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Characterization and comparison of flavor compounds in stewed pork with different processing methods

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ABSTRACT

The objective of this study was to investigate the volatile and non-volatile compounds of stewed pork with different processing methods (TS: traditional stewing, TSE: traditional stewing with enzymatic degradation, TSEM: traditional stewing with enzymatic degradation and Maillard reaction, HS: high-temperature stewing, HSE: high-temperature stewing with enzymatic degradation, HSEM: high-temperature stewing with enzymatic degradation and Maillard reaction). The odor compounds results showed that HS, HSE and HSEM had higher types and contents of volatile compounds than TS, TSE and TSEM, especially HSEM. All stewed pork from traditional and high-temperature stewing methods were classified into two groups using an electronic nose due to different flavor characteristics. Non-volatile compounds results displayed the contents of total amino acids in HS, HSE and HSEM were higher significantly ($P < 0.05$) than those of TS, TSE and TSEM, of which the contents of Asp and Glu related to umami taste were the most in HS and HSEM. In contrast, there were the lower contents of 5′-nucleotides and fatty acids in HS, HSE and HSEM. These findings indicated that the high-temperature stewed pork method could be used as an effective method to improve the flavor of pork, among which HSEM processing method was particularly remarkable.

1. Introduction

The stewed pork is a traditional sauce pickled product in China and processed by boiling hind leg meat with various condiments and spices for a long time. The stewed pork product is popular with consumers owing to the unique aroma and taste profile. Flavor is the most important factor for sensory attributes with regard to eating quality of stewed meat products (Qi, Liu, Zhou, & Xu, 2017) and closely related to volatile compounds and non-volatile compounds (Dashdorj, Amna, & Hwang, 2015; Kosowska, Majcher, & Fortuna, 2017). The volatile compounds including aldehydes, alcohols, ketones, hydrocarbons, esters, ethers, furans, N- and S- containing compounds were generated from many chemical reactions (e. g. lipid oxidation, Maillard reaction and lipid-Maillard interactions) (Aaslyng & Meinert, 2017; Han, Zhang, Fauconnier, & Mi, 2019). The precursors of non-volatile compounds were mainly reducing sugar, free amino acids (FAAs), nucleotides and fatty acids (FAs) (Maughan & Martini, 2012). As reported by Li et al. (2016), the flavor compounds were continuously volatilized and outflowed during the processing of stewed meat to reduce the flavor quality of meat products. Although our research team had proposed the quantitative stewing method to keep its characteristic aroma, in order to obtain the more satisfactory flavor, it is also necessary to find a better way to improve special flavor.

To the best of our knowledge, lots of flavor precursors formed by enzymatic degradation were involved in Maillard reaction. Several studies have reported that the meat flavor was prepared by using Flavourzyme™, Trypsase and Protamex™ to hydrolyze chicken and beef bones, thereby improving the aroma of the meat (Dong et al., 2014; Xu, You, Song, Gong, & Pan, 2018). The Maillard reaction typically occurs between amino acids and reducing sugars, and eventually results in a large number of volatile compounds responsible for the special aroma in meat products (Jayasena, Ahn, Nam, & Jo, 2013). The D-xylose, L-cysteine and thiamin are important precursors to generate meat-flavored sulfur-containing odorants (Aaslyng & Meinert, 2017) in

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the Maillard reaction system. The research has showed that several main flavor compounds were identified in the model systems of D-xylose, L-cysteine and thiamin. It has been reported that 3-mercapto-2-pentanone (MP), 2-methyl-3-furanthiol (MFT), 4,5-dihydro-2-methyl-3-furanthiol and 4-methyl-5-thiazoleethanol have been identified in these model reaction, which presented intense roasted, coffee-like and meat-like notes (Hofmann & Schieberle, 1995). These results indicated that the enzymatic hydrolysis and Maillard model reaction were usually used to generate the dominated aroma compounds. Therefore, this is a feasible method to enhance the flavor of stewed pork by enzymatic hydrolysis and Maillard model reaction.

The aim of this study was to analyze the differences of volatile compounds and non-volatile compounds (FAAs, nucleotides, FAs) in the pork samples from traditional and high-temperature stewing combined with enzymatic degradation and Maillard reaction. Additionally, it could be expected to provide an effective method to improve the aroma of stewed meat products.

2. Materials and methods

2.1. Materials and chemicals

The hind leg muscle of pigs was obtained from Chuying Agro-Pastoral Group Co. Ltd. (Zhengzhou, Henan Province, China) and stored at −20 °C until use. Pigs belonged to Duroc × (Landrace × Yorkshire) pig breed (DLY, aged 5–6 months and with body weights of 90–95 kg). All pigs were fed under the same rearing conditions and slaughtered following routine abattoir procedures. The sodium chloride and mixed spices were obtained from the local market (Beijing, China). Flavourzyme ™ (25000 U/g) was purchased from Novozymes Co. Ltd (Beijing, China). The standard of free amino acids, 5′-nucleotide and 2-methyl-3-heptanone were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA). D-xylose, L-cysteine and thiamine were analytical grade bought from Sinopharm Chemical Reagent Beijing Co. Ltd (Beijing, China).

