# Impact of Low-temperature Storage on Volatile Flavor Compounds in Prepared Pork Products

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Abstract [Objectives] This study was conducted to explore the dynamic changes of volatile flavor compounds in prepared pork during storage at different low-temperature conditions. [Methods] Prepared pork was stored at 4, -4 and -18 °C. The volatile flavor compounds of prepared pork were determined by solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) at days 0, 7, 14, 21 and 28, and relative odor activity value (OAV), principal component analysis (PCA) and cluster analysis (CA) were combined to analyze changes in volatile flavor compounds of prepared pork during storage. [Results] The total number of volatile flavor compounds gradually decreased with the prolongation of the storage period, and OAV analysis identified 22 key flavor compounds ( $OAV \ge 1$ ). The results of PCA and CA showed that 2-methyl-1-butanol, 1-octen-3-ol, linalool, cineole, hexanal and nonanal were the main key flavor components, and the degree of flavor degradation was low under both superchilling and freezing conditions. After 28 days of storage, the alcohol content in the chilling group was significantly higher than other two groups, and the overall content of volatile flavor compounds was also significantly higher than other two groups, indicating that the -4 °C chilling storage was more favorable for maintaining the overall flavor of prepared pork. [Conclusions] This study provides a theoretical basis for finding a better storage method for prepared meat products.

Key words Low-temperature storage; Prepared pork; Volatile flavor component; Principal component analysis; Cluster analysis DOI:10.19759/j.cnki.2164 - 4993.2024.04.017

Prepared pork products are meat products that take pork as the main raw material, which is processed initially, and then subjected to one or more processing methods such as seasoning, pickling, rolling, starching, coating, shaping, and hot processing. They are stored, transported, and sold under low-temperature conditions. There are many storage methods for meat products, such as traditional storage methods including drying (dehydration), salting and smoking, and modern storage methods including lowtemperature storage, modified atmosphere storage, irradiation storage, chemical storage and vacuum packaging. Different storage methods have great influence on the eating quality and storage period of prepared pork products. Low-temperature storage is a common storage method of meat products, mainly including chilling storage, freezing storage, quick freezing and superchilling. In recent years, a large number of researchers have studied the lowtemperature storage of meat products, mainly analyzing their sensory indicators, microorganisms, physical indicators and chemical indicators [1-4].

Flavor is the key parameter for determining the eating quality of meat products<sup>[5]</sup>. The flavor change of meat products is mainly due to the degradation of flavor precursors, Maillard reaction and fat oxidation. Under the condition of low temperature, with the

extension of storage time, the stored meat products will be degraded due to oxidation reaction<sup>[6]</sup>, which will reduce meat quality and affect its flavor, nutritional value, color and texture. In recent years, many scholars have devoted themselves to the study of lowtemperature storage of meat products, which can maintain the original flavor of prepared pork products to the maximum extent<sup>[7]</sup>. Due to the low chemical reaction rate in low-temperature environment, the loss of flavor substances is correspondingly reduced, and hence, meat products can better maintain their unique flavor during storage. Li et al. [8] found that low temperature would affect the freshness and flavor degradation of sea bass fillets by comparing the flavor differences of sea bass fillets at 0 and 4 °C. Chen<sup>[9]</sup> compared the changes of volatile flavor components of grass carp during low-temperature storage at different temperatures, which provides a theoretical basis for the quality changes of fish during low-temperature storage.

In this study, a pork product cooked in vacuum at low temperature for a long time (abbreviated as prepared pork) serving as the research object was determined for volatile flavor components under three low-temperature storage methods, namely chilling, superchilling and freezing, by SPME-GC-MS, and odor activity value (OAV), principal component analysis (PCA) and cluster analysis were combined to explore the variation law of volatile flavor compounds in the prepared pork product under different low-temperature storage methods, so as to provide some reference for finding better storage methods for prepared meat products.

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### **Materials and Methods**

#### Materials and reagents

Pig hind leg meat, provided by Chengdu Hope Food Co., Ltd.;

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dried red peppers, dried Sichuan pepper, cooking wine, green onions and ginger, purchased in Chengdu Shilinghao Tesco Supermarket; 2,4,6-trimethylpyridine (C8H11N), analytically pure, Sigma-Aldrich Company, USA.

