ^c	1.71 ± 0.03 ^b	2.02 ± 0.05 ^a
Val	10.64 ± 0.24 ^b	6.34 ± 0.13 ^d	8.56 ± 0.25 ^c	11.47 ± 0.16 ^a
Met	1.75 ± 0.04 ^b	0.56 ± 0.03 ^d	1.15 ± 0.05 ^c	2.04 ± 0.03 ^a
Ile	4.37 ± 0.10 ^b	2.57 ± 0.05 ^d	3.60 ± 0.14 ^c	5.12 ± 0.08 ^a
Leu	6.56 ± 0.22 ^a	1.99 ± 0.02 ^c	4.00 ± 0.16 ^b	6.92 ± 0.11 ^a
Tyr	7.03 ± 0.23 ^a	3.89 ± 0.03 ^c	5.99 ± 0.20 ^b	7.36 ± 0.09 ^a
Phe	11.97 ± 0.31 ^b	5.07 ± 0.07 ^d	9.79 ± 0.39 ^c	13.28 ± 0.03 ^a
Lys	9.85 ± 0.22 ^b	5.11 ± 0.04 ^d	7.04 ± 0.35 ^c	10.43 ± 0.14 ^a
His	6.75 ± 0.18 ^{ab}	4.40 ± 0.10 ^c	6.35 ± 0.72 ^b	7.52 ± 0.12 ^a
Arg	11.99 ± 0.48 ^b	5.00 ± 0.11 ^c	8.66 ± 0.49 ^d	20.65 ± 0.62 ^a
Pro	2.91 ± 0.16 ^a	0.00 ± 0.00 ^d	1.90 ± 0.13 ^c	2.58 ± 0.10 ^b
Umami FAA	73.03 ± 2.53 ^b	83.34 ± 0.98 ^a	75.16 ± 2.71 ^b	87.02 ± 0.91 ^a
Sweet FAA	132.64 ± 2.86 ^b	70.88 ± 1.57 ^d	107.15 ± 3.26 ^c	140.87 ± 3.06 ^a
Bitter FAA	69.17 ± 1.90 ^b	34.37 ± 0.49 ^d	53.97 ± 2.66 ^c	82.76 ± 1.07 ^a
Total FAA	279.57 ± 7.48 ^b	190.05 ± 3.88 ^d	239.89 ± 8.76 ^c	315.24 ± 4.12 ^a

Mean ± standard deviation. Different letters in the same column indicate significant differences determined by Tukey's HSD test ($P < 0.05$).

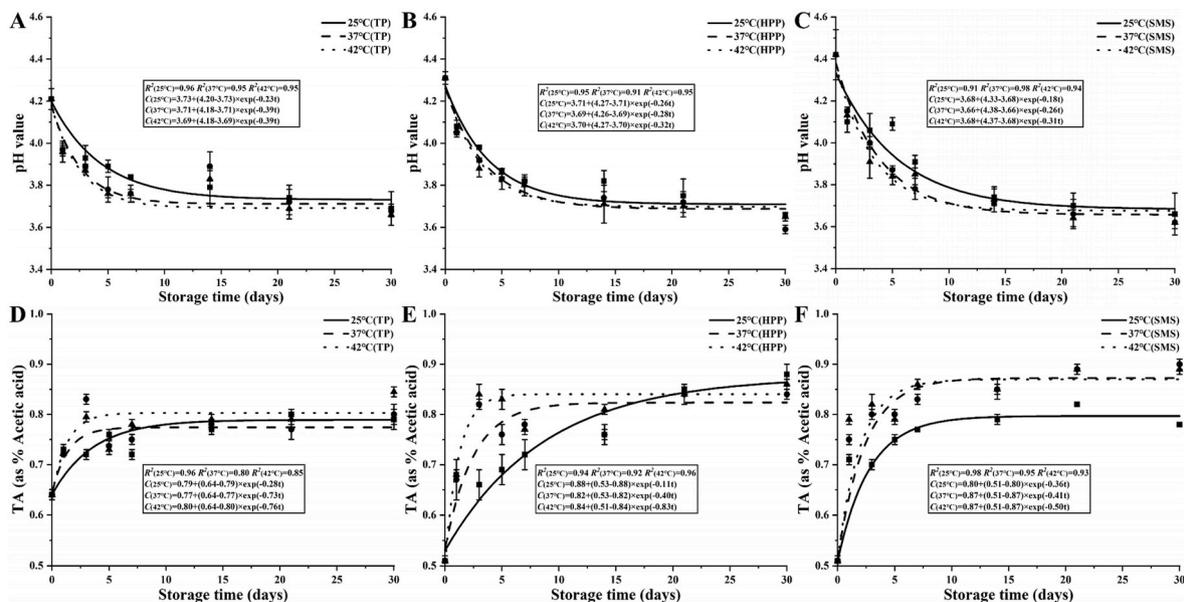


Fig. 1. Changes in pH value and titratable acidity values of acidified chili pepper treated by TP (A and D), HPP (B and E), and SMS (C and F) during storage, respectively. The full lines represent the fitted values by kinetic modelling and the different lines represent the experimental data.

2.8.3. Statistical analysis

Results were analyzed by SPSS statistics software (version 24) and all plots were made by OriginPro 2021 software (Origin Lab Corporation, USA). The data were presented as mean value \pm standard deviation. One-way ANOVA and Tukey's test were performed at a significance level of 0.05 to determine the significant effects of discriminant volatile compound contents.

