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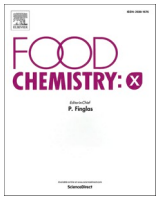

Identification of characteristic flavor quality of ceramic-pot sealed meat after reheating based on HS-GC-IMS, bionic sensory combined chemometrics

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ABSTRACT

This study investigated the impacts of microwave reheating (MR), boil reheating (BR), and steam reheating (SR) on the flavor profile of Ceramic-Pot Sealed Meat (CPSM). Electronic nose and tongue revealed that the microwaving was superior in preserving the original olfactory and gustatory profiles of CPSM compared to the other methods. Headspace- Gas chromatography- ion mobility spectrometry (HS-GC-IMS) detected 48 compounds, encompassing 15 alcohols, 11 aldehydes, 9 ketones, 7 esters, 2 alkenes, and 2 others, 1 acid. Spectral and clustering analysis revealed a significant rise in the content of Warmed-over flavor compounds after boil reheating, culminating in pronounced flavor distortion and a decline in sensory scores. Relative odor activity value (ROAV) and chemometrics identified nine substances as the principal flavor compounds responsible to flavor distortion. In conclusion, all reheating methods induce changes in the original flavor characteristics profiles of CPSM. However, microwave reheating offers superior preservation of the flavor characteristics of CPSM.

1. Introduction

Ready-to-eat meals, referred to as packaged foods or prepared dishes, offer consumers the convenience of immediate consumption with minimal cooking or reheating. China's market for prepared dishes has experienced rapid growth in recent years. The market value increased from USD 22.65 billion in 2018 to USD 62.94 billion in 2022, representing a substantial growth of 177.88% (Yi & Xu, 2023). Current trends and predictions indicate that the market is poised to sustain its robust growth, with the potential to achieve a valuation of USD 144.8 billion by 2026 (Hui & Liu, 2024). Ceramic-Pot Sealed Meat (CPSM) represents a quintessential Sichuan-style dish, notable for its fermented nature and unique saucy flavor, achieved through ceramic-pot fermentation (Xiao et al., 2021). The fermentation process of CPSM involves intricate steps that require strict control over environmental conditions and temperature, making it impractical to prepare at home. Fortunately, prepared CPSM simplifies this process, allowing individuals to recapture the dish's flavor and texture through reheating, which significantly reduces both time and labor costs.

However, previous research found that reheating beef by boiling or microwaving over three days led to a decrease in protein content and an increase in acidity, negatively affecting the meat's flavor and nutritional

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value (Nanje et al., 2024). Parvin et al. (2020) demonstrated that microwaving pre-cooked beef balls improved their oxidation stability, viscosity, and overall acceptability while reducing cooking loss, hardness, and chewiness. Another research examined the effects of various reheating methods on Hongsu chicken, revealing that microwaving yielded the highest sensory scores, with minimal weight loss and a quality akin to fresh meat in terms of aroma quality (Wang et al., 2018). Currently, a lot of investigations focus on the prosperities of prepared dishes from a macroscope, unfortunately, only a few reports have been explored the flavor transitions after reheating. The chemical senses, in particular taste and flavor, serve as crucial deterrents to spoiled or toxic foods, while encouraging the consumption of nutritious and beneficial foods (Breslin & Spector, 2008). Flavor is a multi-sensory experience involving taste, smell and somatosensory inputs (Auvray & Spence, 2008; Small, 2012), it is recognized that taste and retronasal olfaction are central to shaping our sensory perception of food (Gotow et al., 2013). Flavor stands as one of the predominant drivers of food selection, often taking precedence over other influencing factors.

Numerous studies have demonstrated that the sensory characteristics of food, including aroma, flavor, and texture, significantly influence consumer decisions. However, flavor perception is highly complex, involving interactions among hundreds of molecules at both the physiochemical and sensory levels (Chen et al., 2022). Flavor compounds can vary widely in chemical structure and concentration, making them technically challenging and labor-intensive to quantify. Traditional flavor detection methods including Gas Chromatography (Kolomnikov et al., 2018), Gas Chromatography-Mass Spectrometry (Huang et al., 2023; Qian et al., 2023), Liquid Chromatography-Mass Spectrometry (Di Stefano et al., 2012). Gas chromatography-ion mobility spectrometry (GC-IMS) is an innovative analytical technique that offers several advantageous features, including rapid response time, operational simplicity, high sensitivity, without sample pretreatment and efficient separation capabilities (Chen et al., 2024). Notably, GC-IMS enhances the selectivity of analysis methods by incorporating additional separation processes, thereby facilitating the differentiation of isobaric and isomeric compounds (Wang, Chen, & Sun, 2020). Electronic nose (*E*-nose) and electronic tongue (E-tongue) systems emulate the human olfactory and gustatory sensors, respectively, along with their neural communication pathways (Ma et al., 2023) Through the application of feature extraction and pattern recognition algorithms, these instruments can rapidly and accurately identify and classify distinct flavor profiles in a non-destructive manner (Peris & Escuder-Gilabert, 2016).

Although the flavor of prepared dishes plays a crucial role in determining consumers preference and acceptance, relatively few studies have reported the changes in flavor resulting from various reheating methods. Additionally, there is still a lack of methodology to sensitively detect the flavor discrepancies of Ceramic-Pot Sealed Meat after reheating. Therefore, this study aims to develop an innovative approach to investigate flavor discrepancies in Ceramic-Pot Sealed Meat after microwave, boiling, and steam reheating methods based on HS-GC-IMS, bionic sensory evaluation combined chemometric analysis. The transformations of amino acids, sensory attributes, color, and energy content post-reheating are also analyzed. This study can provide some basic knowledge for the regulation and control of the flavor quality of prepared dishes.

2. Materials and methods

2.1. Sample pretreatment

The Ceramic-Pot Sealed Meat (Zhouqu Green Vein Agricultural Science and Technology Co., Gansu Province, China) was carefully transported to the laboratory using ice containers and stored at ambient temperature for 6 h. The samples were then reheated using microwave, boiling, and steam methods. Control group (CG): Fresh CPSM serving as a reference, without any treatment; Microwave reheating (MR): CPSM heated in a microwave-safe container at 700 W for 30 s (Supor, UW30, Zhejiang Supor Co., China); Boiling reheating (BR): CPSM immersed in boiling water, 100 °C for 5 mins (Supor, UW30, Zhejiang Supor Co., China); Steam reheating (SR): CPSM placed in a steamer and heated at 130 °C for 5 mins (Rational iCombi Pro XS, Rational AG, Co., German).

2.2. Assessment of physicochemical characteristics

2.2.1. Sensory evaluation

Sensory evaluation was carried out by 10 panelists who non-smokers and did not have any documented diseases, particularly pertaining to the oral cavity and olfactory system. They carefully assessed the color, aroma, flavor, and texture of the CPSM based on evaluation criteria (Supplementary Table 1), with weighting of 0.3, 0.2, 0.3, and 0.2 for taste, appearance, aroma and texture. The sensory analysis in this study complied with sensory ethical standards, all participants participated voluntarily and their consent and knowledge were obtained. The information and privacy of participants in the study were anonymized and appropriate measures were taken to protect it. Consent was obtained from all participants before the open publication of the experimental data.

2.2.2. Determination of colorimetric analysis

Chromaticity values were obtained using a colorimeter (NR200+, 3nh Intelligent Technology Co., Ltd., Guangzhou City, China), which was precalibrated against a D65 whiteboard standard. Triplicate measurements were performed at three positions near the center of each sample, and the average values were recorded for the following color parameters: L^*(lightness), a^*(red-green component), b^*(blue-yellow component), C^*(chroma), h^*(hue angle), and ΔE (total color difference). The formula (1) for calculating ΔE is as follows:

$$\Delta E = \sqrt{(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2} \quad (1)$$

2.2.3. Determination of nutritional analysis

The energy, protein, fat, carbohydrate, and water of the CPSM was evaluated using a CA-HM Food Calorimetric Component Analyzer (JWP, Tokyo, Japan). The experimentation was conducted on five sets, with each set subjected to triplicate measurements. The final reported values for each sample set were the averages of the three independent measurements, ensuring reproducibility.

2.2.4. Determination of textural prosperity

The detection parameter of textural prosperity was according to a published article, with slight modification (Chen et al., 2024). Texture prosperity was meticulously performed using a TMS-Pro texture analyzer (FTC, Sacramento, CA, USA). Meat samples, precisely cut into 5 mm squares, were examined using a P/5 cylindrical probe with a diameter of 5 mm. The procedure entailed a multi-cycle mode at a speed of 1.0 mm/s, a triggering force of 0.375 N, and a down-pressing distance of 25 mm, with a deformation amount set at 50%. A consistent interval of 30 s was maintained between the cycles. Furthermore, the down-pressing deformation variable was precisely calibrated to 65%. Both the fat and muscle layers of each sample were evaluated. This entire process was systematically repeated six times, discounting the highest and lowest values for heightened accuracy.

2.3. Taste-active substances analysis

2.3.1. Analysis of E-tongue

The detection parameter of E-tongue was according to a published article, with slight modification (Zhao et al., 2023). Assessing comprehensive taste attributes was conducted using an Alpha MOS α-ASTREE electronic tongue (Toulouse, France), equipped with seven sensors, including sourness (AHS), saltiness (CTS), umami (NMS), sweetness

(ANS), bitterness (SCS), and two reference electrodes (PKS and CPS). Before the analysis, each sample was prepared by thoroughly mixing 50 g of CPSM with 200 g of ultrapure water then subjected to sonication at 55 KHz for 25 mins. The sonicated mixture was filtered, and 80 mL of the obtained filtrate was added to a 120 mL beaker for examination. The E-tongue measured each sample for 120 s, while sensors were cleaned with ultrapure water for 20 s to maintain consistent potential readings.

2.3.2. Determination of free amino acids (FAAs)

The detection parameter of amino acids was according to a published article (Qiao et al., 2024). The free amino acid contents were determined using an automatic amino acid analyzer (S433D, Sykam, Munich, Germany). Sample pretreatment involved the following steps: 1) Homogenizing equal parts of the sample (25 g) and ultrapure water; 2) Combining the homogenate with a 7% sulfosalicylic acid solution in a 1:1 mass ratio; 3) Sonicating the mixture at 55 kHz for 40 min; 4) Filtering the sonicated mixture; 5) Centrifuge the filtrate at 1145 *g* maintaing 15 min (MK-16B High Speed Table Centrifuge, Hunan Michael Experimental Instrument Co., China); 6) Filtering the supernatant through a 0.22 μm microporous membrane (Sigma Aldrich Trading Co., Ltd., Shanghai, China). The amino acid analyzer was equipped with a PEEK column (4.6 mm × 150 mm, 7 μm particle size, 10% cross-linking) and operated under the following conditions: temperature gradient from 20 to 99 °C reactor temperature of 130 °C, detection wavelengths of 570 and 440 nm, total analysis time of 57 mins, ninhydrin reagent flow rate of 0.25 mL/min, and injection volume of 40 μL.

2.4. Analysis of characteristic flavor compounds on CPSM

2.4.1. Analysis of E-nose

The E-nose (Alpha MOS Fox 4000, Toulouse, France) was employed for the objective detection of overall flavor profiles. This analytical instrument is equipped with an injection system, eighteen sensor chambers, a mass flow controller, and a microcontroller-based acquisition board. Table 1 exhibits the sensors performance characteristics. TOC-grade synthetic air, at a pressure of 5 psi, was utilized as the carrier gas for the experiments. 1 g of sample was placed into a 10 mL glass vial, which was subsequently sealed and incubated at 50 °C for 5 mins to facilitate volatile organic compounds (VOCs) generation. The duration of the measurement phase was 120 s, allowing the sensors to reach stable signal values. Data from the sensors were recorded by the computer at a rate of one reading per second during the measurement phase and were stored for analysis upon completion.