### Instruments and equipment

Refrigerator BCD-452WDPF, Qingdao Haier Co., Ltd.; precision electronic balance AL-104, Mettler-Toledo Instruments (Shanghai) Co., Ltd.; vacuum packaging machine GY-2B-6202, Jiangxi Ganyun Food Machinery Company; cooking barrel BZZT-IV-150, Jiaxing Aibo Industry Co., Ltd.; constant temperature water bath pot HHS-11-4, Medical Equipment Factory of Shanghai Boxun Industrial Co., Ltd.; gas chromatography-mass spectrometer 5977A-7890B, multifunctional autosampler PARSI 85tcc, chromatographic column DB-WAX (30 m  $\times$ 250  $\mu$ m  $\times$ 0.25  $\mu$ m) and extraction fiber (50/30  $\mu$ m DVB/CAR/PDMS), Agilent Company, USA.

### Sample treatment

Fresh pig hind leg meat was selected as the raw material and cleaned. After removing anadesma, it was cut into 6 cm  $\times$  6 cm pieces, about 300 g. Each piece was added with 2.67% of cooking wine, 0.17% of red pepper, 1.67% of dried Sichuan pepper, 3.33% of ginger and 3.33% of scallion to marinate it for 30 min. The low-temperature vacuum long-term cooking process was adopted. The processed meat pieces were put into high-temperature retort pouches, and the vacuum degree of a vacuum packaging machine was adjusted to 0.09 MPa for vacuum packaging. Next, the temperature of a constant-temperature water bath pot was adjusted to 67 °C , and packaged meat pieces were put into it when the water temperature reached 67 °C. The water level was 1 cm higher than the meat pieces. Timing was started to heat for 4 h. After cooling to room temperature, the meat pieces were stored at 4 °C (L), -4 °C (W), and -18 °C (D) for 28 d, respectively.

### Determination indexes and methods

**Determination of volatile flavor compounds** Meng Weiyi's method [10] was adopted with some modifications. The prepared pork was homogenized, and 3.000 g of the sample was accurately weighed and added into a 15 ml headspace bottle. Next, 1  $\mu$ l of 2  $\mu$ g/ $\mu$ l 2,4,6-trimethylpyridine was added as the internal standard, and the bottle was sealed for testing. The pretreatment conditions of CTC automatic sampler were as follows: heating box temperature 75 °C, heating time 45 min, sample extraction time 20 min and analysis time 5 min.

GC conditions: The capillary column used was HP-5MS (30 m  $\times$  0.25 mm, 0.25  $\mu m$ ). The carrier gas was helium (99.999 9%), and splitless sampling was adopted. Heating program of the column box: The initial temperature was 40 °C, which was kept for 3 min and then heated at 3 °C/min to 65 °C, which was kept for 0 min and then heated at 4 °C/min to 150 °C, which was kept for 2 min and finally heated to 230 °C at 20 °C/min.

MC condition: Electron ionization source; electron energy 70 eV; ion source temperature 230 °C; quadrupole temperature 150 °C; detector voltage 350 V; mass scanning range ( m/z )

40 - 500.

Qualitative analysis: When analyzing compounds, the obtained data were searched and matched in NIST14. L library of the instrument, and the substances with a matching degree higher than 80% were selected.

Quantitative analysis: The peak areas of the total ion chromatogram were normalized for quantification, and the absolute content of each component was obtained according to formula (1).

$$C_i = \frac{\rho \times v \times A_i}{A \times m} \tag{1}$$

In the formula,  $C_i$  is the absolute content of each volatile substance ( $\mu g/kg$ );  $\rho$  is the mass concentration of the internal standard (2  $\mu g/\mu l$ ); v is the volume of the internal standard (1  $\mu l$ );  $A_i$  is the peak area of each component; A is the peak area of the internal standard; and m is the mass of sample (0.003 kg).