2.2. Stewed pork with different processing methods

2.2.1. Traditional stewing (TS) with enzymatic degradation (TSE) or Maillard reaction (TSEM)

The frozen pork was thawed overnight at 4 °C and the skin, visible fat and connective tissues were removed. About 1 kg of pork was cut into 15 pieces (5.0 cm × 4.0 cm × 3.0 cm) with the weight of 60 g and were stewed at 100 °C by adding 2 L deionized water, 30 g sodium chloride and 12 g mixed spices (1.2 g/kg *Cinnamomum cassia* Presl., 0.3 g/kg *Syringa oblata* Lindl., 1.0 g/kg *Myristica fragrans* Houtt., 0.5 g/kg *Glycyrrhiza uralensis* Fisch., 1.0 g/kg *lllicium verum* Hook.f., 0.3 g/kg *Cinnamomum wilsonii* Gamble., 0.3 g/kg *Elettaria cardamomum* (L.) Maton, 1.0 g/kg *Foeniculum vulgare* Mill., 0.3 g/kg *Amomum kravanh* Pierre ex Gagnep., 1.2 g/kg *Citrus reticulata Blanco*, 0.8 g/kg *Alpinia officinarum Hance*, 0.5 g/kg *Trifolium repens* L., 0.6 g/kg *Piper longum* Linn, 0.3 g/kg *Crataegus pinnatifida* Bunge, 0.5 g/kg *Zanthoxylum bungeanum* Maxim., 0.5 g/kg *Keampfera galangal* L., 0.5 g/kg *Amomum tsaoko* Crevost et Lemarie and 1.2 g/kg *Angelica sinensis*). Subsequently, the mixed liquid was obtained by filtering the spices. The flavourzyme was added to mixed liquid at a ratio of 0.075‰ (w/w), then 10% flavourzyme mixture (w/w) was injected into the pork, and finally stirred evenly in the tumbling machine at 23 ± 2 °C for 60 min. The tumbled pork was stewed for 45 min at 98 ± 2 °C in the brine and soaked for 60 min. This brine of TSEM contained sodium chloride (30 g/kg pork), mixed spices (12 g/kg pork), 5‰ D-xylose (w/w, based on pork weight), 1‰ L-cysteine (w/w, based on pork weight) and 3‰ thiamine (w/w, based on pork weight). The brine of TSE included sodium chloride (30 g/kg pork) and mixed spices (12 g/kg pork). The pork processing technology was consistent with TSEM. Compared with TSEM, 10% of deionized water (w/w) was injected into the pork and the brine of TS only contained sodium chloride (30 g/kg pork) and mixed spices (12 g/kg pork). The other processes are the same. The processing flow chart of traditional stewed pork is shown in Figs. S1a–b.

2.2.2. High-temperature stewing (HS) with enzymatic degradation (HSE) or Maillard reaction (HSEM)

The frozen pork was thawed overnight at 4 °C and then cut into small pieces (5.0 cm × 4.0 cm × 3.0 cm). The pork was tumbled at room temperature for 60 min after injected with 10% of the brine (w/w). The brine of HSEM contained sodium chloride (60 g/kg pork), mixed spices (12 g/kg pork), 0.15‰ flavourzyme (w/w, based on pork weight), 5‰ D-xylose (w/w, based on pork weight), 1‰ L-cysteine (w/w, based on pork weight) and 3‰ thiamine (w/w, based on pork weight). The roasting process was 30 min at 90 °C to dry the surface to avoid water exudation from internal tissue. The steaming process was 5 min at 120 °C. Finally, the stewed pork was roasted at 90 °C for 25 min to dry the surface moisture. The brine of HSE was contained sodium chloride (60 g/kg pork), mixed spices (12 g/kg pork) and 0.15‰ flavourzyme (w/w, based on pork weight). The other operations were the same as HSEM. Compared to HSEM, the brine of HS only contained sodium chloride (60 g/kg pork) and mixed spices (12 g/kg pork), and other processes are the same. The processing flow chart of high-temperature stewed pork is shown in Fig. S1c.