3. Results and discussion

3.1. Taste properties

3.1.1. pH and TA

The pH and TA play important roles in the taste of acidified chili pepper. According to previous studies (Cao et al., 2017), the pH value (3.2–4.2) and TA (0.6%–2.4%) of acidified chili pepper were considered to eat. As shown in Table 1, the initial pH and TA values of untreated samples were 4.43 and 0.50%, significantly decreased to 4.21 and increased to 0.64% after TP treatment, respectively ($P < 0.05$). The results showed that TP treatment changed the structure of pepper cells, allowing for facilitated continued acidification during processing (Kamat et al., 2018). Both pH and TA values had no significant changes after HPP and SMS treatments ($P > 0.05$), which were similar to previous studies (Wu et al., 2021). However, the pH and TA values in acidified chili pepper showed remarkable changes during storage, and the changes mainly occurred at the beginning of the storage period (Fig. 1). The pH values were decreased to approximately 3.69 after 14 days in all treatment groups during storage. Accordingly, TA values were observed to increase gradually to approximately 0.80% after 7 days (Fig. 1). This is due to the pH equilibration between acidified liquid and peppers (Tola and Ramaswamy, 2013). Based on our previous study on fermented chili pepper (Ye et al., 2022b), fermented chili pepper and acidified chili pepper have a similar pH value, but fermentation period was lasted 3 months. Therefore, the preparation of acidified chili pepper is much faster than that of fermented chili pepper.

In addition, fractional conversion kinetic modeling was modeled for pH ($R^2 > 0.91$) and TA ($R^2 > 0.80$) of acidified chili peppers during storage (Fig. 1). The change rate (k value) of pH was increased from 0.23 to 0.39 days⁻¹, 0.26 to 0.32 days⁻¹, and 0.18 to 0.31 days⁻¹ in TP, HPP, and SMS groups, respectively, when the storage temperature increased

from 25 °C to 42 °C. Similarly, the k value of TA was increased from 0.28 to 0.76 days⁻¹, 0.11 to 0.83 days⁻¹, and 0.36 to 0.50 days⁻¹ in TP, HPP, and SMS groups, respectively, as the storage temperature increased from 25 °C to 42 °C. The k values of pH and TA increased as the storage temperature increased, indicating that higher storage temperatures lead to the acceleration of the rate of the acidification reaction. The results were similar to the findings of Tola and Ramaswamy (2018) who reported that stored at higher temperatures could enhance the diffusion of acid in solid foods. Therefore, pH and TA values can be considered as potential indicators to evaluate the effect of storage temperatures on the sensory quality of acidified chili pepper during the accelerated storage.

3.1.2. Organic acids

The changes in organic acids of samples are shown in Table 1, including oxalic, lactic, malic, acetic, citric, tartaric, quinic, fumaric, and succinic acids. Compared with fermented chili pepper (Ye et al., 2022b), acidified chili pepper has a similar organic acid profile. However, its total organic acid concentration is lower than that of fermented chili pepper. After TP treatment, the concentration of total organic acids significantly ($P < 0.05$) increased from 8.07 mg/g to 13.33 mg/g, while had no significantly changed after HPP and SMS treatments ($P > 0.05$). The cluster analysis also showed that organic acids of acidified chili peppers after processing were clearly separated into two classifications, one classification included untreated, HPP, and SMS groups, another one included TP group. The results showed that TP can promote the release of organic acids from the food matrix (Qu et al., 2021).

Changes of organic acids in stored groups were investigated via the clustered heatmap, which could be divided into two classes (Fig. 2A). Class A₁ comprised 32 groups, including most of the 1–7 days stored groups. The concentration of organic acids increased within the initial 7 days and then decreased. Meanwhile, the concentration of total organic acids was increased as the storage temperature increased, especially in HPP- and SMS-stored groups. The increase of organic acids could lead to a reduction in pH value and an increase in TA content within the initial 7 days (Fig. 1). The results were similar to the previous study (Wu et al., 2015). However, it seems that the effect of storage time on organic acids was more dominant than that of storage temperature. During the late stage of storage, the losses of organic acids could be related to the oxidation of malic acid into oxaloacetate by the malate dehydrogenase/fumarase enzymes (Salur-Can et al., 2017; Shang et al., 2022b).

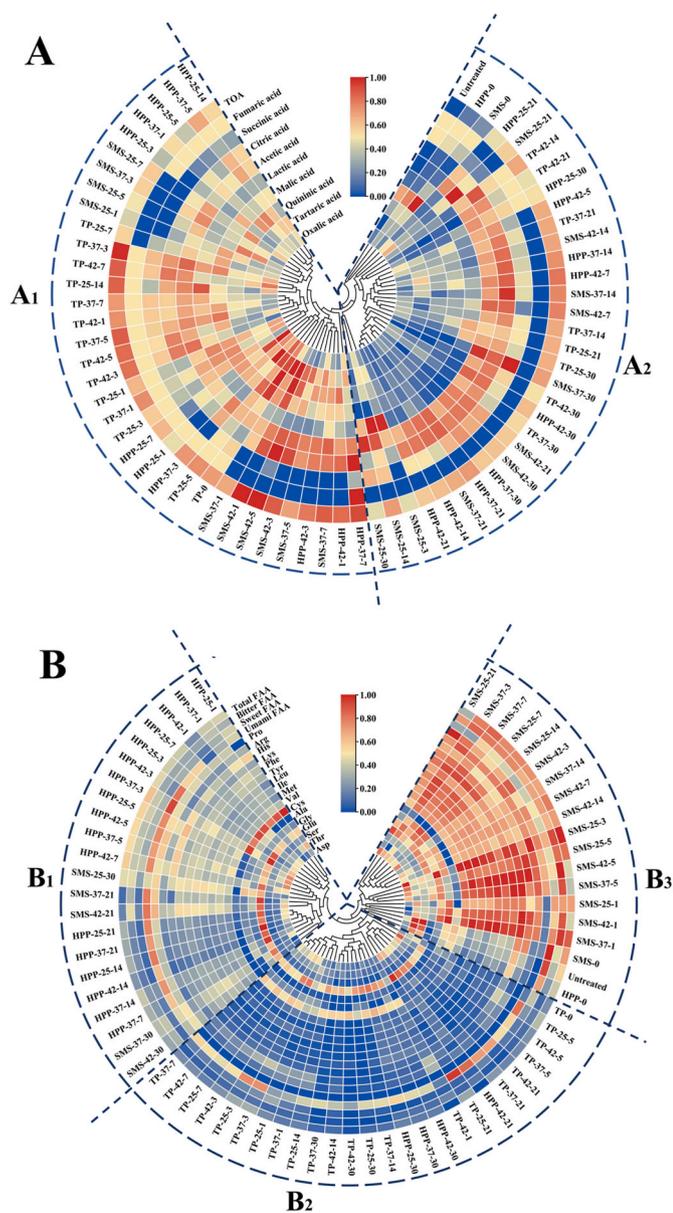


Fig. 2. Hierarchical clustering and heatmap visualization of organic acids (A) and free amino acids (B) content of acidified chili peppers. The colour intensity was based on a normalized scale from a maximum of 1 (red) to a minimum of 0 (blue), which indicated the abundance of the volatiles from high to low. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.1.3. Free amino acids