# Multivariate statistics of key volatile flavor compounds

OAV calculation OAV was used to evaluate the effect of each compound on the overall flavor of the sample. OAV refers to the ratio of the concentration of aroma substances to their threshold. When OAV is less than 1, it means that the substance can improve the overall flavor; when OAV is greater than 1, it means that the substance has a direct effect on the overall flavor; and in a certain range, the greater the OAV, the greater the contribution of the substance to the overall flavor. The OAV value was calculated according to following formula;

$$OAV = \frac{C_i}{F_i} \tag{2}$$

In the formula:  $C_i$  is the mass concentration of compound,  $\mu g / kg$ ;  $F_i$  is the threshold of the compound in water,  $\mu g / kg$ . **Principal component analysis (PCA) and cluster analysis (CA)** Principal component analysis (PCA) and cluster analysis (CA) were conducted referring to the method in reference [11]. **Data processing and analysis** 

All the experimental groups were repeated three times. SPSS 22.0 software was used for data processing and significance analysis, with P < 0.05 indicating a significant difference. SIMCA 14.1 was used for principal component analysis. Tbtools v1.098769 was used for cluster analysis. Origin 2021 was used for correlation analysis.

# **Results and Analysis**

# Effects of low-temperature storage on the types and contents of volatile flavor compounds in prepared pork

The types and contents of volatile flavor compounds in the prepared meat product stored at low temperature are shown in Table 1. During low-temperature storage of the pork product, 7 types of volatile flavor compounds were detected, totaling 59 kinds, including 15 kinds of alcohols, 30 kinds of alkanes, 4 kinds of esters, 5 kinds of ketones, 2 kinds of benzenes, 2 kinds of aldehydes and 1 kind of ether. Among them, alkane flavor compounds were the most diverse, followed by alcohols, esters, and ketones. In group L, 7 types of volatile flavor substances were

identified, totaling 46 kinds, including 21 kinds of alkanes, 13 kinds of alcohols, 4 kinds of esters, 4 kinds of ketones, 1 kind of benzene, 2 kinds of aldehydes and 1 kind of ether, with a total content of 37 614.038  $\mu$ g/kg. Six types of volatile flavor compounds were identified in group W, totaling 46 kinds of including 23 alkanes, 14 alcohols, 2 esters, 3 ketones, 2 benzenes and 2

aldehydes, with a total content of 41 461.91  $\mu g/kg$ . In group D, 6 types of volatile flavor substances were identified, totaling 50 kinds, including 28 kinds of alkanes, 13 kinds of alcohols, 2 kinds of esters, 3 kinds of ketones, 2 kinds of benzene and 2 kinds of aldehydes, with a total content of 38 349.92  $\mu g/kg$ .

Table 1 Effects of low-temperature storage on the types and contents of volatile flavor compounds in prepared pork

C 1		L (4°C)		W ( −4 °C )		D(-18%)
Compound	Number	Total content // µg/kg	Number	Total conten//µg/kg	Number	Total content // µg/kg
Alcohol (15)	13	19 279. 298	14	21 169.15	13	19 264
Aliphatic hydrocarbon (30)	21	3 155.79	23	5 347.9	27	4 991.901
Ester (4)	4	1 110.84	2	605.09	2	789.49
Ketone (5)	4	7 002.11	3	7 363.36	3	6 558.47
Benzene (2)	1	125.03	2	490.61	2	323.86
Aldehyde (2)	2	5 980.71	2	6 485.8	2	6 422.2
Ether (1)	1	960. 26	0	0	0	0
Total (59)	46	37 614.038	46	41 461.91	50	38 349.92

## Multivariate statistical analysis of key flavor compounds in prepared pork stored at low temperature

**Analysis of key flavor compounds** In order to further study the effects of different low-temperature storage methods on volatile flavor compounds of prepared pork, the contribution degrees of various volatile flavor compounds were calculated. When  $OAV \ge 1$ , it shows that the compound contributes greatly to flavor and is considered as a key volatile flavor substance<sup>[27]</sup>. The OAV values and odor description of volatile flavor substances stored at different low temperatures are shown in Table 2. There were 22 kinds of key flavor compounds in total. At storage days 0, 7, 14, 21 and 28, 10, 6, 8, 6 and 9 kinds were detected in group L, respectively; there were 10, 9, 9, 8 and 12 kinds in group W, respectively; and 10, 11, 8, 10 and 6 kinds were detected in group D, respectively. The results showed that with the increase of storage time, the types of key flavor substances of prepared pork decreased and then increased, which is consistent with the research results of Yue<sup>[28]</sup>. It might be due to the slow oxidation of fat in the early stage of low temperature storage, which led to the formation of aldehydes, alcohols and ketones and other substances, forming the unique flavor of pork. With the passage of time, due to the relatively poor air fluidity under the condition of low-temperature storage, the moisture film and frost formed on the surface of pork might limit the volatilization of volatile substances, and some volatile substances might gradually evaporate or lose, resulting in a decrease in their kinds. With the further extension of storage time, the oxidation of fat in pork might be accelerated, and more kinds of volatile flavor substances might be produced. Meanwhile, protein might be slowly decomposed, and nitrogen-containing compounds and other flavor precursors might be produced and converted into volatile flavor substances in subsequent reactions.