2.3. Volatile compounds of different stewed pork

2.3.1. Volatile compounds analysis by gas chromatography-mass spectrometry/olfactometry (GC-MS/O)

The solid-phase micro-extraction (SPME) methods were used to extract the volatile compounds from the stewed pork samples. 5.0 g of the sample was placed into a 40 mL headspace via, and 1 μL of 2-methyl-3-heptanone solution with a concentration of 0.816 μg/μL was added as an internal standard. This via was equilibrated in a thermostatic water bath at 60 °C for 20 min. A 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (Supelco, Inc., Bellefonte, PA, USA) was inserted and the headspace absorption was performed for 40 min. Upon completion, the fiber was inserted into the injection port (250 °C) of the GC instrument to desorb the analyses for 5 min. All samples were extracted in triplicate.

The volatile compounds of stewed pork were analyzed and identified by a GC-MS instrument (7890 A-7000 B, Agilent Technologise, Inc., Santa Clara, CA, USA) equipped with an olfactory detection port (Sniffer 9000; Brechbuhler, Schlieren, Switzerland). Capillary column DB-wax (30 m × 0.32 mm i.d., 0.25 μm film thickness; J & W Scientific, Inc., Folsom, CA, USA) was used with helium (purity of ≥99.999%) as the carrier gas at 1.2 mL/min flow rate. The front inlet temperature was 250 °C with a solvent delay of 4 min. The heating program was as follows: the initial temperature was 40 °C for 3 min, ramped to 200 °C at a rate of 5 °C/min, then ramped to 240 °C at a rate of 10 °C/min with a 3 min final hold. The infector mode was splitless. The transfer line temperature and ion source temperature were kept a 240 °C and 230 °C. Electron-impact mass spectra were generated at 70 eV, with *m/z* scan range from 50 to 400 amu. A sniffing port (Sniffer 9000) coupled to a GC-MS instrument was used for odor-active compound characterization. The effluent from the capillary column was split 1:1 (*v/v*) between the mass spectrometry detector and the olfactory detector port. A panel that contains eight trained staff was utilized for the sniffing test on GC-O.

Volatile compounds were identified based on a comparison of GC retention indices (RI) with authentic compounds, mass spectra (comparison with MS spectra database of NIST 2.0 mass spectra libraries installed in the GC-MS equipment), and odor descriptions in the literature and online databases (http://www.favornet.org; http://www.odour.org.uk). Quantitative data of the identified compounds were obtained by the calibration curves of the GC-peak area and the amount ratios for the target analyte relative to 2-methyl-3-heptanone according to the method of Han, Zhang, et al. (2019).

2.3.2. Electronic nose (E-nose) analysis

E-nose analysis of volatile compounds was carried out according to Yang, Yu, Pei, Mariga, and Hu (2016). The odor profile of stewed pork was analyzed by a portable E-nose (PEN 3, Win Muster Airsense Analytics, Inc., Schwerin, Germany), which included ten types of metal oxide semiconductors for specific recognition of different types of volatile compounds. The performance description and sensitivity of all sensors were present in Table S1. 1.0 g of stewed pork sample was into 10 mL airtight vials and sealed for testing. A filtered and dried air flow (99%, 300 mL/min) was used as a carrier gas for E-nose detection. The measurement time was 60 s, and the standby time was 180 s. The E-nose analysis of each sample was repeated three times.

2.4. Taste compounds of different stewed pork

2.4.1. Determination of free amino acids (FAAs)

FAAs were extracted in accordance with the method of our previous study (Li et al., 2016). Briefly, 5.0 g of stewed pork sample was dissolved in 20 mL of ultra-pure water and homogenized at 0 °C for 1 min in an Ultra Turrax T10 (IKA, Königswinter Germany). Subsequently, 20 mL of 5% cold trichloroacetic acid was added to the homogenate, and the mixture was stored at 4 °C for 12 h. The supernatant was filtered through a 0.45-μm membrane prior to further analysis. Derivation of amino acids was carried out following the procedure described in the "AccQ-Tag" kit. Firstly, 20 μL of the extract or amino acid standards were transferred to a 1.5 mL amber glass vial with a Teflon-lined septum and mixed with 60 μL of AccQ.Fluor borate buffer. Then 20 μL of reconstituted AccQ.Fluor reagent was added and the mixtures were heated at 55 °C for 10 min. Quantification of FAAs was performed using high-performance liquid chromatography (HPLC) (Agilent 1200 series, Agilent Technologies, Palo Alto, CA). The separation of analytes was achieved a Waters AccQ. Tag amino acid analysis column (3.9 mm × 150 mm, 4 μm. Waters, Milford, MA, USA) at 37 °C. The flow rate of the mobile phase was set at 1 mL/min and the UV detection wavelength was 248 nm. Solvent A consisted of AccQ.Tag Eluent A (100 mL AccQ.Tag A concentrate + 1 L ultra-pure water). Solvent B and C were acetonitrile and ultra-pure water respectively. Gradient conditions were shown in Table S2. All experiments were performed in triplicate.