FAAs, which play an important role as precursors for aroma compounds, directly contribute to the taste of acidified chili pepper (Chen et al., 2019). It was reported that proteins could be degraded into various FAAs (Wu et al., 2015). Based on our previous study (Ye et al., 2022b), FAAs are classified into three groups, including umami taste (aspartic acid and glutamic acid), sweetness (serine, threonine, glycine, methionine, and alanine), and bitterness (tyrosine, isoleucine, leucine, phenylalanine, valine, lysine, arginine, and histidine). The changes in FAAs of samples are shown in Fig. 2B. Among them, the FAAs, Thr (sweet), Ser (sweet), Asp (umami), and Glu (umami) were detected to be the main FAAs in acidified chili peppers (Table 1). Similar results were observed in fermented pepper (Ye et al., 2020). The concentration of total FAAs was 279.57 mg/100g, 190.05 mg/100g, 239.89 mg/100g, and 315.24 mg/100g in untreated, TP, HPP, and SMS groups,

respectively. The results indicated that TP treatment could destroy protein structure or promote the degradation of FAAs (Wu et al., 2015). As a result, the content of Thr and Ser decreased by 50.95% 36.97%, respectively. However, HPP and SMS treatments had less effects on FAAs than TP treatment. It was reported that HPP can only disrupt non-covalent bonds rather than small molecules of proteins (Oey et al., 2008). Moreover, high pressure treatments are able to improve the solubility and hydrophobicity of food derived proteins, resulting in the increase of nitrogen index and free sulfhydryl group, which may contribute to the release of taste active protein and peptides (Wu et al., 2019, 2020). Besides, most FAAs had higher concentrations in SMS-stored groups, especially for bitter FAAs. The concentration of FAAs in HPP-stored groups were slightly lower than that in SMS-stored groups, which were higher than that in TP-stored groups. This also confirms the protective effect of HPP and SMS treatments on FAAs.

During storage, the concentration of FAAs increased within the initial 7 days and then decreased. The changes in FAAs were affected by the protein composition and changes in free amino acids, decarboxylation, and deamination reactions (Zhao et al., 2020). Besides, the concentration of FAAs was observed to decrease with increasing storage temperature. It was might due to the cellular biosynthetic activity and indigenous enzyme activity were inhibitory by higher storage temperature (Barba et al., 2017). The results showed that stored at 25 °C made a great contribution to protect the proportion of FAAs in acidified chili pepper.

3.2. Aroma properties

Aromas have been considered as the most influential factor to the acceptability of fermented food (Ye et al., 2020). Volatile compounds of acidified chili pepper after processing and storage were measured by headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS). Meanwhile, considering the complex correlations of the dataset, PLS-DA was used to determine the correlation between processing treatments (Fig. 4A) and storage conditions (Fig. 4B–D) with volatiles. The compounds with an absolute VID value higher than 0.900 was selected as the discriminant volatiles (Ye et al., 2022a).

3.2.1. Processing technologies

The representative total ion chromatogram of samples processed by different treatments were presented in Fig. 3. A total of 99 volatile compounds were detected in all treatment groups (untreated, TP, HPP, and SMS group), including 44 esters, 20 alkanes, 14 terpenes, 8 alcohols, 7 aldehydes, 2 acids, 1 ketone, 1 furan, 1 pyrazine, and 1 ether. Among them, 76, 69, 71, and 80 volatile compounds were detected in untreated, TP, HPP, and SMS groups, respectively. The key aroma compounds marked in total ion chromatogram were also indicated in Table 2. As shown, esters, alkanes, terpenes, alcohols, and aldehydes were the main volatile compounds detected in acidified chili pepper, which contributed to the typical fruity, herbal, sweet, and fresh odors for pickled chili pepper (Ye et al., 2022b). Some of the compounds were also reported in fermented chili pepper, such as esters (e.g., 4-methylpentyl 2-methylbutanoate, 4-methylpentyl 3-methylbutanoate, and 4-methylpentyl 4-methylpentanoate) and terpenes (e.g., (E)- β -ocimene and linalool) (Ye et al., 2020). In addition to the aromatic compounds, alkanes showed high concentration in acidified chili peppers. Even though, alkanes were not considered as an important contributor for the formation of typical odors of samples (Pino et al., 2011).