2.4.2. Analysis of flavor compounds by HS–GC-IMS

The VOCs were scrutinized using HS-GC-IMS (FlavourSpec®, G.A.S., Germany) in thermal mode at 60 °C. The system utilized an MXT-WAX column (15 m × 0.53 mm × 1 μm) for separation, where analytes were ionized using a tritium (^{3}H) source in the IMS ionization chamber maintained at 75 °C. The ionized VOCs were then introduced into a 53 mm IMS drift tube, applying an electric field of 500 V/cm with the drift tube also at 75 °C. Nitrogen (purity: 99.999%) served both as the carrier gas for the GC and the drift gas for the IMS, flowing counter-currently to the analyte ions at 150 mL/min, with the IMS operating in positive ion mode. 5 g of sample was placed into a 20 mL headspace glass vial and incubated at 70 °C for 20 mins. Subsequently, 500 μL of the headspace sample was injected into the GC-IMS system via a heated syringe at 85 °C through the heated inlet port. Each sample was analyzed in triplicate to ensure reproducibility. The retention indices of the VOCs were calculated using N-ketones C4-C9 as external references, and VOC identification was based on comparing RIs and drift times (DT) with NIST and IMS databases. Quantification was performed using VOCal software (G.A.S., Dortmund, Germany, version 0.4.03).

Table 1
Characteristics and performance parameters of E-nose sensor array.

Serial Number	Sensor Name	Performance	Type of Sensitive Substance
1	LY2/LG	Sensitive to gases with strong oxidizing ability	Chlorine, Fluorine, Sulfides
2	LY2/G	Sensitive to toxic gases	Ammonia, Amine compounds
3	LY2/AA	Sensitive to organic compounds	Ethanol, Ammonia
4	LY2/Gh	Sensitive to toxic gases	Ammonia, Amine compounds
5	LY2/gCTI	Sensitive to toxic gases	Sulfides
6	LY2/gCT	Sensitive to flammable gases	Propane, Butane
7	T30/1	Sensitive to polar compounds	Propanol, Hydrogen Chloride
8	P10/1	Sensitive to non-polar compounds	Hydrocarbons, Octane
9	P10/2	Sensitive to non-polar flammable gases	Methane, Heptane
10	P40/1	Sensitive to gases with strong oxidizing ability	Fluorine, Chlorine, Methyl Bran
11	T70/2	Sensitive to aromatic compounds	Xylene, Toluene
12	PA/2	Sensitive to organic compounds, toxic gases	Acetaldehyde, Amine compounds
13	P30/1	Sensitive to flammable gases, organic compounds	Ammonia, Ethanol
14	P40/2	Sensitive to gases with strong oxidizing ability	Chlorine, Methanethiol
15	P30/2	Sensitive to organic compounds	Hydrogen Sulfide, Copper
16	T40/2	Sensitive to gases with strong oxidizing ability	Chlorine
17	T40/1	Sensitive to gases with strong oxidizing ability	Fluorine
18	TA/2	Sensitive to organic compounds	Ethanol

2.4.3. Calculation of relative odor activity value

The relative odor activity value (ROAV) was used to calculate the key VOCs in the samples (Yuan et al., 2024), The ROAV of a VOCs contributes to the overall flavor of CPSM, which was set to 100. The ROAV values of other VOCs were calculated using the following formula (2). ROAV exceeding 1 significantly contribute to the flavors of YSSP. ROAV ranging from 0.1 to 1 indicates a moderate effect on sample flavors.

$$\mathrm{ROAV} \approx 100 \times \frac{C\%_X}{C\%stan} \times \frac{T_{stan}}{T_X} \quad (2)$$

Formula: Where C%*stan* and T*stan* are the percentage (%) and threshold (μg/kg) of the components contributing most to the flavor in YSSP; C%*X* and T*X* are the percentage (%) and threshold (μg/kg) of each VOCs.

2.5. Data processing and statistical analysis

The significance of differences and standard deviation of the ROAV was using SPSS 25.0 (IBM Corporation, Armonk, NY, USA) at a significance level of $P < 0.05$. Radar plots were plotted by Origin 2022 (Origin Lab Corporation, Northampton, MA, USA), and OPLS-DA were performed using SIMCA software (Version 18.1, Umetrics, Umeå, Sweden). Fingerprint spectra were plotted using Reporter plug-in (FlavourSpec®, G.A.S., Germany). Chemical structure diagrams of the identified VOCs were plotted using ChemDraw 20.0 (PerkinElmer, Waltham, MA, USA). Heat maps and hierarchical cluster analysis (HCA) were constructed using TBtools (Version 2.056, China). Matel test and Pearson test were analyzed by Chiplot (https://www.chiplot.online/#Network-plot).

3. Result and discussion

3.1. Physicochemical properties analysis

3.1.1. Sensory analysis

According to Fig. 1 and Supplementary Table 2, the sensory scores of CG, MR, BR, and SR samples were 85.2, 84.0, 75.3, and 79.3, respectively, indicating that different reheating methods significantly affected the original flavor ($P < 0.05$). The total sensory score of MR was significantly higher than other reheating methods, with the flavor and tissue status scores being significantly higher than those of other reheating methods ($P < 0.05$). However, there was no significant difference compared to the CG group ($P > 0.05$). In terms of aroma and taste, microwave reheating performed better than other samples in sensory evaluation, with its score being closest to that of the control group. This result is consistent with the findings of previous studies (Parvin et al., 2020). The minimal effect of microwave reheating on flavor and texture can be attributed to the unique heating mechanism of microwaves. Microwaves typically retain more moisture in food compared to conventional ovens, which can help maintain flavor (Ibrahim et al., 2012). The rapid heating process also reduces the time for flavor compounds to dissipate. Additionally, the electromagnetic energy from microwaves is converted into heat, causing polar molecules in the food to move rapidly. This movement results in friction and heat, which cooks the food quickly without significantly altering its texture (Yong et al., 2019).

3.1.2. Analysis of colorimetric

The color of a product greatly influences the quality of cooked meat products and consumer preferences, with vivid colors promoting positive consumer sentiment (Spence, 2015). Table 2 presents the impact of various reheating methods on the color of CPSM, where the L^* value denotes the brightness of the sample, the a^* value indicates the redness of the sample ($a^* > 0$), and the b^* value signifies the yellowness of the sample ($b^* > 0$). In comparison to the CG, the brightness and yellowness values of all sample groups decreased post-reheating, suggesting that diverse reheating methods expedite the oxidation rate of myoglobin and hemoglobin in pork as well as the non-enzymatic browning rate of amino acids (Bou et al., 2008), thus further diminishing brightness and yellowness. By calculating the ΔE, it is discernible that microwave and steam reheating can more effectively conserve the original color of CPSM, whereas BR exhibits a significant discrepancy in original color compared with the control group ($P < 0.05$).

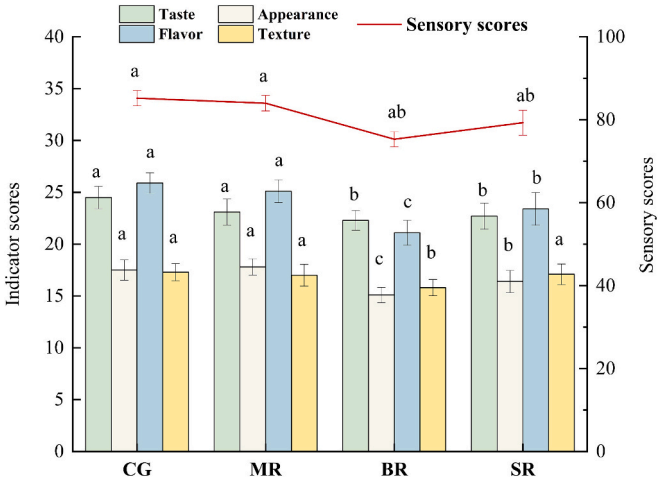


Fig. 1. Sensory socres of Ceramic-Pot Sealed Meat after various reheating methods.
Note: CG, control group; MR, microwave reheating; BR, wboil reheating; SR, steam reheating.

Table 2
Effects of different reheating methods on the color of CPSM.

Samples	L^*	a^*	b^*	C^*	h^*	ΔE
CG	61.67 ± 1.34^{a}	11.20 ± 1.19^{c}	24.75 ± 0.83^{a}	26.65 ± 1.43^{a}	55.81 ± 1.69^{a}	–
MR	59.11 ± 1.02^{b}	12.98 ± 0.18^{a}	20.89 ± 0.55^{a}	27.78 ± 0.54^{a}	53.47 ± 0.39^{b}	3.65 ± 0.38^{b}
BR	55.07 ± 0.25^{c}	12.35 ± 0.38^{ab}	22.24 ± 0.35^{a}	26.55 ± 0.50^{a}	52.79 ± 0.27^{b}	4.24 ± 0.33^{a}
SR	58.70 ± 0.33^{b}	11.48 ± 0.14^{bc}	20.69 ± 0.15^{a}	26.86 ± 0.88^{a}	51.78 ± 0.43^{b}	3.40 ± 0.23^{b}

Note: a, b, c Means with different letters within a row differ significantly ($P < 0.05$).
±: Represents the standard deviation. $n = 3$.

3.1.3. Analysis of textural properties

The texture of cooked meat is a paramount determinant in shaping consumer purchasing decisions (Jeltema et al., 2015). As Fig. 2 shows, reheating methods significantly influenced the hardness, elasticity, adhesiveness, and chewiness of CPSM ($P < 0.05$). BR and SR markedly diminished the hardness of samples ($P < 0.05$), whereas MR contrasted it. This enhancement in hardness can be attributed to the elevated temperatures observed during microwave reheating, leading to water migration and surface evaporation. The subsequent water loss culminates in a harder texture. Such findings align with previous research, which ascribes the heightened hardness to water loss and the contraction of connective tissue during microwaving (Li et al., 2023). It is significant to note that microwave and steaming reduced elasticity, while boiling reheating resulted in minimal alteration. The mean viscosity values for CG, MR, BR, and SR were 5.56 N, 5.09 N, 3.02 N, and 3.59 N, respectively, suggesting that all reheating methods curtail viscosity. But microwave reheating only caused a marginal decrease and exhibited an insignificant effect on the penetration difference ($P > 0.05$). Chewiness displayed a similar phenomenon, with microwaving preserving a superior chewiness. In contrast, boil reheating led to a substantial reduction in chewiness. Sensory evaluation corroborated that the cooked meat post boiling had forfeited its texture and integral shape.

3.1.4. Analysis of nutrient contents

Table 3 shows the nutrition changing of CPSM. Reheating improved the nutritional value of the meat, increasing the energy, fat, and carbohydrate contents while reducing water content to varying degrees ($P < 0.05$). MR caused a major drop in water content, possibly due to the intensified vaporization of internal water molecules, leading to water migration from the inside to the outside and evaporation on the surface. Previous studies have reported similar findings, stating that food ingredients exposed to high microwave frequency experience rapid water evaporation, generating pressure that drives water diffusion and promotes dehydration (Ambros et al., 2018). Water holding capacity is closely related to meat hardness, which may explain the increased hardness of cooked meat after microwave reheating. The protein content in BR and SR was significantly reduced, possibly due to excessive heat treatment promoting protein denaturation. The energy content of BR samples increased significantly to 1866 kJ compared to the control group ($P < 0.05$). The carbohydrate content in SR was significantly enhanced at 39.77 g/100 g, and the fat content was relatively high at 10.97 g/100 g, while the protein content was low at 9.0 g/100 g. These findings suggest that different reheating methods can significantly alter the nutritional composition of CPSM, with microwave reheating having the most notable impact on water content and hardness, while boiling and steaming affect protein content and other nutritional parameters.