2.4.2. Determination of 5′-nucleotide analysis

5′-Nucleotides were extracted and analyzed according to the method of Hou, Liu, Xu, Zhou, & Li. (2018) with some modifications. 5.0 g of stewed pork sample was mixed 20 mL of 5% cold perchloric acid for 1 min and homogenized using an Ultra Turrax T10 (IKA, Königswinter Germany). The mixture was centrifuged at 3000×*g* for 10 min, then the supernatant was filtered and adjusted to pH 6.5 by adding 1 M NaOH. All samples and eluents were filtered through a 0.45 μm filtration membrane before analysis. The filtrate (10 μL) was injected into the Agilent 1200 HPLC fitted with the Intersil ODS-3 column (250 mm × 4.6 mm; Waters) and UV detector (260 nm). The column temperature was set at 30 °C. Methanol (Eluent A) and 0.05% of phosphoric acid (Eluent B) were used as mobile phases at a flow rate of 1.0 mL/min. Gradient elution program was conducted as follows: 5% eluent A for 10 min, linear change to 15% eluent A for 5 min, then to 70% eluent A for 6 min, and finally to 5% eluent A for 4 min. The identification and quantification of nucleotides were assessed by comparison with the retention times and peak areas of nucleotide standards. All samples had three replicates for the same conditions.

2.4.3. Calculation of equivalent umami concentration (EUC)

The EUC is defined as the concentration of the monosodium glutamate (MSG, mg/100 g) equivalent to the umami intensity given by a mixture of MSG and 5′-nucleotides and is calculated following equation (Sun et al., 2014):

$$EUC = \sum a_i b_i + 1218 \left(\sum a_i b_i\right)\left(\sum a_j b_j\right)$$

The unit of EUC of the mixture is g MSG/100 g, a_i is the concentration (g/100 g) of each umami amino acid (Asp or Glu), b_i is the relative umami concentration (RUC) for each umami amino acid to MSG (Glu, 1; Asp, 0.077), a_j is the concentration (g/100 g) of each umami 5′-nucleotide (5′-IMP, 5′-GMP or 5′-AMP), b_j is the RUC for each umami 5′-nucleotide to 5′-IMP (5′-IMP, 1; 5′-GMP, 2.3; 5′-AMP, 0.18) and 1218 is a synergistic constant based on the concentration used.

2.4.4. Determination of fatty acids (FAs)

FAs were extracted from the freeze-dried pork samples with chloroform-methanol according to the method reported by Folch, Lees, and Stanley (1957). Fatty acid methyl esters (FAME) were analyzed using a GC-7890 Agilent gas chromatography and a capillary column DB-5 MS (30 m × 0.25 mm × 0.25 μm, Agilent Technologies, Santa Clara, USA). Individual fatty acid was identified by the comparison of retention time of the FAME mixture (Sigma-Aldrich, Germany) and quantitated using the external standard method. Each sample was analyzed in triplicate.

2.4.5. Electronic tongue (E-tongue) analysis

The taste composition of stewed pork was detected according to the method of Liu et al. (2017). An E-tongue system (ASTREE, Alpha MOS, France) consisting of 7 chemical sensors (AHS, SCS, ANS, CPS, NMS, CTS, PKS) with cross-selectivity was applied for taste measurements of stewed pork samples. To ensure the reliability and stability of the test data, the E-tongue was first self-tested, and then the sensors were activated, calibrated and diagnosed. The sample solution was measured for 120 s, and the measurement data was recorded every 1 s. The distilled water was used to clean the sensors for 30 s to ensure the stable potentials. The experiment was implemented at room temperature and each sample was analyzed in triplicate.

2.5. Statistical analysis

Statistical analysis was performed by SPSS 19.0 (IBM, Armonk, NY, USA), and one-way analysis of variance was used for the significant difference test ($P < 0.05$). The data were presented as the mean ± standard deviation. Principal component analysis (PCA) of E-nose and E-tongue was conducted using the software XLSTAT (2016) from Addinsoft (Barcelona, Spain).

3. Results and discussion

3.1. Volatile compounds profiling of stewed pork with different processing methods

3.1.1. Volatile components analysis of stewed pork by GC-MS/O

The types and concentrations of volatile components in stewed pork with different processing techniques were detected by GC-MS/O. As shown in Table 1, there were about 60 volatile compounds were identified in different stewed pork samples, including aldehydes, alcohols, ketones, esters, hydrocarbons, ethers, phenols and heterocyclic compounds. These classes of compounds agreed with the previous studies of pork flavor (Han, Zhang, et al., 2019; Zhao et al., 2017). Compared with the concentrations of aldehydes in TS, TSE and TSEM samples, these compounds in HS, HSE and HSEM samples increased significantly ($P < 0.05$) indicating that the high-temperature treatment of stewed pork may contribute to the production of more aldehydes induced by lipid oxidation (Yang, Sun, Pan, Wang, & Cao, 2018). On the contrary, TS, TSE and TSEM samples presented the higher concentration of ketones, ester and phenols than that observed in HS, HSE and HSEM samples. The reason might be that the high-temperature treatment could covert ketones and phenols into intermediates for heterocyclic compounds or

Table 1
The concentrations and types of volatile components in stewed pork with different processing methods.