As can be seen from Table 2, for group L, the variance contribution rate of the first principal component was 45.3%, and the variance contribution rate of the second principal component was 29.1%, and the cumulative variance contribution rate was 74.4%. For group W, the variance contribution rate of the first

principal component was 44.6%, and the variance contribution rate of the second principal component was 37.3%, and the cumulative variance contribution rate was 81.9%. As to group D, the variance contribution rate of the first principal component was 47.1%, and the variance contribution rate of the second principal component was 31%, and the cumulative variance contribution rate was 78.1%.

Through the principal component analysis based on the OAV value of the prepared pork, the separation situation of volatile flavor substances in the prepared pork at different storage temperatures at different time points could be seen from the PCA score chart in Fig. 1. In the 4 °C score chart of Fig. a1, the 0 and 28 d groups are obviously separated, and the distance between the 7, 14 and 21 d groups is close, indicating that the flavor was significantly different between 0 and 28 d at 4 °C, and the volatile flavor substances in the 7, 14 and 21 d groups were similar. There was certain correlation between the principal component score chart and the position of the load chart. In Fig. a2, it can be seen that nonanal, 2-methyl butanol,  $\alpha$ -terpinene, linalool and (1R)-()- $\alpha$ -pinene had a high contribution to the 7, 14 and 21 d groups; sabinene, camphene, 3-hydroxy-2-butanone, pinene and terpinen-4-ol contributed highly to the 0 d group; and octane, 3-octanone, ethyl isovalerate, isoamyl acetate and (2R,3R)-(-)-2,3-butanediol had a high contribution to the 28 d group. In the −4 °C score chart of Fig. b1, the 0 and 7 d groups are obviously separated, and the distances between the 14, 21 and 28 d groups are close, indicating that the flavor was significantly different between the 0 and 7 d groups at -4 °C, and the volatile flavor substances in the 14, 21 and 28 d groups were similar. Combined with the load diagram b2, it could be seen that (E)-β-ocimene, phellandrene, αterpinene, nonanal, sabinene, d-limonene and linalool had a high contribution to the 7 d group; isoamylol, 2-methyl-1-butanol, pinene, camphene, methyl heptenone, 3-hydroxy-2-butanone and terpinene had a high contribution to the 0 d group; and α-terpineol, octane and terpinen-4-ol contributed a lot to the 14, 21 and 28 d groups. In Fig. c1, it can be seen that the separation between the 0 and 14 d groups is obvious, and the distances among the 7, 21 and 28 d groups are close, indicating that under the condition of storing at  $-18\,^\circ\text{C}$ , the difference in flavor between the 0 and 14 d groups was significant, and the volatile flavor substances in the 7, 21 and 28 d groups were similar. From the load chart c2, it can be seen that 2-methyl butanol, nonanal, terpinen-4-ol,  $\alpha$ -terpinene,  $\alpha$ -terpineol, phellandrene and linalool had a high contribution to the 7, 21 and 28 d groups, while isoamylol, sabinene, 2-methyl-1-butanol, 3-hydroxy-2-butanone and pinene had a high contribution to the 0 d group, and (E)- $\beta$ -ocimene, phellandrene, myrcene and (2R,3R)-(-)-2,3-butanediol had a high contribution to the 14 d group.