3.2. Analysis of E-nose

Fig. 3(a) delineates the electronic nose signal radar diagram of CPSM. The overall aroma profile of the samples exhibits significant

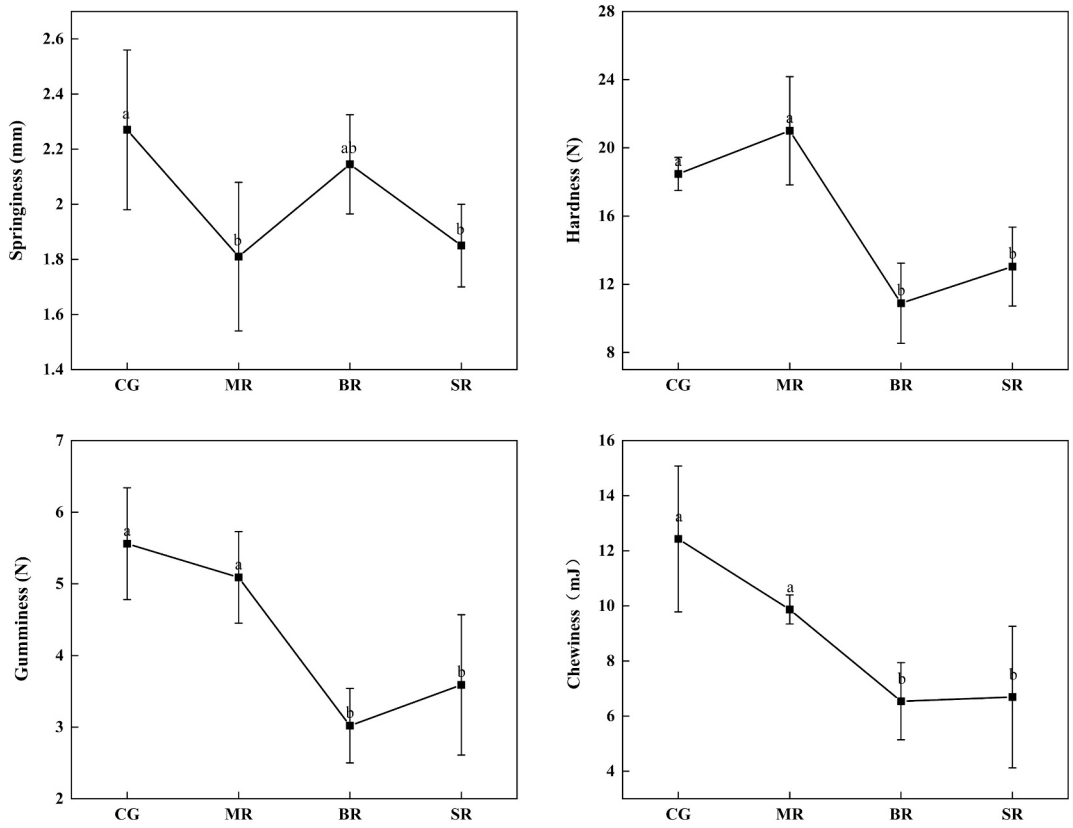


Fig. 2. Textural properities analysis of Ceramic-Pot Sealed Meat after various reheating methods.

Table 3
Nutrient contents in CPSM treated with different reheating methods.

Samples	Energy(kJ/100 g)	Protein (g/100 g)	Fat(g/100 g)	Carbohydrate (g/100 g)	Water(g/100 g)
CG	1444.67 ± 1.67^{d}	18.23 ± 0.29^{b}	7.20 ± 0.12^{b}	27.03 ± 0.09^{c}	39.57 ± 0.09^{a}
MR	1672.33 ± 18.35^{c}	22.23 ± 0.57^{a}	8.07 ± 0.38^{b}	30.93 ± 0.55^{b}	28.60 ± 0.1^{d}
BR	1866.00 ± 2.31^{a}	12.23 ± 0.18^{c}	11.33 ± 0.23^{a}	39.1 ± 0.06^{a}	36.23 ± 0.35^{b}
SR	1831.00 ± 9.07^{b}	9.0 ± 0.71^{d}	10.97 ± 0.56^{a}	39.77 ± 0.33^{a}	33.13 ± 0.12^{c}

Note: a, b, c Means with different letters within a row differ significantly ($P < 0.05$).
±: Represents the standard deviation. $n = 5$.

variance, indicating that different reheating methods attenuate the original aroma of CPSM, aligning with prior research findings. Among the 18 sensors in the response array, 12 sensors displayed conspicuous response intensity, while the response intensity of LY2/LG, LY2/G, LY2/AA, LY2/Gh, LY2/gCT1, and LY2/gCT sensors was nearly identical. This implies that without fluorine, chlorine, ammonia, sulfide, amine compounds, ethanol, butane, or propane are produced during the reheating process. The response values of sensors TA/2, T40/1, P30/2, P40/2, P30/1, PA/2, P40/1, P10/2, P10/1, and T30/1 to the heat-sensitive substances in samples are relatively high, with PA/2, P30/1, and P30/2 exhibiting the most robust response signals. These sensors, sensitive to aldehydes, alcohols, and organic compounds, can serve as evaluation indicators to monitor the characteristic flavor profile post-reheating. The electronic nose analysis offers invaluable insights into the impact of reheating methods on the volatile compounds and overall flavor of CPSM, underscoring the significance of opting for an appropriate reheating method to preserve the original flavor profile.

3.3. Analysis of E-tongue

The E-tongue, analogous to the E-nose, simulates the human taste system through an array of chemical sensors to discern the overall taste profile of food. It serves as a rapid, unbiased, and cost-efficient substitute to the gustatory system of human (Schlossareck & Ross, 2019). Fig. 3(b) suggests that the taste profiles following three distinct reheating methods are relatively comparable, especially for MR and BR. Nonetheless, in the case of SR, the signal intensity of the CTS sensor escalates significantly after steaming. Fig. 3(c) unveils that the flavor of CPSM undergoes substantial transformations after different reheating treatments. The original sour, salty, and umami flavors of CG were significantly intensified after reheating, while the sweetness and bitterness were markedly attenuated. The taste profiles of CPSM altered post-reheating, with SR notably augmenting the sour, salty, and umami flavors while diminishing the sweet and bitter signal intensity values ($P < 0.05$), culminating in a greater deviation from the CG. BR reduces sweetness and saltiness while enhancing sourness. Conversely, the MR results in increased umami, decreased sourness and saltiness.

3.3.1. Analysis of FAAs

Amino acids, serving as the precursors of flavor compounds, play a vital role in flavor development. Table 4 records the amino acid content in CPSM exposed to different reheating methods. A total of 20 amino acids were detected, inclusive of 8 essential ones. Microwave reheating amplified the levels of sweet and umami amino acids, such as threonine, serine, glutamic acid, glycine, alanine, and arginine. The total free amino acids in the CG were 385.2 mg/g, while SR exerted negligible effect on this content. Conversely, BR diminished the total free amino acids, possibly due to lipid degradation, Maillard reactions during cooking, or the solubilization of free amino acids in water. MR escalated the total free amino acids to 411.41 mg/g. Glutamic acid exhibited the highest content in MR, BR, and SR samples, recorded at 87.42 mg/g,

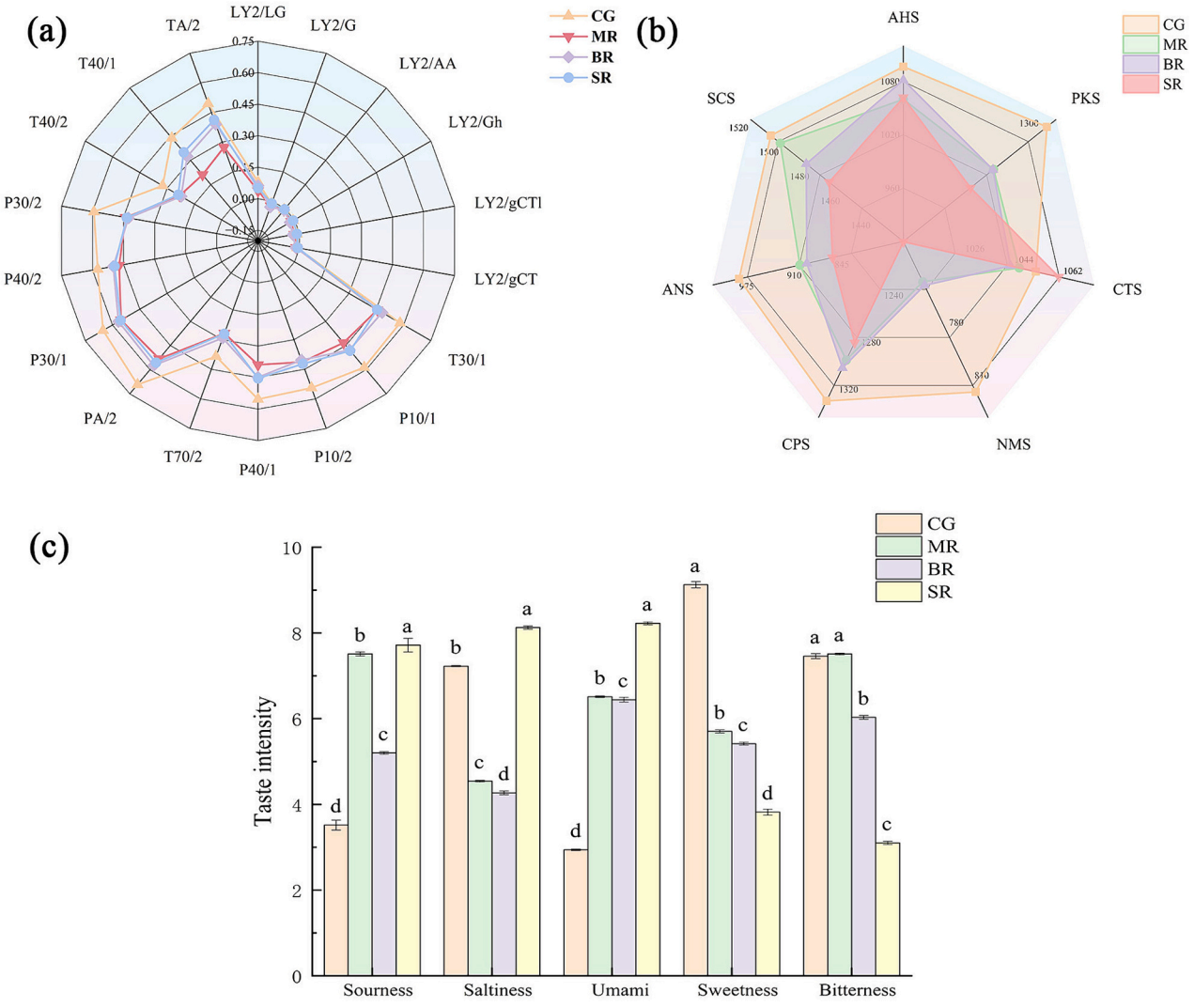


Fig. 3. Analysis of E-nose combined E-tongue after different reheating methods.
Note: (a) The radar chart of the E-nose, (b) The radar chart of the E-tongue, (c) Taste intensity value by E-tongue.

84.22 mg/g, and 85.43 mg/g, respectively. Glutamic acid crucially contributes to the umami flavor of CPSM and demonstrates a synergistic effect when amalgamated with other amino acids, thereby intensifying the umami taste (Bellisle, 1999). Increased concentrations of aspartic acid and glutamic acid enhance the overall flavor, while elevated levels of glycine and alanine amplify the sweetness of the pork. Alanine, glycine, glutamic acid, and aspartic acid collectively dictate the umami flavor of pork, with glutamic acid playing a pivotal role in generating a rich umami taste. Boiling decreased the TFAA, EAA and DAA, yielding a less appealing flavor compared to microwave and steam reheating. These findings corroborate a strong correlation between amino acid content and sensory score results.

3.4. Analysis of HS-GC-IMS

Fig. 4(A) exhibits a 3D topographic map of the overall volatile flavor distribution profile of CPSM. The X-axis denotes the ion migration time (ms), the Y-axis signifies the retention time (s), and the *Z*-axis represents the intensity value of compounds (v). The red vertical line on the left signifies the reactive ion peak, while each dot corresponds to a specific compound. The color depth symbolizes the peak intensity of compound, with blue indicating a peak of relatively low intensity and red indicating a peak of high intensity. To facilitate a more lucid comparison of the differences in the peak areas of compounds post-reheating, CG was utilized as the reference spectrum. The blue background of other samples was eliminated to obtain the subtracted spectra of MR, BR, and SR, as depicted in Fig. 4(B). In the non-reference spectrum, red implies that the compound content exceeds CG, while blue signifies that the compound content is inferior to CG. A qualitative analysis of compounds was conducted by comparing the NIST database and IMS database, in combination with retention time and ion migration time. As presented in Table 5, 48 compounds (including monomers and dimers) were identified, comprising 15 alcohols, 11 aldehydes, 9 ketones, 7 esters, 3 olefins, 1 acid, and 2 other types. The retention time of most volatile compounds falls between 200 and 800 s.