Classes of components	Concentrations (μg/kg) (quantities)					
	Traditional stewed pork			High-temperature stewed pork		
	TS	TSE	TSEM	HS	HSE	HSEM
Aldehydes	4234.6^{c} (9)	2033.2^{d} (5)	4335.2^{c} (12)	5601.5^{b} (9)	4333.3^{c} (8)	8438.6^{a} (16)
Alcohols	1147.2^{c} (8)	884.6^{e} (7)	1196.3^{c} (10)	1055.0^{d} (6)	1411.0^{b} (8)	2213.0^{a} (9)
Ketones	101.4^{c} (1)	161.3^{a} (2)	147.1^{b} (3)	N.D.	81.6^{d} (2)	62.9^{e} (1)
Esters	32.5^{d} (1)	73.9^{a} (1)	39.9^{c} (1)	N.D.	N.D.	46.4^{b} (1)
Hydrocarbons	1008.9^{d} (10)	3134.7^{b} (17)	1602.1^{c} (17)	1585.7^{c} (10)	3556.1^{a} (17)	2900.2^{b} (18)
Ethers	1250.7^{c} (4)	2269.9^{a} (4)	1958.1^{b} (4)	999.7^{d} (4)	1967.6^{b} (4)	1256.8^{c} (3)
Phenols	474.1^{c} (2)	1298.0^{a} (2)	857.9^{b} (2)	154.1^{e} (1)	249.2^{d} (2)	260.0^{d} (2)
Heterocyclic compounds	262.6^{e} (2)	700.8^{b} (3)	334.0^{d} (3)	393.3^{c} (3)	419.1^{c} (3)	872.6^{a} (3)
Total	8512.0^{e} (37)	10556.4^{c} (41)	10470.6^{c} (52)	9789.3^{d} (33)	12017.9^{b} (44)	16050.5^{a} (53)

Note: Each value is expressed as mean ± SD; N.D. = not detected. $^{a–e}$ Different letters in the same row indicate that there is significant difference ($P < 0.05$, along the lines). TS, traditional stewing; TSE, traditional stewing with enzymatic degradation; TSEM, traditional stewing with enzymatic degradation and Maillard reaction; HS, high-temperature stewing; HSE, high-temperature stewing with enzymatic degradation; HSEM, high-temperature stewing with enzymatic degradation and Maillard reaction.

volatilize, and also hydrolyze esters into acids and alcohols (Zou et al., 2018). Furthermore, in term of stewed pork with traditional processing methods (TS, TSE and TSEM), the total concentration of volatiles in TS samples was significantly lower ($P < 0.05$) than that in TSE and TSEM samples. Among the stewed pork with the high-temperature processing methods (HS, HSE and HSEM), it was displayed that the lowest concentration of volatile compounds in HS samples. This may be that the hydrolysis of protein could produce more amino acids, peptides and small molecule compounds to promote Maillard reaction process (Kosowska et al., 2017). For the stewing pork with enzymatic hydrolysis or Maillard reaction, although there was no significant difference ($P > 0.05$) in the total concentration of volatile compounds in TSE and TSEM samples, the total concentration of volatiles in HSEM samples were significantly higher ($P < 0.05$) than that in HSE samples. This result indicated that more complex compounds were produced by various chemical reactions, especially Maillard reaction, whereas these chemical reactions may be weakened at a lower temperature (Aaslyng & Meinert, 2017).

3.1.2. Odor-active compounds of stewed pork

To estimate the contribution of the individual compound to the overall aroma profile of stewed pork with different processing methods, their odor activity values (OAV_S) were calculated. The odor-active compounds ($OAV_S \geq 1$) of stewed pork were shown in Table 2. A total of 26 compounds were selected as odor-active compounds in different stewed pork. These volatile compounds of stewed pork belonged to 6 chemical classes: 13 aldehydes, 5 alcohols, 4 hydrocarbons, 2 ethers, 1 phenol and 1 furan. The hexanal (OAV at 139.6–491.8), heptanal (OAV at 28.8–99.4), octanal (OAV at 380.8–1166.4), nonanal (OAV at 781.8–2628.6), 1,8-cineole (OAV at 135.2–178.6), 1-octen-3-ol (OAV at 167.1–531.8), D-limonene (12.0–78.9), β-phellandrene (OAV at 10.2–32.0), estragole (OAV at 22.0–35.4), anethole (OAV at 55.9–125.0), eugenol (OAV at 21.7–171.6) and 2-pentylfuran (OAV at 19.9–96.4) with a higher level of OAVs had played a role in stewed pork, which had a great contribution to the whole flavor in all pork samples. In contrast, the 1-octanol (OAV at 0.6–2.0), styrene (OAV at 0.0–1.1) and naphthalene (OAV at 0.6–1.8) showed the lower OAVs, indicating that they might have less contribution to pork flavor characteristic.