Fig. 4A clearly showed that four treatment groups of acidified chili pepper presented distinct separations, indicating different processing approaches led acidified chili peppers to different aroma profiles. Most of the compounds (shown with small open circles) were clustered around the untreated group, indicating that these compounds decreased clearly after processing, particularly for methyl salicylate, isoamyl isobutanoate, and isopentyl hexanoate. However, a few compounds

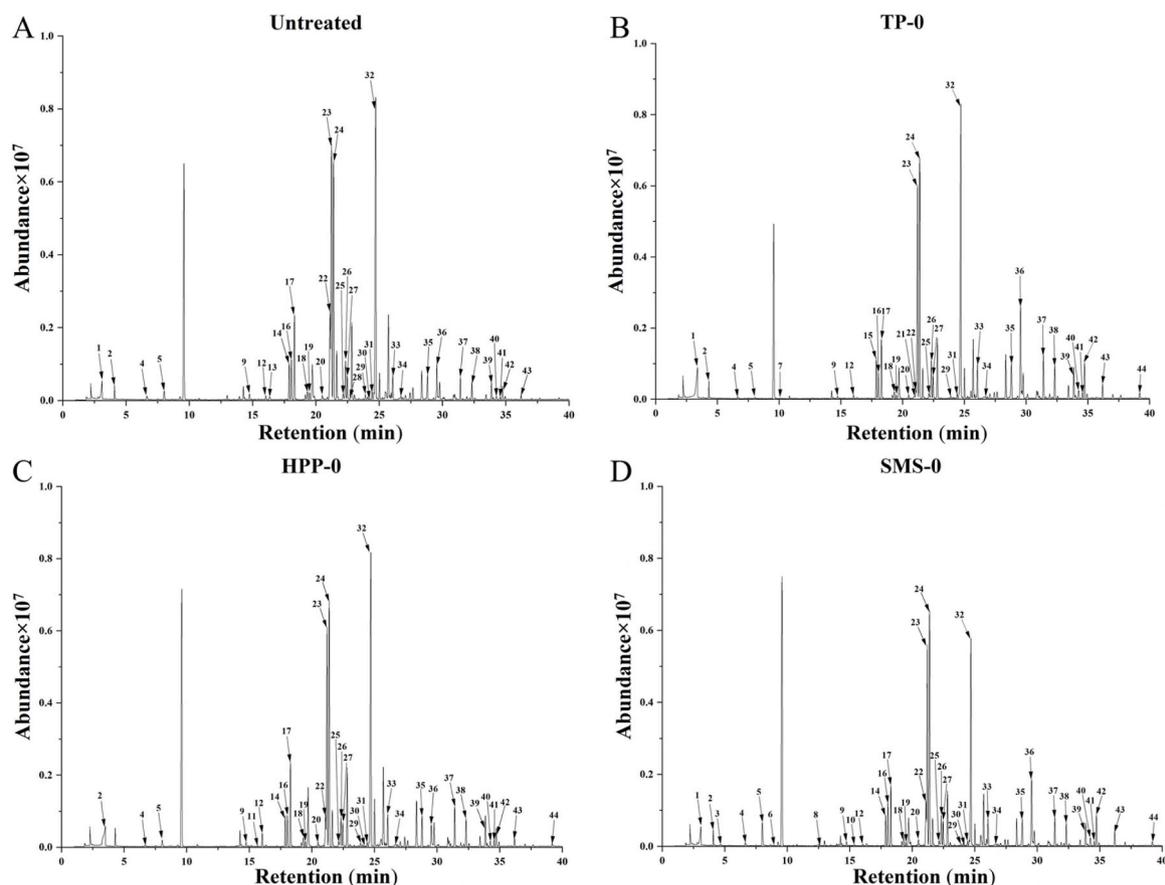


Fig. 3. Representative total ion chromatogram of the headspace volatile compounds of untreated (A) acidified chili pepper and that after TP (B), HPP (C) and adding SMS (D). Per chromatogram, the main volatile compounds are sorted by retention time and shown in [Table 2](#).

clustered around different treatment groups, demonstrating that the concentration of these compounds increased after corresponding processing technologies. For example, 2-heptanone were located closer at TP group and it was detected with high positive VID values (VID = 0.973) in TP group, indicating TP promoted an increase in content of 2-heptanone. The 2-heptanone formed after TP could be linked to thermal-induced unsaturated fatty acid degradation reactions ([Song et al., 2021](#)).

Esters were the abundant volatiles found in acidified chili peppers, but were largely influenced by processing. As shown in [Table 2](#), the sum concentration of ester compounds significantly decreased by 41.85%, 9.76%, and 24.12% after TP, HPP, and SMS treatments, respectively ($P < 0.05$). It demonstrated that esters tended to largely change during pasteurization processing, particularly for TP. Among the ester compounds, 3-methylbutyl 2-methylbutanoate, methyl salicylate, 4-methylpentyl 2-methylbutanoate, 4-methylpentyl 3-methylbutanoate, and 4-methylpentyl 4-methylpentanoate decreased by 100%, 92.99%, 32.90%, 28.88%, and 42.97% after TP ($P < 0.05$), respectively. Oxidative reactions and acid catalyzed hydrolysis induced by TP might be the main reason resulting in the content decrease of these compounds ([Roobab et al., 2021](#)). The decrease in ester contents would result in loss of fruity odor in acidified chili peppers ([Zhang et al., 2019](#)). In addition, compared to TP and SMS, HPP could better retain ester compounds in acidified chili peppers. A similar phenomenon has been observed in pineapple fruit juice treated by HPP ([Wu et al., 2021](#)).

According to VID values, cis- β -ocimene (VID = 0.975), (E)- β -ocimene (VID = 0.948), linalool (VID = 0.978), and α -terpineol (VID = 0.953) were detected in HPP and TP groups with positive VID values, respectively. The β -ocimene were significantly increased in samples after HPP, while some terpenoids (α -terpineol and linalool) were formed after TP ($P < 0.05$), compared to the untreated. The increase in β -ocimene

induced by HPP might due to the terpene synthase catalysis ([Degenhardt et al., 2009](#)). Monoterpene synthase could convert the substrate geranyl diphosphate to acyclic products, such as (E)- β -ocimene ([Degenhardt et al., 2009](#)). On the other hand, it seems that TP could promote the acid hydrolysis of glycosides, thereby leading to the rise in terpenoids, e.g., linalool and α -terpineol ([Marsol-Vall et al., 2019](#)). It might contribute more herbal notes to acidified chili peppers pasteurized by TP ([Ye et al., 2022a](#)).