The subtracted spectra in Fig. 4B (f, h) displays many red spots, indicating an augmentation in certain compounds, while Fig. 4B (g) reveals an increase in blue spots, suggesting a decrease in flavor compounds following boil reheating. This decrease might be ascribed to the potent heat transfer power of the boiling, resulting in protein denaturation and fat degradation into other precursor substances, thereby diminishing the content of volatile substances. To delve deeper into the disparities in VOCs content, we established a fingerprint spectrum, As Fig. 4C shows, The fingerprint pattern is divided into four categories, labeled a (yellow dashed frame), b (green), c (orange), and d (pink), respectively. Zone (a) exhibits nearly no significant alteration in

Table 4
Amino acid content of CPSM under different reheating methods.

Amino acids	Taste characteristics	CG	MR	BR	SR
Aspartic acid (Asp)	Umami	5.47 ± 0.89^{ab}	6.17 ± 0.63^{ab}	7.80 ± 1.74^{a}	5.25 ± 0.95^{b}
Threonine (Thr)	Sweet	18.58 ± 0.10^{b}	19.38 ± 0.64^{ab}	17.53 ± 0.32^{c}	19.63 ± 0.77^{a}
Serine (Ser)	Sweet	3.54 ± 0.57^{a}	2.28 ± 1.80^{a}	3.69 ± 0.88^{a}	4.54 ± 0.29^{a}
Asparagine (Asn)	Umami	15.02 ± 0.58^{a}	13.40 ± 1.20^{ab}	12.36 ± 1.30^{b}	13.77 ± 0.21^{ab}
Glutamic acid (Glu)	Umami	86.11 ± 0.14^{ab}	87.28 ± 0.16^{a}	82.67 ± 1.34^{c}	84.56 ± 1.09^{b}
Glycine (Gly)	Sweet	22.57 ± 0.03^{a}	23.02 ± 0.04^{a}	21.71 ± 0.72^{ab}	21.05 ± 1.18^{b}
Alanine (Ala)	Sweet	35.79 ± 0.25^{a}	36.43 ± 0.20^{a}	35.98 ± 0.53^{a}	35.93 ± 0.67^{a}
Citrulline	Sweet	1.69 ± 0.83^{a}	1.88 ± 0.81^{a}	1.30 ± 0.37^{a}	1.18 ± 0.43^{a}
Methionine (Met)	Sweet	13.59 ± 0.61^{ab}	14.80 ± 0.63^{a}	13.84 ± 0.72^{a}	12.56 ± 0.59^{b}
Isoleucine (Ile)	Bitter	20.06 ± 0.44^{a}	20.97 ± 0.50^{a}	18.24 ± 0.41^{b}	19.05 ± 0.76^{b}
Leucine (Leu)	Bitter	30.27 ± 0.62^{ab}	31.77 ± 0.47^{a}	28.10 ± 2.08^{b}	32.59 ± 1.74^{a}
Tyrosine (Tyr)	Bitter	18.58 ± 0.71^{b}	19.80 ± 0.59^{a}	16.81 ± 0.30^{b}	18.22 ± 0.32^{c}
Phenylalanine (Phe)	Bitter	18.47 ± 0.36^{b}	20.90 ± 0.28^{a}	17.43 ± 0.54^{b}	18.53 ± 0.87^{c}
Histidine (His)	Bitter	2.66 ± 0.02^{a}	2.50 ± 0.90^{a}	2.38 ± 0.44^{a}	1.22 ± 1.30^{a}
Tryptophan (Trp)	Bitter	34.24 ± 0.34^{b}	43.43 ± 0.26^{a}	31.70 ± 0.86^{c}	32.94 ± 1.34^{bc}
Ornithine (Orn)	Umami	11.56 ± 1.43^{b}	13.77 ± 1.10^{a}	0.44 ± 0.14^{c}	13.56 ± 0.72^{a}
Lysine (Lys)	Bitter	18.91 ± 0.88^{b}	19.32 ± 0.67^{b}	19.87 ± 0.39^{b}	21.36 ± 0.05^{a}
Arginine (Arg)	Sweet	3.41 ± 0.23^{b}	5.39 ± 0.19^{a}	4.14 ± 1.06^{b}	3.51 ± 0.37^{b}
Hydroxyproline (Hyp)	Sweet	0.57 ± 0.69^{a}	0.38 ± 0.80^{a}	1.36 ± 0.55^{a}	1.54 ± 0.71^{a}
Proline (Pro)	Sweet	21.87 ± 0.64^{b}	23.35 ± 0.62^{a}	20.59 ± 0.35^{c}	21.77 ± 0.41^{b}
Total Free Amino Acid Content		382.93 ± 2.76^{b}	406.23 ± 4.90^{a}	357.95 ± 2.41^{c}	382.77 ± 4.80^{b}
Total essential amino acids (EAA)		156.79 ± 0.30^{b}	173.07 ± 1.47^{a}	149.09 ± 2.09^{c}	157.88 ± 2.61^{b}
Flavor amino acid content (DAA)		369.12 ± 1.48^{b}	390.18 ± 4.34^{a}	354.84 ± 2.62^{c}	366.50 ± 4.74^{b}
DAA/TFAA(%)		96.39	96.05	99.13	95.75

Note: a, b, c Means with different letters within a row differ significantly ($P < 0.05$).
±: Represents the standard deviation. $n = 3$.

substance content, predominantly composed of alcohols. In zone (b) all compounds exhibit a certain degree of loss post-heating, primarily comprising small molecule oxidation products such as esters. The relative contents of total esters in CG, MR, BR, and SR are 5.46, 4.92, 4.05, and 4.11, respectively, with the boiling being the most destructive to esters. Esters typically possess floral and fruity aromas, playing an integral role in the flavor of dishes. Conversely, the relative content of ketones (10.47) in zone (c) escalated after microwave reheating, aligning with previous research findings (Xiao et al., 2019). Zone (d) encompasses aldehydes and ketones, including heptaldehyde-D, heptaldehyde-M, 2-butanone-M, 2-heptanone, and cyclohexanone-D. While these compounds are present in relatively low quantities in CG, but their contents increased following both boil and steam reheating. Fig. 5(a) exhibits a cluster heat map of peak area, lucidly illustrating that alcohols and esters undergo the most drastic transformations after boil reheating, while SR has a greater impact on aldehydes. Notably, microwave reheating better preserves the original flavor content. Cluster analysis uncovers that BR and SR are classified into the same category, and CG and MR are classified into the same category, indicating that the flavor substances post-microwave reheating are relatively close to the original samples.

3.5. Multivariate statistical analysis

3.5.1. Analysis of ROAV

In addition to examining the peak intensity of VOCs, it is crucial to consider their threshold concentration, as these two factors collectively determine the true extent of their flavor contribution (Bi et al., 2024). The relative content of 1-nonenal-M is relatively high, with a threshold value of 1 μg/kg, indicating its remarkable contribution to the overall flavor. The ROAV of 1-nonenal-M is designated as 100. As Table 6 and Fig. 5(b) shows, 26 key VOCs were identified, including 4 alcohols, 11 aldehydes, and 5 ketones, 4 esters, 1 acid and 1 alkene.

A total of 11 aldehydes were detected, with 9 serving as key flavor compounds (ROAV>1) across all three reheating methods. Notably, 7 aldehydes exhibited an ROAV>20, establishing them as the dominant flavor compounds in CPSM. The low thresholds and high volatility of aldehydes enable them to play a crucial role in shaping the distinctive flavors of various livestock and poultry species while also imparting a characteristic grassy aroma to fermented meat products (Bozkurt & Erkmen, 2002). Aldehydes are primarily formed through two pathways: (1) the decomposition of unsaturated fatty acids, such as linoleic acid, linolenic acid, and arachidonic acid, catalyzed by lipoxygenase and fatty acid hydroperoxide lyase, yielding hexanal and branched aliphatic aldehydes like valeraldehyde; and (2) the Maillard and Strecker reactions between carbohydrates and reducing sugars (Qian et al., 2021). The flavor contribution of aldehydes follows the order: 1-nonanal-M > 2-methylbutanal-D > hexanal-D > hexanal-M > butanal-M > heptaldehyde-M > 2-methylbutanal-M. By calculating the change rate of ROAV, using CG as the control group, it was determined that the change rates following MR, BR, and SR were 14.52%, 24.57%, and 23.43%, respectively. Microwave heating demonstrated the least impact on flavor substances, while boil reheating had the most pronounced effect, followed by steam reheating.

1-Nonanal-M, characterized by its low threshold, emerged as the compound with the most significant flavor contribution. It imparts a fatty and fresh citrus aroma and can act synergistically with other flavor compounds or precursor substances to enhance the meaty flavor (Weng et al., 2024). These findings are consistent with previous studies that have identified 1-Nonanal as a characteristic flavor substance in fermented meat products, sausages, and fish, further validating the results of the current research (He et al., 2024; Zhang et al., 2024; Zhou et al., 2023). 2-methylbutanal, known for its nutty and cocoa aroma, is primarily formed from valine, leucine, and isoleucine through the Strecker reaction, which is one of the Maillard reaction pathways. 2-methylbutanal undergo condensation reactions in meat and other foods to form volatile flavor products with important flavor properties (Smit et al., 2009). Boil reheating significantly enhanced the flavor intensity of 2-methylbutanal ($P < 0.05$), while steam and microwave reheating reduced its flavor intensity ($P < 0.05$). Although aldehydes contribute greatly to the flavor of livestock and poultry products, however, excessive content can lead to unpleasant greasy and putrid smells (Yuan et al., 2019). Combined with sensory evaluation found that the flavor intensity after boil reheating was significantly lower than others. It is hypothesized that 2-methylbutanal may work synergistically with other compounds, such as hexanal, nonanal, and heptanal, to cause unpleasant odors increasing. High temperatures during boil heating promote the Strecker reaction, thereby converting flavor precursors (leucine and isoleucine) into 2-methylbutanal-M and 2-methylbutanal-D. This