Additionally, some aldehydes, such as 2-methylbutanal (nutty odor), 3-methylbutanal (almond and nutty odor), (*E*)-2-octenal (green, nut and fatty odor), decanal (soap and orange peel odor), dodecanal (herbaceous and fatty odor) and (*E,E*)-2,4-decadienal (fried, wax and fatty odor) were only detected in TSEM and HSEM samples. This might be the main reason that stewed pork with the above methods promoted degradation reaction of amino acid, oxidation and degradation of lipid (Yang et al., 2018; Zou et al., 2018) to produce more important pork flavor. The OAVs of pentanal, hexanal, heptanal, octanal, nonanal, benzaldehyde and (*E*)-2-nonenal in HSEM samples were significantly higher ($P < 0.01$) than those of TS, TSE, TSEM, HS and HSE samples. This showed that HSEM samples had an advantage in contributing more pleasant fatty and grass aroma (Li et al., 2016) to the overall flavor.

For the identified alcohols, one linear alcohol (1-octanol) and three branched alcohols (1,8-cineole, 1-octen-3-ol and linalool) were mainly derived from the degradation of lipid (Pham et al., 2008). The lower odor thresholds of alcohols (except for 1-octanol) were observed, so they contribute significantly to mushroom, flower and mint note (Lorenzo, Carballo, & Franco, 2013) to the stewed meat products. The OAVs of alcohols in HSEM samples were the highest, indicating that the extent of lipid oxidation was greatest. In term of the dominated hydrocarbons, ethers and phenols, the D-limonene, β-phellandrene, estragole, anethole and eugenol could be mainly formed from a small amount of lipid oxidation and various spices, such as anise, cardamun and other spiceries (Liu, Xu, & Zhou, 2007). The OAVs of these compounds with herbaceous and clove odor in TSE and HSE samples were significantly higher than those in TS, TSEM, HS and HSEM samples. The 2-pentrylfuran, with the buttery odor, was usually an important volatile compound in cooked meat products (Benet et al., 2015) and had the highest OAVs in HSEM samples, which could be due to linoleic acid oxidization (Aparicio, Morales, & Alonso, 1996). These findings showed that high-temperature stewed pork with enzymatic hydrolysis and Maillard reaction presented more aroma components.

3.1.3. Volatile composition analysis of stewed pork using E-nose

The E-nose is very sensitive to the odor information of samples, minor changes in volatile compounds may cause differences of sensor responses (Yang et al., 2016), and it had been also widely used in meat as an important method (Tian, Wang, & Cui, 2013). According to the response values of all sensors (Fig. 1a), sensor W1W was the most sensitive to the volatile compounds from the stewed pork, followed by sensor W1C, W5S, W3C, W6S, W5C, W2W and W3S, and finally sensor W2S and W1S. This result indicated that pork samples had large numbers of terpenes and sulfur-containing organic compounds. The response values of W5S, W6S, W1S, W1W, W2S, W2W and W3S for HS, HSE and HSEM samples were significantly higher ($P < 0.05$) than those for TS, TSE and TSEM samples. However, the response values of W1C and W3C for TS, TSE and TSEM samples were significantly lower ($P < 0.05$) than those for HS, HSE and HSEM samples. The result demonstrated that the processing technology of high-temperature stewed pork had the significant effect on the formation of furans, N- and S-containing compounds, the processing technology of traditional stewed pork was more sensitive to aromatic compounds.

To evaluate the overall flavour characteristic of stewed pork samples, PCA was applied to analyze the E-noes data. As shown in Fig. 1b, it showed a good discrimination from the different stewed pork samples,

Table 2
Odor-active compounds (OAVs ≥ 1) in stewed pork with different processing methods.