An increase in the alcohol compounds of acidified chili peppers with an addition of SMS was observed. As shown in [Table 2](#), the 3-methyl-1-butanol, 4-methyl-3-pentanol-1-ol, and 2-ethyl-1-hexanol increased from 0 to 34.01, 18.57, and 25.95 $\mu\text{g}/\text{kg}$ in SMS group, respectively. In addition, the 4-methyl-1-pentanol increased by 127.74% in acidified chili pepper after SMS processing ($P < 0.05$). The increase in alcohols could be related to the conversion of aldehyde to the alcohol induced by the SMS reducibility ([Ren et al., 2022](#)) and alcohol dehydrogenase ([Wang et al., 2022](#)). Correspondingly, a significant decrease in aldehyde was observed, including (E)-2-heptenal, (E)-2-octenal, and hexenal ($P < 0.05$). It was reported that alcohol dehydrogenase could promote the transformation of C6 aldehydes into C6 alcohols ([Wang et al., 2022](#)). Furthermore, 13-methyltetradecanal was observed with a higher concentration in TP, HPP, and SMS groups, respectively, which could relate to lipid oxidation ([Xia et al., 2020](#)).

In general, esters seem the most affected volatile compounds during processing. Among these processing technologies, HPP and SMS have less effects on volatile compounds than TP. TP clearly reduced the concentration of esters and aldehydes but promoted the production of some terpenoids.

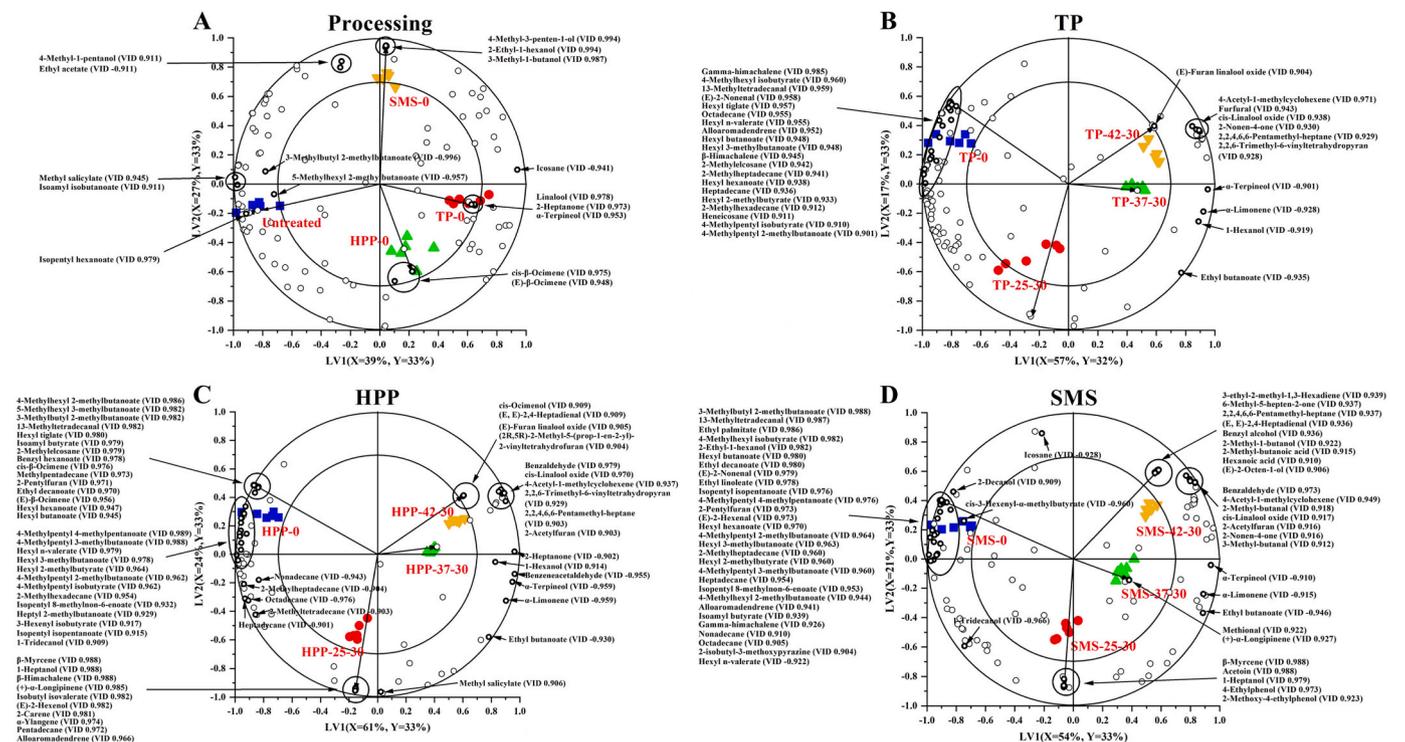


Fig. 4. Bi-plots visualizing the difference on volatile compounds during processing and storage by PLS-DA models. (A) Acidified chili pepper after different processing; (B) Acidified chili pepper pasteurized by TP during 30 days' storage at different temperature; (C) Acidified chili pepper pasteurized by HPP during 30 days' storage at different temperature; (D) Acidified chili pepper added with SMS during 30 days' storage at different temperature. The volatile compounds with VID higher than 0.900 (in absolute value) are named and marked in bold. All volatiles are represented by small and open circles. The different treatment groups are represented by differently shaped symbols. The X- and Y-variance explained by each LV are indicated in the respective axes.

3.2.2. Storage conditions

Storage time and temperature are the main factors affecting the volatile compounds of samples during storage (Kebede et al., 2015). According to Fig. 4B–D, stored groups and non-stored groups were clearly separated, indicating that storage time and temperature affected the volatiles of the acidified chili peppers. It can be seen that most volatiles are projected to the non-stored groups, showing that most volatiles in acidified chili peppers decreased during storage. Similar results were reported in other study, where large numbers of volatiles decreased in pineapple fruit juice during storage (Wu et al., 2021).