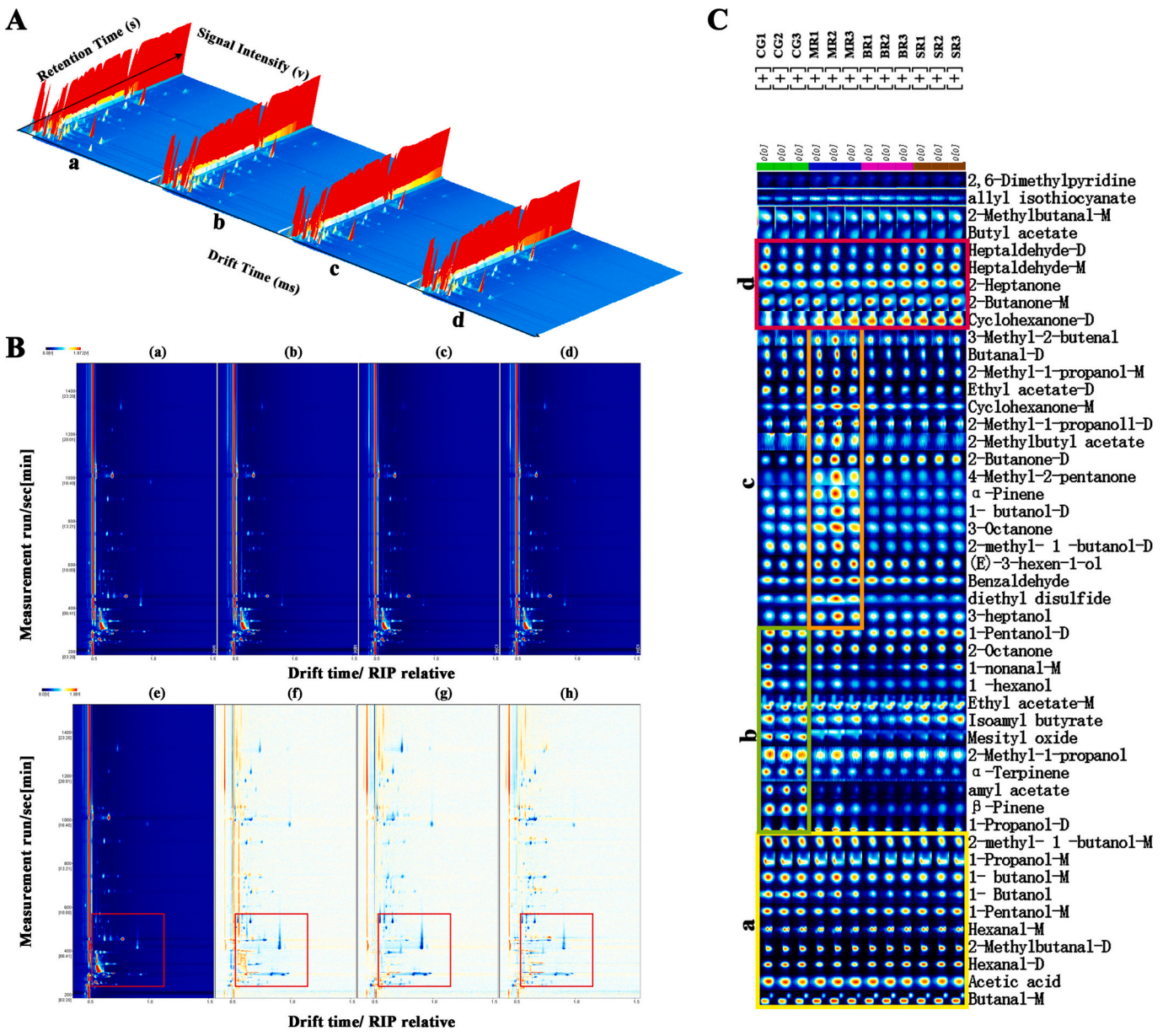


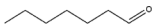
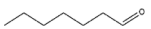
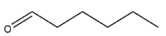
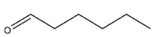
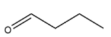
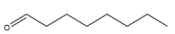
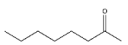
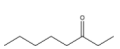
Fig. 4. Analysis of HS-GC-IMS after different reheating methods.
Note: CG, control group; MR, microwave reheating; BR, water bath reheating; SR, steam reheating; M, monomer; D, dimer.
(A). The three-dimensional spectrum of volatile compounds in CG, MR, BR, SR, (a) Gontrol group, (b) microwave reheating, (c) boil reheating, (d) steam reheating; (B). Composition spectrum (top view) and difference spectrum of volatile compounds in CG, MR, BR, SR; (a) 2D qualitative chromatogram of CG, (b) 2D qualitative chromatogram of MR, (c) 2D qualitative chromatogram of BR, (d) 2D qualitative chromatogram of SR (e) reference chromatogram of CG, (f) Deducition chromatogram of MR (g) Deducition chromatogram of BR, (h) Deducition chromatogram of SR. (C). Fingerprint spectrum of samples gallery plot.

finding is consistent with the detection results of free amino acids. The decrease in the content of 2-methylbutanal after microwave treatment may be attributed to the short microwave heating time and insufficient reaction of the precursor substances. This observation is in agreement with the findings of Luo, who also reported that the content of this substance after microwave reheating was significantly lower than that after boil reheating (Luo et al., 2022).

Hexanal, a long-chain aldehyde substance, serves as a crucial indicator of quality deterioration in cooked meat products and in evaluating the intensity of Warmed-over flavor (WOF), an unfavorable flavor characteristic (Tikk et al., 2008). Hexanal arises from the rapid oxidative spoilage of precooked, refrigerated, and reheated meat. Initially, the formation of hexanal was attributed to the oxidation of membrane phospholipids during heating (Ang & Lyon, 1990). However, recent years have seen the proposition of two additional synthetic pathways: (1) n-6 hydrogen peroxide, formed from n-6 polyunsaturated fatty acids and n-7 unsaturated fatty acids via a typical lipid autooxidation mechanism, can be cleaved to an alkoxy group and further cleaved to hexanal by β-cleavage on the carboxylic acid terminal side; and (2) lipid oxidation products, such as 2,4-nonadienal and 2,4-decadienal, may undergo further cleavage to produce hexanal through the reverse aldol reaction (Wang, Zhen, et al., 2020). We observed an increase of 17.08 and 17.62 in hexanal-M and hexanal-D content, respectively, after boil reheating. Therefore, BR significantly exacerbates the WOF in CPSM, leading to quality deterioration.

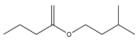
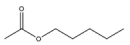
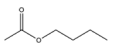
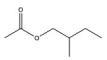
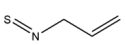
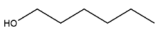
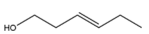
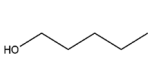
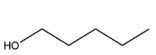
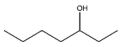
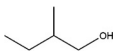
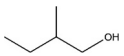
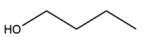
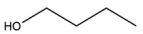
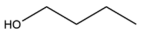
However, both steam and microwave reheating reduced the WOF resulting from lipid oxidation, thereby aiding in maintaining or enhancing the product's original flavor. These observations align with the sensory score results. Butanal-M, heptaldehyde-D, heptaldehyde-M, and hexanal share similar synthesis pathways, all produced through

Table 5
Volatile components of CG, MR, BR and SR samples.

Count	Classification	Compound name	CAS#	Formula	Structural type	Molecular weight	Retention time	Retention indexes	Drift time	Peak intensity			
										CG	MR	BR	SR
1	Aldehydes	Benzaldehyde	100–52-7	C7H6O		106.1	1510.1	1663.168	1.15341	3679.2 ± 459.5	4894.6 ± 521.7	4366.7 ± 304.2	21,287.6 ± 30,328.7
2		Heptaldehyde-D	111–71-7	C7H14O		114.2	1189.4	677.345	1.69616	333.6 ± 46.7	306.3 ± 54.3	317.6 ± 37.4	427.1 ± 38.0
3		Heptaldehyde-M	111–71-7	C7H14O		114.2	1188.3	674.818	1.32691	1538.7 ± 118.5	1379.1 ± 117.1	1446.0 ± 167.8	1715.5 ± 52.3
4		hexanal-M	66–25-1	C6H12O		100.2	1091.9	460.228	1.2623	6076.3 ± 149.7	5594.6 ± 261.9	5972.8 ± 31.6	6089.8 ± 90.7
5		hexanal-D	66–25-1	C6H12O		100.2	1089	456.606	1.56433	7785.7 ± 213.6	5973.6 ± 657.1	7628.9 ± 325.7	8118.6 ± 173.1
6		2-Methylbutanal-D	96–17-3	C5H10O		86.1	920.7	299.902	1.40707	3338.5 ± 204.9	3499.2 ± 100.3	3665.3 ± 61.9	3547.8 ± 92.6
7		2-Methylbutanal-M	96–17-3	C5H10O		86.1	917.7	298.188	1.15542	861.8 ± 59.9	698.9 ± 66.2	778.1 ± 30.4	707.5 ± 14.3
8		Butanal-D	123–72-8	C4H8O		72.1	843	255.216	1.27997	371.9 ± 2.7	362.1 ± 6.6	253.9 ± 17.2	268.8 ± 14.1
9		Butanal-M	123–72-8	C4H8O		72.1	848.4	258.33	1.11561	11,055.7 ± 237.4	12,610.3 ± 538.8	13,064.0 ± 102.3	12,880.5 ± 195.7
10		1-Nonanal-M	124–19-6	C9H18O		142.2	1400.3	1334.244	1.46427	2447.6 ± 629.1	2475.7 ± 173.3	1868.9 ± 427.8	2966.6 ± 504.0
11		3-Methyl-2-butenal	107–86-8	C5H8O		84.1	1206.5	730.514	1.09527	381.9 ± 25.5	385.3 ± 24.5	369.0 ± 13.5	363.2 ± 19.0
12	Ketones	Cyclohexanone-M	108–94-1	C6H10O		98.1	1308.3	1058.231	1.15833	847.5 ± 49.2	1261.3 ± 69.4	1006.7 ± 74.1	901.6 ± 18.0
13		2-Octanone	111–13-7	C8H16O		128.2	1294	1015.136	1.32623	8977.5 ± 1687.7	9361.3 ± 438.2	8433.4 ± 80.5	8102.1 ± 94.3
14		3-Octanone	106–68-3	C8H16O		128.2	1210.9	744.611	1.30828	719.1 ± 22.6	1014.1 ± 97.0	647.6 ± 21.8	662.1 ± 13.2
15		2-Heptanone	110–43-0	C7H14O		114.2	1184.7	666.606	1.25808	836.8 ± 18.7	846.4 ± 71.1	908.5 ± 23.4	917.1 ± 25.1
16		Mesityl oxide	141–79-7	C6H10O		98.1	1131	543.952	1.11571	2873.4 ± 19.4	2435.7 ± 111.0	1604.4 ± 38.2	1899.1 ± 6.7
17		2-Butanone-D	78–93-3	C4H8O		72.1	910.9	294.281	1.24601	2215.6 ± 261.3	3734.3 ± 386.4	3295.1 ± 45.7	3327.1 ± 189.5
18		2-Butanone-M	78–93-3	C4H8O		72.1	910.2	293.875	1.05721	745.4 ± 75.2	750.3 ± 27.0	838.1 ± 16.4	837.9 ± 17.0
19		4-Methyl-2-pentanone	108–10-1	C6H12O		100.2	1017.4	369.345	1.18055	203.4 ± 14.5	369.8 ± 51.7	238.6 ± 13.5	240.3 ± 10.1

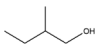
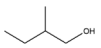
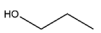
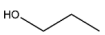
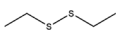
(*continued on next page*)

Table 5 (*continued*)