Table 2 Characteristic roots and variance contribution values of various principal components under low-temperature storage

<u> </u>	Principal	Variance	Cumulative
Group	component	contribution rate $/\!/\%$	contribution rate $/\!/\%$
L (4 °C)	1	45.3	74.4
	2	29.1	
W ( −4 °C)	1	44.6	81.9
	2	37.3	
D ( −18 °C)	1	47.1	78.1
	2	31.0	

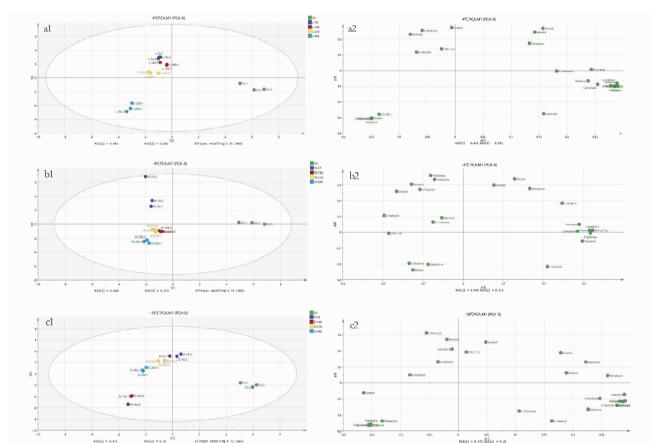


Fig. 1 PCA score and load charts of volatile flavor compounds in prepared pork stored at low temperatures

Cluster analysis of key flavor compounds Cluster analysis is a multivariate statistical method which reflects differences between samples. In order to judge the flavor differences between groups, the volatile flavor substances with  $OAV \ge 1$  were clustered, and the results are shown in Fig. 2. The horizontal axis represents 22 flavor compounds with  $OAV \ge 1$ , and the vertical axis represents three groups of samples at different time points. The red, yellow and green colors in the heatmap represent the contents of different substances. The darker the red color, the higher the content of the compound, and the darker the green color, the lower the content of the compound. The color of unique flavor compounds among different groups is the darkest<sup>[29]</sup>. It can be seen from Fig. 2 that alcohols and aldehydes played a key role in the overall flavor during

low-temperature storage, mainly including 2-methyl butanol, 1-octen-3-ol, linalool, cineole, hexanal and nonanal. Among them, the key flavor substances in group L were isoamyl acetate and hexyl isovalerate; and hexanal was the key flavor substance in group W and myrcene in group D. In addition, it was also found that 2-methyl butanol in group L at days 7, 14 and 21 d and in group L at day 28 was significantly different from other groups.

From the clustering results, it could be seen that day 0 was clustered with day 14 of group W and day 14 of group D, indicating that the flavor at day 14 of groups W and D during storage was not significantly different from that of day 0, and the attenuation was not obvious, while day 14 of group L was clustered with day 28 of groups W and D, indicating that there was no significant difference

Table 2 Odor description and OAV value of key volatile flavor substances in prepared pork products during low-temperature storage