Among the compounds, esters were the main compounds decreasing during storage, particularly hexyl 2-methylbutyrate, hexyl hexanoate, hexyl butanoate, and hexyl 3-methylbutanoate. Comparing different processing technologies, the highest numbers of esters (21) decreased in HPP samples, followed by SMS groups (16) and TP groups (9), during storage. The fewest ester decreased in TP groups might be because that the main changes of esters had occurred during processing. In other words, for TP groups, the effects of processing on ester changes were more dominant than that of storage conditions. The decrease in esters observed might be related to esterase and acids catalyzed hydrolysis reactions (Yi et al., 2017). In addition, α -limonene and α -terpineol were selected with negative VID values in all non-stored groups, indicating that α -limonene and α -terpineol were mainly affected by storage time. In other words, the formation of α -limonene and α -terpineol could be selected as indicators to evaluate the influence of storage time on aroma properties. Some previously reported study found that α -limonene of chili peppers increased during storage (Korkmaz et al., 2020). While the increased in α -terpineol could be related to the acid-catalyzed hydration of linalool to α -terpineol (Teribia et al., 2021).

As shown in Fig. 4B–D, samples stored at high temperatures (37 °C and 42 °C) were located closer to each other compared to the 25°C-stored groups. It shows that storage temperature clearly affected volatile

changes in acidified chili peppers, especially high storage temperature. The changes in concentration of key aroma compounds affected by storage temperature were also illustrated displayed in Fig. 5. According to the VID procedure, aldehydes (2-methyl-butanal and 3-methyl-butanal), ketones (2-nonen-4-one, 6-methyl-5-hepten-2-one, and 2-heptanone), furans (2-acetylfuran, furfural, and (2R,5R)-2-methyl-5-(prop-1-en-2-yl)-2-vinyltetrahydrofuran), and oxides (cis-linalool oxide and (E)-furan linalool oxide) have been detected in 37°C- and 42°C-stored groups with positive VID values, indicating that high storage temperature resulted in these compounds increasing. Some of them have been considered as off-flavors of vegetable products. For example, sensorial attributes of furans are described as a toasted note in guacamole (Alañón et al., 2021). Therefore, the presence of 2-acetylfuran, furfural, and (2R,5R)-2-methyl-5-(prop-1-en-2-yl)-2-vinyltetrahydrofuran could detract freshness of acidified chili peppers. It was reported that the presence of furan compounds might be related to the non-enzymatic Maillard reactions induced by elevated temperature and low pH values (Alañón et al., 2021). Moreover, the increased of cis-linalool oxide and (E)-furan linalool oxide might be related to the formed upon oxidation of linalool (Golombek et al., 2021). The increase in 2-methyl-butanal and 3-methyl-butanal could be related to Strecker degradation, which were the reaction products from valine, leucine, and isoleucine (Korkmaz et al., 2020). In addition, the thermal oxidation or amino acid degradation of polyunsaturated fatty acids could lead to the increased in ketones (Song et al., 2021). On the other hand, Fig. 5 showed that esters were the main compounds clearly decreasing with the increase of storage temperature, including hexyl isobutyrate, methyl salicylate, 4-methylpentyl 2-methylbutanoate, 4-methylpentyl 3-methylbutanoate, hexyl 2-methylbutyrate, hexyl 3-methylbutanoate, 4-methylpentyl 4-methylpentanoate, and 4-methylpentyl 8-methylnon-6-enoate. It has been reported that esters were easily hydrolyzed when increased storage temperature (Yi et al., 2017).

Table 2
The main volatile compounds identified in acidified chili peppers after different processing.