Compounds	[a]DB-Wax	[b]Identification	[c]Odor description; [d]odor threshold ($\mu g \cdot kg^{-1}$)	Traditional stewed pork			High-temperature stewed pork			*p* value
				TS	TSE	TSEM	HS	HSE	HSEM	
2-Methylbutanal	906	MS, RI, O	Nutty; 1	N.D.	N.D.	11.7 ± 1.4	N.D.	N.D.	N.D.	N.D.
3-Methylbutanal	910	MS, RI, O	Almond, nutty; 4	N.D.	N.D.	12.8 ± 2.1	N.D.	N.D.	N.D.	N.D.
Pentanal	969	MS, RI, O	Almond, pungent; 9	13.5 ± 1.2^{b}	6.1 ± 0.3^{de}	5.2 ± 0.2^{e}	11.1 ± 0.7^{c}	7.0 ± 0.4^{d}	17.0 ± 0.5^{a}	0.000
Hexanal	1076	MS, RI, O	Grass, fat; 4	426.7 ± 7.2^{b}	176.9 ± 5.0^{e}	139.6 ± 7.0^{f}	353.0 ± 2.8^{c}	215.6 ± 6.9^{d}	491.8 ± 4.2^{a}	0.000
Heptanal	1177	MS, RI, O	Fat, citrus; 3	55.8 ± 7.1^{cd}	28.8 ± 1.3^{e}	68.0 ± 1.9^{b}	62.4 ± 0.4^{bc}	55.1 ± 5.3^{d}	99.4 ± 2.6^{a}	0.000
Octanal	1284	MS, RI, O	Fat, lemon, green; 0.578	380.8 ± 9.1^{e}	390.8 ± 19.7^{e}	555.7 ± 49.9^{d}	883.9 ± 31.7^{b}	605.3 ± 20.0^{c}	1166.4 ± 9.3^{a}	0.000
Nonanal	1391	MS, RI, O	Fat, citrus, green; 1	987.7 ± 24.5^{e}	781.8 ± 18.1^{f}	1446.7 ± 63.2^{d}	2339.4 ± 31.1^{b}	1560.9 ± 28.7^{c}	2628.6 ± 28.1^{a}	0.000
(*E*)-2-Octenal	1425	MS, RI, O	Green, nut, fat; 3	N.D.	N.D.	N.D.	N.D.	N.D.	36.2 ± 2.1	N.D.
Decanal	1490	MS, RI, O	Soap, orange peel; 2	N.D.	N.D.	N.D.	N.D.	N.D.	76.8 ± 5.8	N.D.
Benzaldehyde	1517	MS, RI, O	Bitter almond, 41.7	8.7 ± 0.5^{d}	N.D.	26.3 ± 0.4^{a}	12.2 ± 0.4^{c}	20.9 ± 0.4^{b}	25.9 ± 0.1^{a}	0.000
(*E*)-2-Nonenal	1534	MS, RI, O	Cucumber, green; 1	63.1 ± 12.9^{b}	N.D.	62.6 ± 13.4^{b}	77.9 ± 7.2^{b}	N.D.	133.0 ± 35.3^{a}	0.008
Dodecanal	1708	MS, RI, O	Herbaceous, fatty; 2	N.D.	N.D.	N.D.	N.D.	N.D.	22.8 ± 1.1	N.D.
(*E,E*)-2,4-Decadienal	1808	MS, RI, O	Fried, wax, fat; 0.07	N.D.	N.D.	N.D.	N.D.	N.D.	708.4 ± 73.0	N.D.
1,8-Cineole	1204	MS, RI, O	Mint, sweet; 1	158.5 ± 2.6^{b}	135.2 ± 13.9^{c}	144.6 ± 3.8^{c}	160.4 ± 2.2^{b}	178.6 ± 6.3^{a}	173.4 ± 3.8^{a}	0.000
1-Octen-3-ol	1445	MS, RI, O	Mushroom; 2	268.2 ± 8.9^{bc}	179.0 ± 4.6^{d}	167.1 ± 7.3^{d}	256.7 ± 6.5^{c}	275.6 ± 11.3^{b}	531.8 ± 7.8^{a}	0.000
Linalool	1541	MS, RI, O	Flower, lavender; 6	8.8 ± 1.0^{c}	11.3 ± 0.7^{b}	9.4 ± 0.5^{c}	8.6 ± 0.5^{c}	12.2 ± 0.9^{ab}	13.3 ± 1.1^{a}	0.000
1-Octanol	1554	MS, RI, O	Herbal, green; 110 green	0.6 ± 0.0^{c}	1.0 ± 0.1^{c}	1.6 ± 0.2^{b}	1.8 ± 0.1^{b}	1.8 ± 0.1^{b}	2.0 ± 0.1^{a}	0.000
(*E*)-2-Octen-1-ol	1610	MS, RI, O	Soap, plastic; 3	22.4 ± 1.4^{cd}	N.D.	19.1 ± 6.1^{d}	28.6 ± 2.0^{b}	27.3 ± 1.1^{bc}	46.4 ± 2.3^{a}	0.000
D-Limonene	1187	MS, RI, O	Citrus, mint; 10	14.4 ± 1.5^{e}	40.3 ± 0.4^{b}	12.0 ± 0.7^{f}	32.9 ± 0.7^{c}	78.9 ± 0.7^{a}	17.5 ± 1.7^{d}	0.000
β-Phellandrene	1197	MS, RI, O	Turpentine, mint; 8 mint	10.2 ± 0.8^{e}	17.8 ± 1.2^{c}	14.9 ± 0.3^{d}	13.5 ± 0.5^{d}	32.0 ± 1.5^{a}	25.6 ± 2.6^{b}	0.000
Styrene	1246	MS, RI, O	Herbaceous, fatty; 65	N.D.	1.1 ± 0.0^{b}	0.8 ± 0.0^{c}	N.D.	N.D.	1.3 ± 0.1^{a}	0.000
Naphthalene	1737	MS, RI, O	Camphoric; 60	0.6 ± 0.1^{d}	1.4 ± 0.1^{b}	1.2 ± 0.2^{c}	1.8 ± 0.0^{a}	1.8 ± 0.0^{a}	1.4 ± 0.1^{b}	0.000
Estragole	1665	MS, RI, O	Licorice, anise; 6	18.8 ± 1.5^{e}	32.3 ± 1.9^{b}	25.7 ± 0.7^{c}	22.0 ± 0.5^{d}	35.4 ± 1.3^{a}	24.2 ± 0.9^{cd}	0.000
Anethole	1822	MS, RI, O	Rubber, paint; 15	71.2 ± 2.1^{c}	125.0 ± 0.7^{a}	113.0 ± 2.1^{b}	55.9 ± 0.8^{d}	112.3 ± 1.4^{b}	71.2 ± 1.2^{c}	0.000
Eugenol	2155	MS, RI, O	Clove, honey; 7.1	61.4 ± 2.0^{c}	171.6 ± 3.3^{a}	110.2 ± 1.7^{b}	21.7 ± 0.8^{e}	30.2 ± 0.3^{d}	31.3 ± 2.1^{d}	0.000
2-Pentylfuran	1222	MS, RI, O	Green bean, butter; 6	36.9 ± 2.0^{d}	21.7 ± 0.4^{e}	19.9 ± 0.2^{e}	55.8 ± 0.2^{b}	45.4 ± 2.9^{c}	96.4 ± 2.3^{a}	0.000
Total				2608.3 ± 71.3^{e}	2122 ± 12.6^{f}	2968 ± 118.4^{d}	4399.6 ± 29.6^{b}	3296.3 ± 20.6^{c}	6442.2 ± 133.8^{a}	0.000