No.         Compound         μg/kg         Other description           1         Cincede         4.6         Citrus fragrance, mint fragrance           2         Linabol         6         Fragrant fruit fragrance           4         Isoamylol         2.0         Fruit and floral fragrance           5         1-Octen-3-cd         1         Mushroom, lavender, rise and hay aroma           6         Terpinert-4-ol         4 370         Pepper aroma, wine aroma, sweet aroma           7         n-Hexanal         2.0         Fruit and floral fragrance, and has seen of aged wood           7         n-Hexanal         2.0         Fruit and floral fragrance, the smell of camphor           10         Sabinene         2.14         Girus fragrance of citrus and lemon florers           11         Terpinene         2.14         Girus fragrance of citrus and lemon florers           12         α-Terpinene         85         Fragrance of citrus and lemon florers           13         4 (IR)-(-)-α-Finene         34         Girus fragrance of citrus and lemon florers           14         (IR)-(-)-α-Finene         38         Spicy smell           15         Phellandene         200         Mixed fragrance of lemon florers           16         (E)-β-Ocimene		0 d 7 d 372.79 448. 21.89 74. 0.00 1531. 6.83 1. 338.62 0. 0.05 0. 0.05 0. 0.00 0. 0.08 0. 56.26 0. 26.26 0.	90 07 93 3	1 2		l p	7 d	W [14 d	21 d	28 d	7 d			
Eineole         4.6           Linabool         6           2-Methyl butarol         0.4           I-Octen-3-ol         1           Terpiner.4-ol         4 370           n-Hexanol         250           2-Methyl-1-butanol         6           Sabinene         1 500           Terpinere         2.14           α-Terpinene         85           d-Limonene         2.000           Phellandrene         2 900           Phellandrene         2 900           Phellandrene         33           (IS)-(·)-β-Finene         34           Myrcene         1.2           Pinene         58           Pinene         58			- m			p p	7 d	14 d	21 d	28 d				
Cincole       4.6         Linabol       6         2-Methyl butarol       0.4         1-Octen-3-ol       1         Terpiner-4-ol       4370         n-Hexanol       250         α-Terpinerol       340         Sabinene       1 500         Terpinere       85         d-Limonene       33         Phellandrene       2 900         Phellandrene       10         (1S)-(·)-β-Finene       34         Myrcene       1.2         Pinene       58         Pinene       58		1	3									14 d	21 d	28 d
Linabol 6  2-Merhyl butarol 0.4  Isoamylol 250  1-Octen-3-ol 1  Terpinen-4-ol 4370  n-Hexanol 250  α-Terpinen 6  Sabinene 1500  Terpinene 85  d-Limonene 34  (IR)-()-α-Finene 33  Phellandrene 2900  Phellandrene 10  (E)-β-Venene 34  Myrcene 1.2  Pinene 58  Pinene 58		=	$\epsilon$		132. 73 22	22.40 4	458.43 17	178.13	112. 18	139.46	559.49 10	100.22 25	252.67 12	129.50
2-Methyl butanol 0.4  Isoamylod 250  1-Octen-3-ol 1  Terpinen.4-ol 4370  n-Hexanol 250  α-Terpireol 340  2-Methyl-1-butanol 6  Sabinene 1500  Terpinene 85  d-Limonene 2900  Phellandrene 2900  Phellandrene 10  (IS)-(·)-β-Pinene 34  (IS)-(·)-β-Pinene 34  Myrcene 1.2  Pinene 58  Phellandrene 2900  Phellandrene 38			$\epsilon$	25. 77 22	22.09	7.62	151.31 2	20.53	45.31	86.20	25.47	7.50 4	43.28	21.89
Lyounylod   250    -Octen-3-ol   1    -Octen-3-ol   4 370    -Ordersamol   250    -Ordersamol   250    -Ordersamol   340    -Ordersamol   250    -Ordersamol   250    -Ordersamol   2 14    -Ordersamol   2 14    -Ordersamol   34    -Ordersamol   10    -Ordersamol				446.13 484	484.58 (	0.00 13	386. 70	0.00 47	474.30 1	402.45 1 4	473.23	0.00 87	874.83 59	594.30
1-Octen-3-ol 1  Terpiner 4-ol 4370  n-Hexanol 250  α-Terpireol 340  2-Mehly!-1-butanol 6  Sabinene 1500  Terpinere 85  d-Limonene 85  d-Limonene 33  Phellandrene 2900  Phellandrene 140  (1R)-(·)-α-Finene 33  Phellandrene 170  Phellandrene 180  Phellandrene 2900  Phellandrene 2900  Phellandrene 180  Phellandrene 180  Phellandrene 1900				1.63 0	0.52	5.47	1.92	0.99	0.42	2.05	2.27	0.80	1.03	0.74
Terpiner.4-ol 4370 n-Hexamol 250 α-Terpireol 340 2-Methyl-1-butanol 6 Sabinene 1500 Terpinene 2.14 α-Terpinene 85 d-Limonene 33 Phellandrene 2900 Phellandrene 10 (1S)-(·)-β-Pinene 34 Myrcene 1.2 Pinene 58 President 1.0	·		0.00	0.00	0.00	0.00	263.92 11	112.34 20	201.56	173.23	78.43 (	63.42 5	56.40	0.00