Count	Classification	Compound name	CAS#	Formula	Structural type	Molecular weight	Retention time	Retention indexes	Drift time	Peak intensity			
										CG	MR	BR	SR
20		Cyclohexanone-D	108–94-1	C6H10O		98.1	1294.1	1015.518	1.15501	984.3 ± 69.6	1273.8 ± 100.9	1312.8 ± 19.4	1397.9 ± 49.6
21	Esters	Isoamyl butyrate	106–27-4	C9H18O2		158.2	1298.3	1028.156	1.40609	494.1 ± 49.4	459.0 ± 23.0	399.0 ± 51.6	512.3 ± 12.8
22		amyl acetate	628–63-7	C7H14O2		130.2	1127.6	536.281	1.32132	906.7 ± 33.9	349.3 ± 33.5	279.8 ± 4.9	399.9 ± 30.2
23		Butyl acetate	123–86-4	C6H12O2		116.2	1074.1	438.468	1.24304	292.4 ± 13.8	286.5 ± 2.7	269.8 ± 12.7	268.4 ± 7.8
24		Ethyl acetate-D	141–78-6	C4H8O2		88.1	895.5	285.421	1.33647	371.9 ± 2.7	362.1 ± 6.6	253.9 ± 17.2	268.8 ± 3.4
25		Ethyl acetate-M	141–78-6	C4H8O2		88.1	892.8	283.864	1.1002	11,055.7 ± 237.4	12,610.3 ± 538.8	13,064.0 ± 102.3	12,880.5 ± 14.1
26		2-Methylbutyl acetate	624–41-9	C7H14O2		130.2	1128.8	539.006	1.29223	134.6 ± 2.3	169.2 ± 17.7	115.2 ± 10.7	120.8 ± 1.7
27		Allyl isothiocyanate	1957/6/7	C4H5NS		99.2	1377.5	1265.768	1.08992	431.4 ± 19.4	450.7 ± 24.3	434.4 ± 3.7	420.1 ± 4.8
28	Alcohols	1 -hexanol	111–27-3	C6H14O		102.2	1364.2	1225.895	1.33305	778.8 ± 204.0	610.2 ± 73.8	523.8 ± 18.2	579.5 ± 22.1
29		(E)-3-hexen-1-ol	928–97-2	C6H12O		100.2	1309	1060.333	1.23406	692.2 ± 60.4	856.6 ± 46.2	780.1 ± 50.7	664.1 ± 22.9
30		1-Pentanol-M	71–41-0	C5H12O		88.1	1261.8	910.321	1.2525	2993.3 ± 106.7	3071.1 ± 15.4	2846.0 ± 17.5	3043.2 ± 58.5
31		1-Pentanol-D	71–41-0	C5H12O		88.1	1260.5	905.99	1.50712	3516.1 ± 59.5	4145.8 ± 347.0	2540.6 ± 79.7	3280.9 ± 69.2
32		3-heptanol	589–82-2	C7H16O		116.2	1260.5	905.99	1.32315	626.7 ± 29.0	793.0 ± 117.1	639.0 ± 12.6	668.3 ± 26.9
33		2-methyl-1-butanol-M	137–32-6	C5H12O		88.1	1210.9	744.764	1.2436	3231.0 ± 109.6	3149.1 ± 287.9	2580.3 ± 26.1	2763.8 ± 43.4
34		2-methyl-1-butanol-D	137–32-6	C5H12O		88.1	1211.8	747.823	1.48288	885.4 ± 26.9	1033.4 ± 200.5	607.8 ± 23.6	678.9 ± 30.0
35		1- butanol-M	71–36-3	C4H10O		74.1	1144.4	574.704	1.17967	1302.6 ± 38.2	1235.4 ± 109.8	1024.4 ± 11.5	1112.0 ± 25.3
36		1- butanol-D	71–36-3	C4H10O		74.1	1143.6	572.868	1.37955	189.2 ± 1.2	229.4 ± 46.6	138.0 ± 8.1	152.1 ± 4.2
37		1- Butanol	71–36-3	C4H10O		74.1	1131.4	544.948	1.18065	1299.1 ± 25.4	1043.6 ± 71.9	722.6 ± 13.7	901.6 ± 46.3

(*continued on next page*)

Table 5 (*continued*)

Count	Classification	Compound name	CAS#	Formula	Structural type	Molecular weight	Retention time	Retention indexes	Drift time	Peak intensity			
										CG	MR	BR	SR
38		2-Methyl-1-propanol-M	137–32-6	C5H12O		74.1	1099	470.982	1.17234	1106.7 ± 37.6	1166.5 ± 83.0	952.0 ± 19.0	1018.8 ± 18.1
39		2-Methyl-1-propanol-D	137–32-6	C5H12O		74.1	1093.5	462.115	1.35857	3806.1 ± 91.7	4542.4 ± 383.3	3914.8 ± 21.5	4043.3 ± 36.5
40		1-Propanol-M	71–23-8	C3H8O		60.1	1041.5	398.751	1.11336	2993.3 ± 106.7	3071.1 ± 15.4	2846.0 ± 17.5	3043.2 ± 58.5
41		1-Propanol-D	71–23-8	C3H8O		60.1	1041.1	398.164	1.25334	3516.1 ± 59.5	4145.8 ± 347.0	2540.6 ± 79.7	3280.9 ± 69.2
42		2-Methyl-1-propanol	78–83-1	C4H10O		74.1	1116.9	511.876	1.16665	89.1 ± 3.6	70.4 ± 4.5	70.7 ± 1.4	65.1 ± 3.9
43	Acids	Acetic acid	64–19-7	C2H4O2		60.1	1512	1669.073	1.05307	13,153.0 ± 811.0	14,115.8 ± 415.1	13,496.4 ± 426.7	15,180.7 ± 39,038.4
44	Alkenes	α-Terpinene	586–62-9	C10H16		136.2	1160	610.172	1.21593	442.4 ± 28.3	372.3 ± 47.5	291.5 ± 18.5	280.4 ± 14.4
45		β-Pinene	127–91-3	C10H16		136.2	1116.6	511.168	1.22076	483.2 ± 8.6	326.1 ± 34.5	220.9 ± 4.2	259.2 ± 9.4
46		α-Pinene	80–56-8	C10H16		136.2	1025.6	379.332	1.22316	471.3 ± 10.5	596.6 ± 57.9	415.4 ± 19.2	418.5 ± 16.1
47	Others	diethyl disulfide	110–81-6	C4H10S2		122.2	1230.9	809.866	1.14096	933.8 ± 24.8	1382.6 ± 171.6	820.9 ± 18.3	850.3 ± 21.7
48		2,6-Dimethylpyridine	108–48-5	C7H9N		107.2	1274.7	952.246	1.08417	776.9 ± 42.8	980.6 ± 112.2	842.7 ± 15.7	868.6 ± 28.9

±: Represents the standard deviation. n = 3.

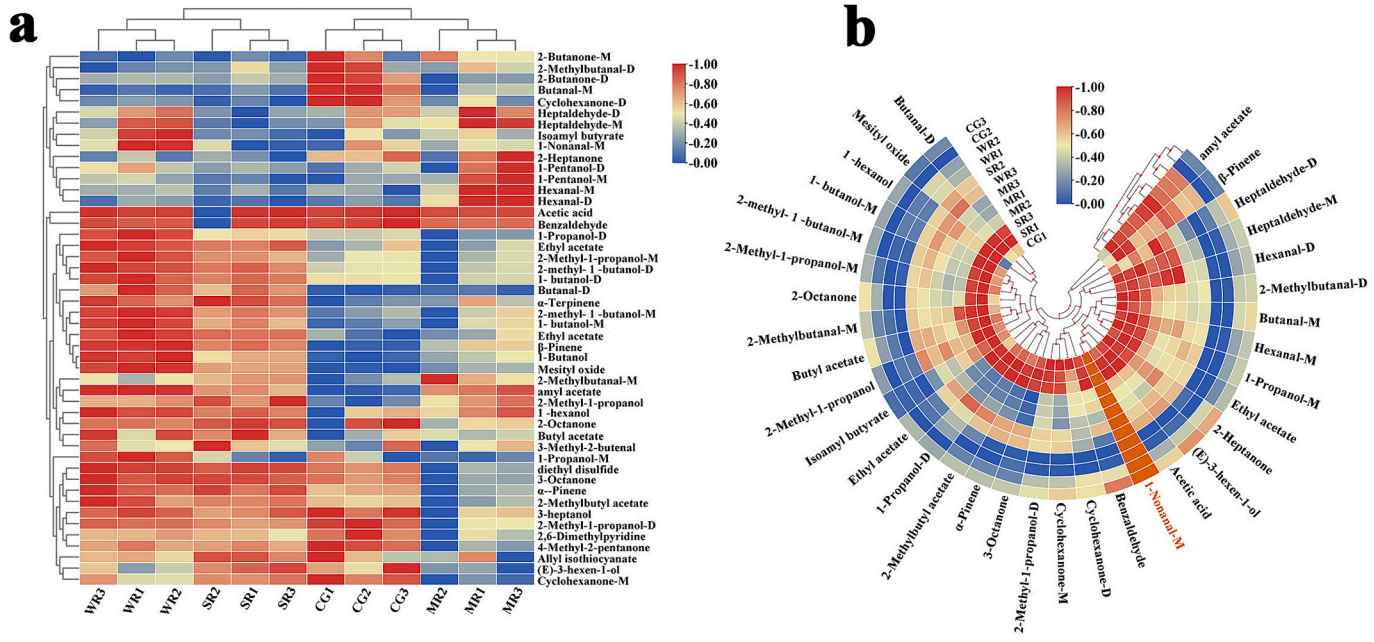


Fig. 5. The cluster heat map of volatile organic compounds.
Note: (a) The cluster heat map of peak intensity, (b) The cluster heat map of ROAV values.
CG, control group; MR, microwave reheating; BR, water bath reheating; SR, steam reheating; M, monomer; D, dimer.

lipid oxidation reactions. Collectively, they are key factors in WOF development (Yang et al., 2020). We found butanal-M, heptaldehyde-D, heptaldehyde-M exhibited a similar upward trend to hexanal, with a significant increase after boil reheating, and a non-significant decrease following microwave and steam reheating ($P < 0.05$). This suggests that prolonged heating in boil reheating increases lipid oxidation. Through analysis of the above aldehydes, we found that boil heating significantly increases lipid oxidation in CPSM, converting certain amino acid precursors, like leucine and isoleucine, into lipid oxidation products, including hexanal, nonanal, and heptanal. An increase in these products can yield a putrefaction-like odor, thereby spoiling the original flavor. Conversely, microwave and steam reheating can mitigate lipid oxidation and reduce the content of WOF, thus better preserving the original flavor of the cooked meat products. This finding may explain the popularity of microwave and steam reheating methods.

A total of 15 alcohols were identified. However, only 2-Methyl-1-propanol-M and 2-Methyl-1-propanol-D (ROAV>1) made a significant contribution to the flavor profile. Alcohols are frequently found in fermented foods and are typically produced by the oxidative degradation of lipids. They can also act as precursors of esters. Due to their high thresholds, alcohols have a lesser contribution to the overall sample flavor. However, certain unsaturated alcohols can enhance the fruity and floral aroma of food through the synergistic effects of various precursor substances and compounds (Kaur & Singh, 2000). 2-methyl-1-propanol, characterized by its fruity and floral aromas, can impart a unique, rich, and refreshing flavor to fermented products. Both 2-Methyl-1-propanol-M and 2-Methyl-1-propanol-D are key flavor compounds in esters, with ROAV values of 1.42 and 5.24 in the control group, respectively. Following microwave reheating, the contribution level of these compounds remained relatively stable. This may be because microwave treatment can effectively inactivate lipase activity, thus reducing the degree of lipid oxidation and the generation of free radicals, thereby preventing the conversion of lipid into alcohols (Suri et al., 2020). However, the content of these compounds exhibited significant fluctuations after boil and steam reheating ($P < 0.05$).

We identified seven ester compounds, ethyl acetate-D, ethyl acetate-M, 2-methylbutyl acetate, and isoamyl butyrate are key flavor substances. Esters primarily originate from the interaction between alcohol and free fatty acids present in pork. Esters created from short-chain fatty acids and alcohols exhibit floral and fruity aromas (Feng et al., 2014; Zhang et al., 2022); given their relatively low olfactory threshold, they play a crucial role in the overall flavor. Typically, ethyl acetate is produced through the esterification reaction between acetic acid and ethanol. It often presents a pleasant floral, fruity, or wine aroma and can synergize with other esters to contribute to the fruity flavor of CPSM (Xu et al., 2022). Boil reheating significantly enhanced the flavor contribution of ethyl acetate-D and ethyl acetate-M ($P < 0.05$), whereas steam reheating significantly diminished their contribution. Microwave reheating also decreased the flavor contribution of these monomer substances. Previous studies have discovered, boiling can elevate the ester content in food, while microwaving can impede the production of esters (Sun et al., 2019; Tang et al., 2024). Interestingly, this study found that 2-Methylbutyl acetate and ethyl acetate-D, demonstrated an increased flavor contribution after microwave reheating ($P < 0.05$). This variance may be attributable to vitamin degradation, lipid oxidation, the interaction of lipid oxidation products with Maillard reaction products during various reheating methods, and thermal reactions instigated by microwave heating during the Maillard reaction.

Ketones typically originate from lipid oxidation or the Maillard reaction, and most exhibit a subtle meaty aroma. However, due to their high thresholds, they contribute minimally to the overall flavor of livestock and poultry foods (Qian et al., 2021). cyclohexanone-M and 2-octanone possess caramel and mushroom flavors respectively. Post microwave and boil reheating, the levels of cyclohexanone-M and 2-octanone increased significantly ($P < 0.05$), whereas steam reheating predominantly decreased their content ($P < 0.05$). As for cyclohexanone-D, feeble changes were observed between microwave reheating and steam reheating, although boil reheating also notably increased its content. Given that cyclohexanone is believed to be produced through lipid oxidation, this again suggests that water bath reheating accelerates the rate of lipid oxidation. Microwave reheating, due to its unique heating mechanism, exerts a lower impact on lipid oxidation, while steam reheating better preserves the original flavor level and reduces the lipid oxidation rate. Mesityl oxide, with its honey aroma, saw a reduction in content after reheating in all samples. Currently, there is no relevant literature to explain this phenomenon. It

Table 6
Volatile organic flavor compounds and ROAV values.