No.	Compounds ^a	RI ^b	Odor description ^c	Concentration (µg/kg)				Identification ^d
				Untreated	TP	HPP	SMS	
Esters								
1	Ethyl acetate	615	Fruity, sweet, green	40.53 ± 6.38 ^b	34.83 ± 6.29 ^b	nd	54.73 ± 8.45 ^a	MS, RI
9	Isoamyl isobutanoate	1016	Fruity, green	126.76 ± 11.84 ^a	55.96 ± 4.27 ^c	79.66 ± 8.73 ^b	86.09 ± 3.12 ^b	MS, RI
14	3-Methylbutyl 2-methylbutanoate	1102	Fruity, sweet	631.29 ± 47.63 ^a	nd	513.05 ± 43.28 ^b	514.37 ± 46.99 ^b	MS, RI
16	Isopentyl isopentanoate	1107	Sweet, fruity, green	746.92 ± 55.38 ^a	388.05 ± 38.63 ^c	542.89 ± 51.37 ^b	744.50 ± 73.37 ^a	MS, RI
17	4-Methylpentyl isobutyrate	1115	–	1517.79 ± 140.85 ^a	895.03 ± 27.97 ^b	1399.80 ± 90.59 ^a	980.02 ± 99.82 ^b	MS, RI
18	3-Hexenyl isobutyrate	1147	Fruity, apple	181.42 ± 15.55 ^a	138.72 ± 3.97 ^b	185.51 ± 17.14 ^a	188.11 ± 18.35 ^a	MS, RI
19	Hexyl isobutyrate	1152	Green, fruity, apple	139.22 ± 16.00 ^a	81.71 ± 8.88 ^{b, c}	94.93 ± 6.28 ^b	69.23 ± 9.43 ^c	MS, RI
22	Methyl salicylate	1199	Peppermint, sweet	2050.16 ± 118.59 ^a	143.75 ± 6.29 ^d	562.40 ± 65.72 ^c	896.45 ± 32.95 ^b	MS, RI
23	4-Methylpentyl 2-methylbutanoate	1202	Fruity	5013.96 ± 232.73 ^a	3364.22 ± 140.90 ^a	4374.40 ± 272.18 ^b	3992.71 ± 443.90 ^b	MS, RI
24	4-Methylpentyl 3-methylbutanoate	1208	Fruity	4698.60 ± 274.21 ^{a, b}	3341.72 ± 116.01 ^c	5042.14 ± 325.62 ^a	4437.12 ± 242.37 ^b	MS, RI
25	cis-3-Hexenyl-α-methylbutyrate	1234	Fresh, sweet, fruity	155.57 ± 11.53 ^a	117.70 ± 7.12 ^b	132.75 ± 14.73 ^b	124.89 ± 15.90 ^b	MS, RI
26	Hexyl 2-methylbutyrate	1240	Pineapple, spicy	757.07 ± 49.44 ^a	669.57 ± 54.16 ^{a, b}	652.39 ± 39.67 ^b	647.79 ± 70.68 ^b	MS, RI
27	Hexyl 3-methylbutanoate	1245	Sweet, green, fruity	450.58 ± 30.18 ^{a, b}	417.60 ± 32.92 ^b	498.87 ± 44.50 ^a	482.28 ± 70.65 ^{a, b}	MS, RI
28	Isopentyl hexanoate	1253	Fruity	42.84 ± 4.07 ^a	nd	nd	nd	MS, RI
29	Hexyl n-valerate	1291	Fruity, green	112.47 ± 6.02 ^a	72.86 ± 5.41 ^b	83.26 ± 8.75 ^b	46.79 ± 5.43 ^c	MS, RI
30	5-Methylhexyl 2-methylbutanoate	1299	–	28.92 ± 3.31 ^a	nd	26.08 ± 4.85 ^{a, b}	21.14 ± 3.13 ^b	MS, RI
31	4-Methylhexyl 2-methylbutanoate	1308	–	264.65 ± 15.92 ^a	94.89 ± 9.18 ^c	172.54 ± 12.59 ^b	199.39 ± 32.50 ^b	MS, RI
32	4-Methylpentyl 4-methylpentanoate	1317	Fruity	6651.59 ± 99.55 ^a	3793.26 ± 223.16 ^c	6856.48 ± 345.65 ^a	4398.20 ± 410.11 ^b	MS, RI
33	Hexyl hexanoate	1388	Fruity, sweet	95.92 ± 4.22 ^a	78.72 ± 5.74 ^b	86.08 ± 8.95 ^{a, b}	51.79 ± 7.84 ^c	MS, RI
41	4-Methylpentyl 8-methylnon-6-enoate	1693	Fruity	46.05 ± 6.50 ^c	122.23 ± 15.47 ^a	130.61 ± 15.50 ^a	88.25 ± 9.88 ^b	MS, RI
Terpenoids								
11	cis-β-Ocimene	1043	Floral, herbal	nd	nd	26.22 ± 3.63 ^a	nd	MS, RI
12	(E)-β-Ocimene	1053	Sweet, herbal	78.14 ± 12.66 ^b	47.81 ± 5.60 ^b	237.56 ± 52.38 ^a	42.30 ± 4.89 ^b	MS, RI
15	Linalool	1102	Floral, lavender	nd	671.16 ± 23.54 ^a	nd	nd	MS, RI
21	α-Terpineol	1196	Citrusy, woody	nd	61.55 ± 13.42 ^a	nd	nd	MS, RI
37	Gamma-himachalene	1492	–	809.22 ± 45.06 ^c	1903.34 ± 151.14 ^a	538.78 ± 32.76 ^d	1615.70 ± 134.87 ^b	MS, RI
Alcohols								
3	3-Methyl-1-butanol	736	Fruity, whiskey	nd	nd	nd	34.01 ± 3.47 ^a	MS, RI
5	4-Methyl-1-pentanol	844	Nutty	206.51 ± 11.17 ^b	67.24 ± 9.18 ^d	169.59 ± 11.42 ^c	470.30 ± 36.02 ^a	MS, RI
6	4-Methyl-3-penten-1-ol	868	–	nd	nd	nd	18.57 ± 0.79 ^a	MS, RI
10	2-Ethyl-1-hexanol	1033	Citrusy, fresh, floral	nd	nd	nd	25.95 ± 1.66 ^a	MS, RI
20	2-Decanol	1181	–	95.44 ± 5.96 ^b	67.26 ± 2.48 ^d	80.70 ± 7.33 ^c	114.21 ± 3.18 ^a	MS, RI
Aldehydes								
4	Hexanal	801	Fresh, green, fatty	104.70 ± 4.04 ^a	56.00 ± 13.62 ^c	54.04 ± 6.84 ^c	84.72 ± 7.47 ^b	MS, RI
8	(E)-2-Heptenal	963	Vegetables	28.97 ± 5.01 ^a	nd	nd	25.43 ± 3.23 ^a	MS, RI
13	(E)-2-Octenal	1062	Fresh, green	38.33 ± 4.06 ^a	nd	nd	32.97 ± 1.96 ^b	MS, RI
40	13-Methyltetradecanal	1681	–	70.11 ± 9.58 ^c	141.54 ± 23.51 ^b	135.62 ± 16.09 ^b	173.30 ± 19.60 ^a	MS, RI
Acids								
2	Acetic acid	655	Sour, vinegar	544.74 ± 106.81 ^{b, c}	1160.79 ± 168.38 ^a	712.47 ± 136.29 ^b	492.89 ± 24.81 ^c	MS, RI
Ketones								
7	2-Heptanone	895	Herbal, woody	nd	18.43 ± 1.61 ^a	nd	Nd	MS, RI
Alkanes								
33	2-Methyltridecane	1366	–	413.43 ± 40.85 ^b	409.53 ± 59.95 ^b	596.19 ± 53.36 ^a	606.67 ± 69.78 ^a	MS, RI
35	2-Methyltetradecane	1465	–	484.13 ± 31.89 ^c	765.50 ± 40.88 ^a	640.42 ± 59.83 ^b	754.92 ± 93.65 ^a	MS, RI
37	2-Methylpentadecane	1565	–	359.65 ± 42.35 ^c	547.21 ± 70.53 ^b	827.18 ± 131.25 ^a	586.36 ± 74.54 ^b	MS, RI
38	Hexadecane	1600	–	262.11 ± 35.19 ^c	463.06 ± 45.16 ^b	541.77 ± 31.61 ^a	464.05 ± 65.55 ^b	MS, RI
39	2-Methylhexadecane	1665	–	255.75 ± 35.47 ^c	576.62 ± 67.50 ^a	606.28 ± 87.43 ^a	426.55 ± 53.30 ^b	MS, RI
42	Heptadecane	1700	–	215.01 ± 32.33 ^d	452.97 ± 21.14 ^b	349.62 ± 36.87 ^c	536.57 ± 81.99 ^a	MS, RI
43	2-Methylheptadecane	1765	–	71.08 ± 8.88 ^d	213.78 ± 44.67 ^b	152.70 ± 16.38 ^c	266.67 ± 19.29 ^a	MS, RI
44	Icosane	2000	Waxy	nd	35.52 ± 11.62 ^a	28.04 ± 2.33 ^a	26.54 ± 3.05 ^a	MS, RI

Mean ± standard deviation. Different letters in the same column indicate significant differences determined by Tukey's HSD test ($P < 0.05$). nd: Not detected.