a-Terpireol 340 2-Methyl-1-butanol 6 Sabinene 1 500 Terpinene 2. 14 α-Terpinene 85 d-Limonene 34 (1R)-(.)-α-Finene 33 Phellandrene 2 900 Phellandrene 10 (1S)-(.)-β-Pinene 34 Myrcene 1.2 Pinene 58 Pinene 58	· · · · · · · · · · · · · · · · · · ·		0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.01	1.00	00.00	0.00	0.38
α-Terpireol       340         2-Methyl-1-butand       6         Sabinene       1 500         Terpinere       2 14         α-Terpinene       85         d-Limonene       34         (1R)-( )-α-Finene       2 900         Phellandrene       2 900         Phellandrene       10         (1S)-(·)-β-Pinene       140         (E)-β-Ocimene       34         Myrcene       1.2         Pinere       58         Pinere       58			0.00	0.56 0	0.67	0.32	0.00	3.92	1.43	4.94	0.00	4.48	3.00	0.72
2-Methyl-1-butanol 6 Sabinene 1500  Terpinene 2.14 α-Terpinene 85 d-Limonene 34 (1R)-(.)-α-Finene 33 Phellandrene 2900 Phellandrene 10 (1S)-(.)-β-Pinene 34 Myrcene 1.2 Pinene 58 Pinene 58	4		0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.10	0.00	0.03	0.10	0.00
Sabinene 1500  Terpinene 2.14  α-Terpinene 85  d-Limonene 34  (1R)-(.)-α-Finene 2900  Phellandrene 10  (E)-β-Pinene 140  (E)-β-Ocimene 140  Myrcene 1.2  Pinene 58			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	00.00	0.00	0.00
Terpinene       2.14         α-Terpinene       85         d-Limonene       34         (1R)-( )-α-Finene       33         Phellandrene       2 900         Phellandral       10         (1S)-(·)-β-Pinene       140         (E)-β-Ocimene       34         Myrcene       1.2         Pinere       58         Pinere       58			0.00	0.00	0.04	0.00	0.13	0.09	0.03	0.14	0.02	0.03	0.02	0.00
a. Terpirene 85 d-Limonane 34 (1R)-( )-α-Finene 33 Phellandrene 2 900 Phellandral 10 (1S)-(·)-β-Pinene 140 (E)-β-Ocimene 34 Myrcene 1.2 Pinere 58			0.00	44.07 0	0.00	0.00	00.00	0.00	0.00	18.28	36.67	0.00	58.70	0.00
d-Limonene 34  (1R)-( )-α-Finene 33  Phellandrene 2 900  Phellandral 10  (1S)-(-)-β-Pinene 140  (E)-β-Ocimene 34  Myrcene 1.2  Pinere 58			0.00	0.19 0	0.25 (	0.00	0.67	0.33	0.30	0.00	0.00	0.00	0.45	0.00
(1R)-( )-α-Finene 33  Phellandrene 2 900  Phellandral 10  (1S)-(-)-β-Pinene 140  (E)-β-Ocimene 34  Myrcene 1.2  Pinere 58			0.92	3.39	1.56	1.17	5.10	6.78	4.31	3.89	3.23	4.02	2.24	1.51
Phellandrene 2 900 Phellandral 10 (1S)-(·)-β-Pinene 140 (E)-β-Ocimene 34 Myrcene 1.2 Pinere 58		0.00	0.00	3.18 0	0.00	0.00	2.45	1.47	0.00	4. 12	00.00	0.57	0.00	0.00
Phellandral 10 (1S)-(·)-β-Pinere 140 (E)-β-Ocimene 34  Myrcene 1.2  Pinere 58	tii	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	00.00	0.02	0.00	0.00
(IS)-(·)-β-Pinene 140 (E)-β-Ocimene 34  Myrcene 1.2  Pinere 58		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.40	0.00	0.00	0.00
(E) β-0-cimene 34  Myrcene 1.2  Pinere 58		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	00.00	0.73	0.00	0.00
Myrene 1.2 Pinere 58	sam fragrance, and pine wood fragrance	0.00	0.00	0.00	0.00	0.00	0.69	0.00	0.00	0.00	0.00	0.87	0.00	0.00
Pinere 58		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	89.78	0.00	0.00
1 960		2.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Campriene 1 900		0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
23 Tetradecane 1 000 –		0.09	0.12	0.07 0	0.02	0.02	0.07	0.00	0.00	0.00	90.0	0.00	0.05	0.00
24 Octane 10 -		0.00	0.00	0.00	0.00	9.28	0.00	6.01	0.00	13.76	0.00	3.77	0.00	9.32
25 Isoamyl acetate 5 Banana fragrance, pear fragrance		0.00	0.00	0.00	0.00	38.18	0.00	0.00	0.00	0.00	0.00	00.00	0.00	0.00
26 Ethyl isovalerate 0.2 Aroma of apples and mulberries		0.00	0.00	0.00	0.00 292	292.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
27 3-Octanone 28 Mold fragrance, betone fragrance, Fragrant aroma, wax fragrance, and vegetable fragrance	roma, wax fragrance, and vegetable fragrance	0.00	0.00	0.00	0.00	3.08	00.00	0.00	0.00	0.00	00.00	00.00	0.00	0.00
28 3-Hydroxy-2-butanone 750 Sweet aroma and dairy aroma		7.43	0.00	1.09 0	0.00	0.01	00.00	1.43	0.35	0.00	00.00	0.59	0.00	0.00
29 (2R,3R)-(-)-2,3-Butanediol 400 -		0.00	0.00	0.00	0.00	0.19	0.00	0.00	0.00	0.00	0.00	0.24	0.00	0.00
30 Methyl heptenone 300 Fragrant aroma, citrus fragrance		1.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
31 Hexanal 5 The smell of fat, grease and grass	2	291.56 36	369.44 233	233.06 168	168.23 112	112.64 3	366. 22 28	286. 22 19	191.33	145.07	316.49 22	226.48 21	211.74 20	208.87
32 Nonanal 1 Aroma of roses and citrus, strong grease smell	e smell	0.00	99'.	0.00 38	38.37 (	0.00	54.99	0.00	28.82	0.00	72.49	0.00	74.03	0.00