Classification	Chemical compounds	Flavor description[a]	Odor Threshold value(μg/kg) [b]	ROAV			
				CG	MR	BR	SR
Aldehydes	Benzaldehyde	Bitter almonds	350	0.44 ± 0.11^{a}	0.56 ± 0.05^{a}	0.70 ± 0.18^{a}	2.47 ± 3.70^{a}
	Heptaldehyde-D	Nutty	3	4.63 ± 0.55^{b}	4.10 ± 0.45^{b}	6.09 ± 0.23^{a}	4.88 ± 0.82^{b}
	Heptaldehyde-M	Nutty	3	23.90 ± 0.78^{a}	18.56 ± 0.29^{b}	26.20 ± 2.66^{a}	18.01 ± 0.74^{b}
	hexanal-M	Oily, grassy	4.5	64.17 ± 3.25^{b}	50.27 ± 1.23^{c}	81.25 ± 1.06^{a}	40.98 ± 1.35^{d}
	hexanal-D	Oily, grassy	4.5	83.38 ± 3.58^{b}	53.53 ± 2.21^{c}	101.74 ± 0.64^{a}	55.48 ± 1.92^{c}
	2-Methylbutanal-D	Nutty, cocoa	2	81.12 ± 1.07^{b}	70.81 ± 3.02^{c}	111.29 ± 1.36^{a}	54.56 ± 2.09^{d}
	2-Methylbutanal-M	Nutty, cocoa	2	16.58 ± 1.93^{b}	15.55 ± 0.26^{b}	23.87 ± 1.14^{a}	10.88 ± 0.28^{c}
	Butanal-D	Smoky, fish	9	1.76 ± 0.42^{a}	1.63 ± 0.10^{a}	1.55 ± 0.24^{ab}	1.03 ± 0.25^{b}
	Butanal-M	Smoky, fish	9	58.96 ± 2.58^{b}	56.67 ± 1.63^{b}	89.08 ± 0.26^{a}	44.68 ± 1.97^{c}
	1-Nonanal-M	Citrus, grease	1	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
Ketones	Cyclohexanone-M	Caramel	1.1	34.06 ± 0.93^{c}	46.37 ± 1.79^{b}	57.70 ± 2.01^{a}	24.92 ± 0.25^{d}
	2-Octanone	Mushroom	50.3	7.39 ± 0.74^{b}	7.53 ± 0.21^{b}	10.26 ± 0.18^{a}	5.89 ± 0.89^{c}
	3-Octanone	Fruit, flower	18	1.71 ± 0.44^{b}	2.27 ± 0.06^{a}	2.33 ± 0.13^{a}	1.27 ± 0.22^{b}
	2-Heptanone	Fruit, flower	140	0.25 ± 0.06^{b}	0.24 ± 0.01^{b}	0.36 ± 0.07^{a}	0.23 ± 0.04^{b}
	Mesityl oxide	Honey	17	7.85 ± 0.61^{a}	5.79 ± 0.15^{b}	5.53 ± 0.37^{b}	3.85 ± 0.71^{c}
	Cyclohexanone-D	Caramel	1.1	43.50 ± 0.35^{b}	46.82 ± 3.09^{b}	72.44 ± 0.99^{a}	43.83 ± 3.15^{b}
Esters	Isoamyl butyrate	Banana, pear	15	1.38 ± 0.20^{a}	1.24 ± 0.09^{a}	1.44 ± 0.13^{a}	1.18 ± 0.21^{a}
	amyl acetate	Fruity	43	0.89 ± 0.19^{a}	0.33 ± 0.02^{b}	0.36 ± 0.07^{b}	0.32 ± 0.06^{b}
	Butyl acetate	Fruity	66	0.19 ± 0.04^{ab}	0.18 ± 0.01^{ab}	0.23 ± 0.05^{a}	0.14 ± 0.03^{b}
	Ethyl acetate-D	Pineapple, fruity	5	17.96 ± 1.55^{b}	21.02 ± 1.41^{a}	22.16 ± 1.08^{a}	11.72 ± 0.24^{c}
	Ethyl acetate-M	Pineapple, fruity	5	31.90 ± 2.04^{b}	26.78 ± 1.65^{c}	38.21 ± 1.18^{a}	21.97 ± 4.10^{c}
	2-Methylbutyl acetate	Apples, bananas	1.57	3.66 ± 0.90^{ab}	4.35 ± 0.23^{a}	4.66 ± 0.28^{a}	2.65 ± 0.51^{b}
	Allyl isothiocyanate	Spicy, kohlrabi	3800	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.01 ± 0.00^{a}	0.00 ± 0.00^{a}
Alcohols	1-hexanol	Fruity, grass	250	0.13 ± 0.01^{a}	0.10 ± 0.01^{bc}	0.12 ± 0.03^{ab}	0.08 ± 0.02^{c}
	(E)-3-hexen-1-ol	Flower, grass	70	0.42 ± 0.11^{ab}	0.50 ± 0.05^{ab}	0.62 ± 0.16^{a}	0.33 ± 0.07^{b}
	1-Pentanol-M	Fruity, spice	4000	0.03 ± 0.01^{a}	0.03 ± 0.00^{a}	0.04 ± 0.01^{a}	0.03 ± 0.01^{a}
	1-Pentanol-D	Fruity, spice	4000	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
	2-methyl-1-butanol-M	Banana, wine	500	0.27 ± 0.06^{a}	0.25 ± 0.1^{b}	0.28 ± 0.16^{a}	0.19 ± 0.04^{c}
	2-methyl-1-butanol-D	Banana, wine	500	0.08 ± 0.02^{b}	0.08 ± 0.0^{b}	0.07 ± 0.02^{b}	1.27 ± 0.22^{a}
	1- butanol-M	Fruity	480	0.12 ± 0.03^{a}	0.10 ± 0.00^{a}	0.12 ± 0.02^{a}	0.08 ± 0.02^{a}
	1- butanol-D	Fruity	480	0.02 ± 0.00^{ab}	0.02 ± 0.00^{a}	0.02 ± 0.00^{ab}	0.01 ± 0.00^{b}
	1-Butanol	Fruity	500	0.11 ± 0.03^{a}	0.08 ± 0.00^{ab}	0.08 ± 0.02^{ab}	0.06 ± 0.02^{b}
	2-Methyl-1-propanol-M	Fruity, flower	33	1.42 ± 0.29^{ab}	1.43 ± 0.04^{ab}	1.59 ± 0.31^{a}	1.06 ± 0.19^{b}
	2-Methyl-1-propanol-D	Fruity, flower	33	5.24 ± 0.57^{b}	5.56 ± 0.11^{b}	7.22 ± 0.20^{a}	4.22 ± 0.76^{c}
	1-Propanol-M	Apple, cognac	240	0.53 ± 0.13^{a}	0.52 ± 0.04^{a}	0.65 ± 0.13^{a}	0.44 ± 0.07^{a}
	1-Propanol-D	Apple, cognac	240	0.62 ± 0.15^{a}	0.70 ± 0.01^{a}	0.58 ± 0.11^{a}	0.47 ± 0.10^{a}
	2-Methyl-1-propanol	Fruity, flower	33	0.12 ± 0.03^{a}	0.09 ± 0.00^{ab}	0.12 ± 0.02^{a}	0.07 ± 0.01^{b}
Acids	Acetic acid	Sourness	99	5.62 ± 0.15^{b}	5.77 ± 0.29^{b}	8.56 ± 0.12^{a}	4.01 ± 0.16^{c}
Alkene	β-Pinene	Rosin, resin	33	0.62 ± 0.14^{a}	0.40 ± 0.01^{b}	0.37 ± 0.07^{b}	0.27 ± 0.06^{b}
	α-Pinene	pine, tea, grass	18	1.11 ± 0.24^{ab}	1.34 ± 0.05^{a}	1.28 ± 0.31^{a}	0.80 ± 0.13^{b}

Note: a: Represents the flavor descriptions are obtained from the technology of food flavoring (Sun, 2017) and http://www.odour.org.uk
b: Represents the aroma threshold of flavor compounds is mainly derived from the technology of food flavoring (Sun, 2017).
±: Represents the standard deviation. n = 3.

is hypothesized that it may undergo an addition reaction with other compounds in the food or function as a precursor for aldehydes, participating in the synthesis reaction between compounds, thereby reducing its content (Tian et al., 2014). Acetic acid and 2-pinene, with their sour and grassy flavors respectively, are considered off-flavors in cooked meat products. Microwave reheating had no markable impact on acetic acid and 2-pinene; however, boil reheating significantly amplified the odor level of acetic acid ($P < 0.05$). Conversely, steam reheating crucially reduced the content of these two compounds, thereby decreasing the sourness in cooked meat.

3.5.2. Analysis of combined PLS—DA and OPLS-DA

Partial Least Squares Discriminant Analysis (PLS—DA) and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) are both supervising methods with robust multivariate discriminant analysis ability, which excels in discriminating complex variables. Fig. 6 The left half (a, b, c, d) which belongs to PLS—DA model and the right half part (e, f, g, h) belongs to OPLS-DA model.

Fig. 6(a, b) and Fig. 6(e, f) represent score plots and inner relation plots for the PLS—DA and OPLS-DA models, respectively, where the degree of flavor change was determined by judging the distance between samples, with greater distance representing more severe flavor change. These plots show the similar trend that MR is closest to the CG, SR is next closest, and BR results in the most severe change. The PLS—DA model shows scattered distances between the same samples, whereas the OPLS-DA model is more concentrated, which is due to the fact that it can more accurately screen out the discrepant variables by discarding the variables that are not related to Y. Fig. 6(c, g) indicates the variable importance in projection (VIP) values screened by the OPLS-DA and PLS—DA models, with VIP values>1 representing a significant contribution to distinguishing differences. Supplementary Table 4 shows the volatiles screened by the two models with VIP >1. Nine recurring compounds were jointly identified by comparison with the ROAV values, which were amyl acetate, mesityl oxide, heptaldehyde-D, hexanal-D, butanal-D, 3-octanone, ethyl acetate, heptaldehyde-M, and cyclohexanone-D. Most of these compounds are produced after lipid oxidation, suggesting that the reason for the large variation in flavor after reheating is due to the WOF flavor caused by lipid oxidation. Subsequent work could be directed at controlling the growth of such substances during reheating, thereby possibly reducing the flavor loss caused by the reheating process. Fig. 6(d, h) shows the validation plot of the model after 200 permutations. The intersection of the regression line Q^2 with the Y-axis is below zero, indicating that there is no overfitting and the model are robust.