^a The reliability of the identification proposal is carried out: mass spectrum and retention index agreed with database or literature.

^b Calculated retention index (RI) on HP-5MS column.

^c Odor description were obtained from <https://www.femaflavor.org/Flavor-Library>.

^d Identification methods: MS, mass spectrometry; RI, retention indices; -: not detected.

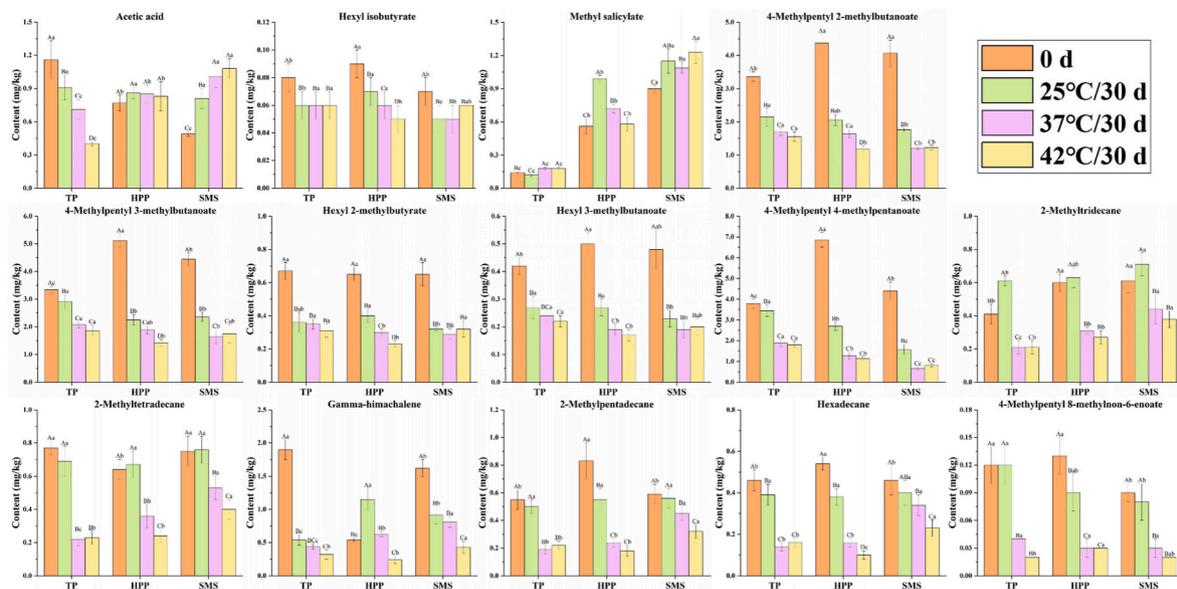


Fig. 5. Discriminative headspace volatile components changed in acidified chili peppers during storage. Significant differences ($P < 0.05$) are indicated with different letters. Error bars represent the standard error of analysis ($n = 6$).

4. Conclusion

Different flavor changes were observed among HPP, TP, and SMS treated acidified chili peppers. Among processing technologies, the largest numbers of flavor attributes of acidified chili pepper changed in TP groups, such as an increase in organic acid contents and TA values but a decrease in FAA concentrations. No clear changes on pH, TA values, and organic acid concentrations were observed after HPP and SMS. While the addition of SMS contributed more bitter FAAs to samples than other processing technologies. Less clear effects of HPP were found in taste changes of acidified chili peppers. As for aroma compounds, esters, alkanes, terpenes, alcohols, and aldehydes were the main volatile compounds detected in acidified chili pepper. Esters as the abundant volatiles, were largely influenced by processing, particularly after TP. TP clearly reduced the concentration of esters and aldehydes, but promoted the production of some terpenoids.

Storage conditions were another main factor affecting flavor changes of acidified chili peppers. The pH, TA values, organic acid concentrations, and FAA contents in acidified chili pepper showed remarkable changes during storage. The samples became acid at the beginning of the storage and then remained stable during followed storage period. While, the concentration of FAAs increased within the initial 7 days, but then decreased in the later storage period. As for volatile compounds, esters and terpenes were the main compounds decreasing during storage. In addition, high storage temperature accelerated and enlarged the effects of storage time on the flavor properties of acidified chili pepper. Furthermore, some off-flavor related compounds were produced in high temperature stored samples, such as furans, aldehydes, and oxides.

In general, HPP and SMS treated acidified chili peppers exhibited better flavor properties than TP samples. Besides, the effects of storage time were more dominant compared to storage temperature. The outcome of this study could provide new insights into the effects of processing and storage conditions on flavor changes and guide for the acidified chili pepper production industry.

CRedit authorship contribution statement

Xi Bao: Methodology, Investigation, Writing – review & editing. **Shiyao Zhang:** Methodology, Investigation. **Xueting Zhang:** Funding acquisition. **Yongli Jiang:** Writing – review & editing. **Zhijia Liu:** Supervision, Writing – review & editing. **Xiaosong Hu:** Supervision,

Funding acquisition. **Junjie Yi:** Conceptualization, Supervision, Project administration, 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.

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