in flavor between day 14 of cold storage and day 28 of slight freezing and freezing conditions. Therefore, with the extension of the storage time of prepared pork, superchilling and freezing conditions were more conducive to maintaining the flavor of prepared pork and delaying flavor attenuation than chilling conditions. The key flavor substances could be divided into two categories. The first category included nonanal, 2-methyl-1-butanol, terpinene, isoamyl acetate, ethyl isovalerate, octane, isoamyl alcohol, n-hexanol, d-limonene, myrcene, 3-octanone, terpinen-4-ol, phellandral, (1R)-()- $\alpha$ -pinene, 3-hydroxy-2-butanone, pinene, and methyl heptenone, totaling 17 kinds. The second category of flavor substances included 2-methyl butanol, 1-octen-3-ol, linalool, cineole and hexanal, totaling 5 kinds.

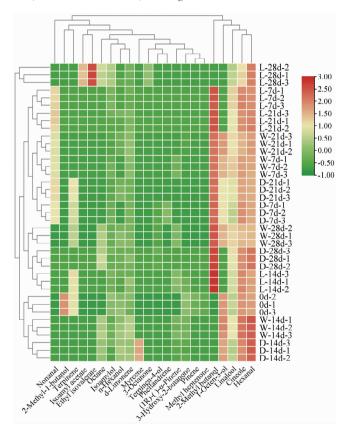


Fig. 2 Heat map of volatile flavor compound *OAV* in prepared pork during low-temperature storage

### Conclusions

In this study, the volatile flavor compounds of prepared pork under three low-temperature storage methods, namely chilling storage, superchilling and freezing, were analyzed at days 0, 7, 14, 21 and 28. The results of SPME-GC-MS showed that 46, 46 and 50 kinds of volatile flavor components were detected under the three low-temperature storage conditions, and among them, alcohols contributed the most to pork flavor, followed by aldehydes, ketones and aliphatic hydrocarbons. OAV analysis showed that there were 22 key volatile flavor compounds (OAV > 1), among which 2-methyl butanol, 1-octen-3-ol, linalool, cineole, hexanal and nonanal contributed greatly to the overall flavor of prepared

pork stored at low temperatures. Combined with principal component analysis and cluster analysis, it was found that there were four characteristic flavor substances under different low-temperature storage conditions, namely isoamyl acetate, hexyl isovalerate, hexanal and myrcene. On the whole, the overall flavor of prepared pork decreased during low-temperature storage, but compared with the conditions of 4  $^{\circ}\mathrm{C}$  and -18  $^{\circ}\mathrm{C}$ , the alcohol substances in the -4  $^{\circ}\mathrm{C}$  group were significantly higher than those in other two groups after 28 days of storage, and the overall content of volatile flavor substances was also significantly higher than that in other two groups, indicating that superchilling storage at -4  $^{\circ}\mathrm{C}$  may be more beneficial to maintain the overall flavor of prepared pork.

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