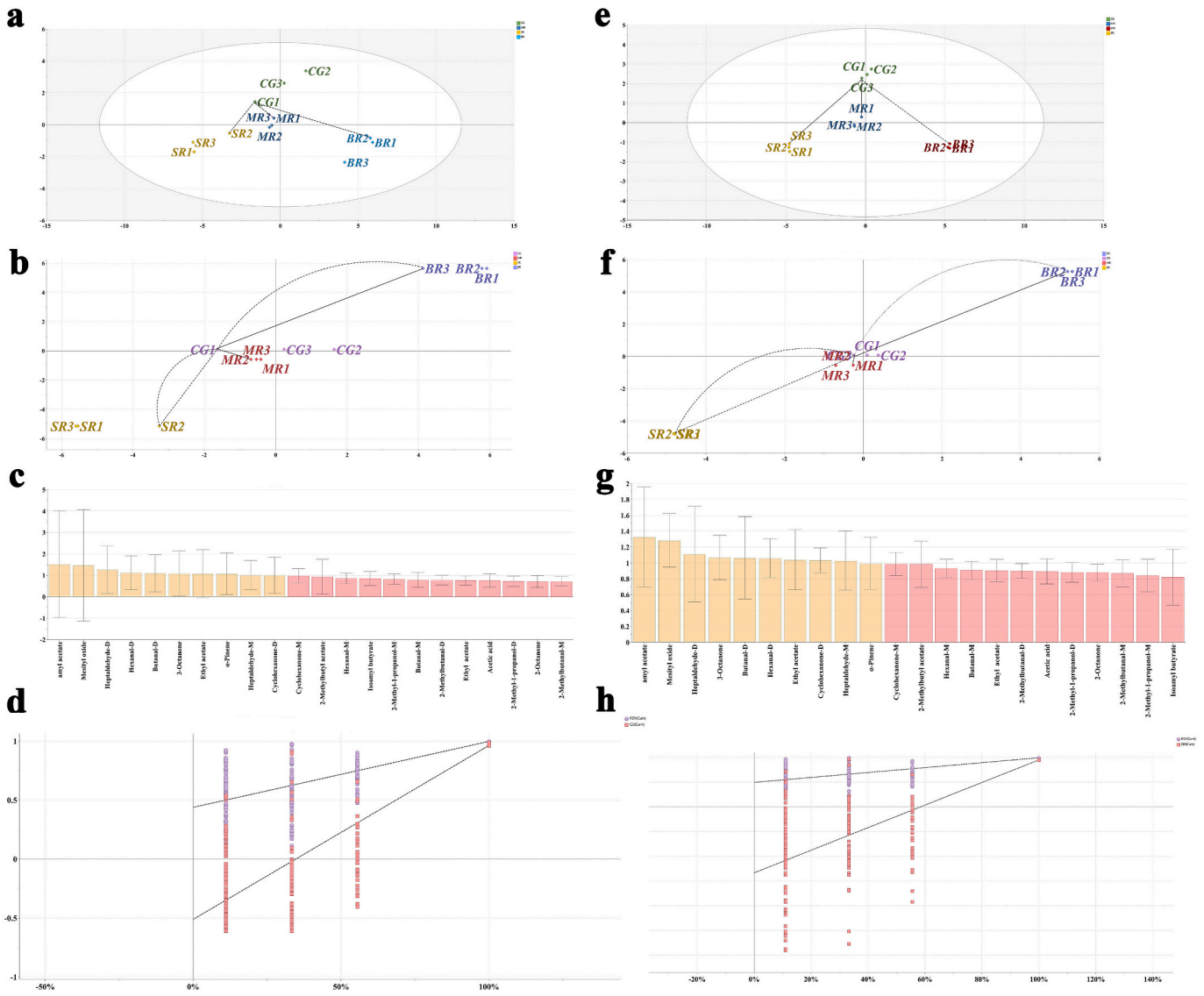


Fig. 6. OPLS-DA model of volatile compounds from different reheating methods.
Note: (a) Scores plot by PLS—DA, $R^2X = 0.979$, $R^2Y = 0.987$, $Q^2 = 0.929$; (b) Inner relation plot by PLS-DA; (c) VIP scores. Yellow corresponds to compounds with VIP > 1, and pink represents compounds with VIP < 1. (d) cross-validation plot for the PLS—DA model with 200 calculations in a permutation test: $R^2 = (0.0, 0.438)$, $Q^2 = (0.0, -0.512)$.
(e) Scores plot by OPLS-DA, $R^2X = 0.981$, $R^2Y = 0.985$, $Q^2 = 0.906$; (f) inner relation plot by OPLS-DA; (g) VIP scores, yellow corresponds to compounds with VIP > 1, and pink represents compounds with VIP < 1. (h) cross-validation plot for the OPLS-DA model with 200 calculations in a permutation test: $R^2 = (0.0, 0.492)$, $Q^2 = (0.0, -0.134)$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.5.3. *Analysis of correlations between HS-GC-IMS and TAAs*

Fig. 7 presents a correlation test between volatiles and free amino acids. To further explore the correlation analysis between the *E*-nose, E-tongue, and sensory scores, the correlation between these three factors was examined using the Pearson test and the Mantel test. The Mantel test is a statistical method used to test the correlation between two or more matrices and can be used to analyze the correlation between different variables (Legendre & Fortin, 2010). Lines of different colors and widths represent statistical significance and the *r*-statistic of the Mantel test, respectively. Red lines indicate highly significant correlations ($P \leq 0.001$), yellow lines mean very significant correlations ($P \leq 0.01$), and green lines represent significant correlations ($P \leq 0.05$). Pearson correlation analysis revealed that Asp was significantly positively correlated with almost all the key volatiles, whereas Thr, Orn, Leu, Tyr, and Pro were negatively correlated with some of the products of lipid oxidation, such as cyclohexanone-M, butanal-M, 2-methylbutanal-D, 2-methylbutanal-M, heptaldehyde-M, hexanal-M, and hexanal-D. Mantel's test showed that sensory scores were significantly and positively correlated with Asn (Umami), Glu (Umami), and Orn (Umami) ($P \leq 0.05$, Mantel's $r \geq 0.3$), as well as significant associations with heptaldehyde-D, hexanal-D, and butanal-M ($P \leq 0.05$, Mantel's $r \geq 0.4$). The electronic tongue had a correlation with Asn (Umami) and Arg (Sweet) ($P \leq 0.05$, Mantel's $r \geq 0.3$), while the electronic nose had a highly significant association with mesityl oxide ($P \leq 0.001$, Mantel's $r = 0.849$) and a significant relationship with amyl acetate ($P = 0.03$, Mantel's $r = 0.68$).

The flavor compounds screened by the Mantel test, including amyl acetate, mesityl oxide, heptaldehyde-D, and hexanal-D, all had VIP values with ROAV >1, indicating that these four flavor compounds made excellent contributions to the flavor differentiation ability after reheating. This also suggests that OPLS-DA, PLS—DA, and ROAV were all effective in identifying the key compounds contributing to the flavor differentiation after reheating.

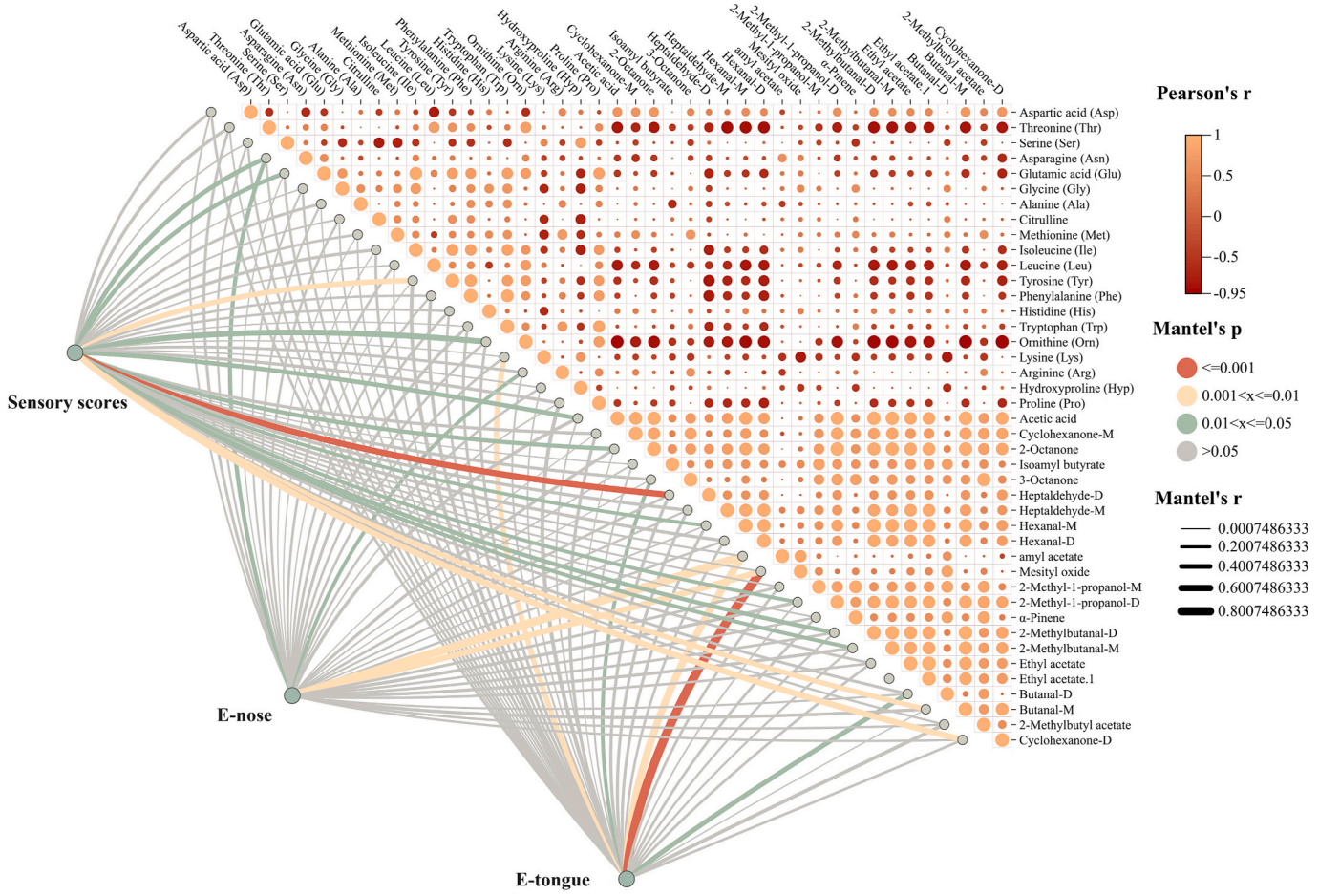


Fig. 7. Analysis of the correlation between amino acids and volatile organic compounds.

4. Conclusion

This study investigated the effects of different reheating methods (microwave, steam, and water bath) on the taste, flavor, and other physicochemical parameters of CPSM. Sensory scores were used to evaluate the outcomes, with microwave reheating yielding the highest scores and being closest to the original flavor, followed by steam reheating, and boil method yielding the lowest scores. A total of 48 flavor compounds were detected by HS-GC-IMS, which included 15 alcohols, 11 aldehydes, 9 ketones, 7 esters, 3 olefins, 1 acid, and 2 others. ROAV and multivariate statistical analysis revealed that nine key compounds, including amyl acetate, mesityl oxide, and various forms of heptaldehyde, hexanal, butanal, and cyclohexanone, were responsible for the flavor changes after reheating. Furthermore, Mantel and Pearson tests showed a close relationship between sensory scores and the presence of fresh amino acids, and a negative correlation with the presence of aldehydes. In conclusion, all reheating methods altered the odor and flavor profile of CPSM, with BR causing the most damage to the original flavor due to the intensification of lipid oxidation. On the other hand, MR was more conducive to preserving the original flavor of CPSM, closely followed by SR. This article establishes a theoretical foundation for the identification of characteristic flavor substances in Ceramic-Pot Sealed Meat and provided basic knowledge for the regulation and control of the flavor quality of Ceramic-Pot Sealed Meat product. Future research could focus on further improving the flavor recoverability of reheated CPSM by exploring the addition of specific spices or natural antioxidants to counteract the effects of lipid oxidation.

Ethical statement

The sensory analysis in this study complied with sensory ethical standards. Additionally, all sensory evaluators were professionally trained and their rights and privacy were adequately respected and protected. The purpose, process, risks and benefits of the study were adequately explained to all participants and their informed consent was obtained. Participation was voluntary and participants could withdraw from the study at any time.

CRediT authorship contribution statement

Chunyuan Ping: Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Yuanqi Liu:** Methodology, Data curation. **Jicai Bi:** Validation, Supervision. **Xuemei Cai:** Supervision, Software. **Xiang Li:** Validation. **Mingfeng Qiao:** Supervision, Methodology, Funding acquisition.

Declaration of competing interest

Authors declare that they have no conflicts of interest.

Data availability

The data that support the findings of this study are available on request from the corresponding author.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101640.

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