Note: Each value is expressed as mean ± SD; N.D. = not detected. [a–f] Different letters in the same row indicate that there is significant difference ($P < 0.05$, along the lines). TS, traditional stewing; TSE, traditional stewing with enzymatic degradation; TSEM, traditional stewing with enzymatic degradation and Maillard reaction; HS, high-temperature stewing; HSE, high-temperature stewing with enzymatic degradation; HSEM, high-temperature stewing with enzymatic degradation and Maillard reaction.

[a] Linear retention index calculated on DB-Wax capillary column.
[b] Means of identification: MS, mass spectrum comparison using NIST libraries; RI, retention index compared with literature value; O, aroma description (odor).
[c] Odor thresholds were mainly obtained from online database, (http://www.flavornet.org, http://www.odour.org.uk).
[d] Odor descriptions were mainly gathered from online database, (http://www.flavornet.org).

since the first two PCs accounted for more than 95% of the total variances. PC1 explained 88.65% of sample variance and PC2 explained only 8.46%. Therefore, the major variation resulting from PC1 was employed to distinguish the differences among stewed pork samples. The traditional stewed pork samples (TS, TSE and TSEM) and high-temperature stewed pork samples (HS, HSE and HSEM) were located on the negative and positive axis respectively and could be also divided into two different groups on PC1. This result illustrated that the stewed pork from traditional and high-temperature processing methods had significantly different flavour profiles, TS, TSE and TSEM samples had similar aroma compositions, and HS, HSE and HSEM samples were the same. TS, TSE and TSEM samples on PC1 were highly associated with sensor W1C and W3C, and HS, HSE and HSEM samples on PC1 were highly related to sensor W6S, W1W, W2W, W3S, W2S, W5S and W1S. The stewed pork samples on PC2 were depended on W5C, which was sensitive to alkane compounds. Due to the little variance contribution rate of PC2, the alkane compounds had no important influence on the odor of stewed pork. Corresponds to the result of Fig. 1a, aromatic compounds, ammonia, nitrogen oxides, broad alcohols, sulfur organic compounds, terpenes and organic sulphides were the dominant components of stewed pork odor.

3.2. Non-volatile compounds of stewed pork with different processing methods

3.2.1. FAAs analysis of stewed pork

The concentrations of FAAs of stewed pork with different processing methods were shown in Table 3. A total of 16 amino acids were detected

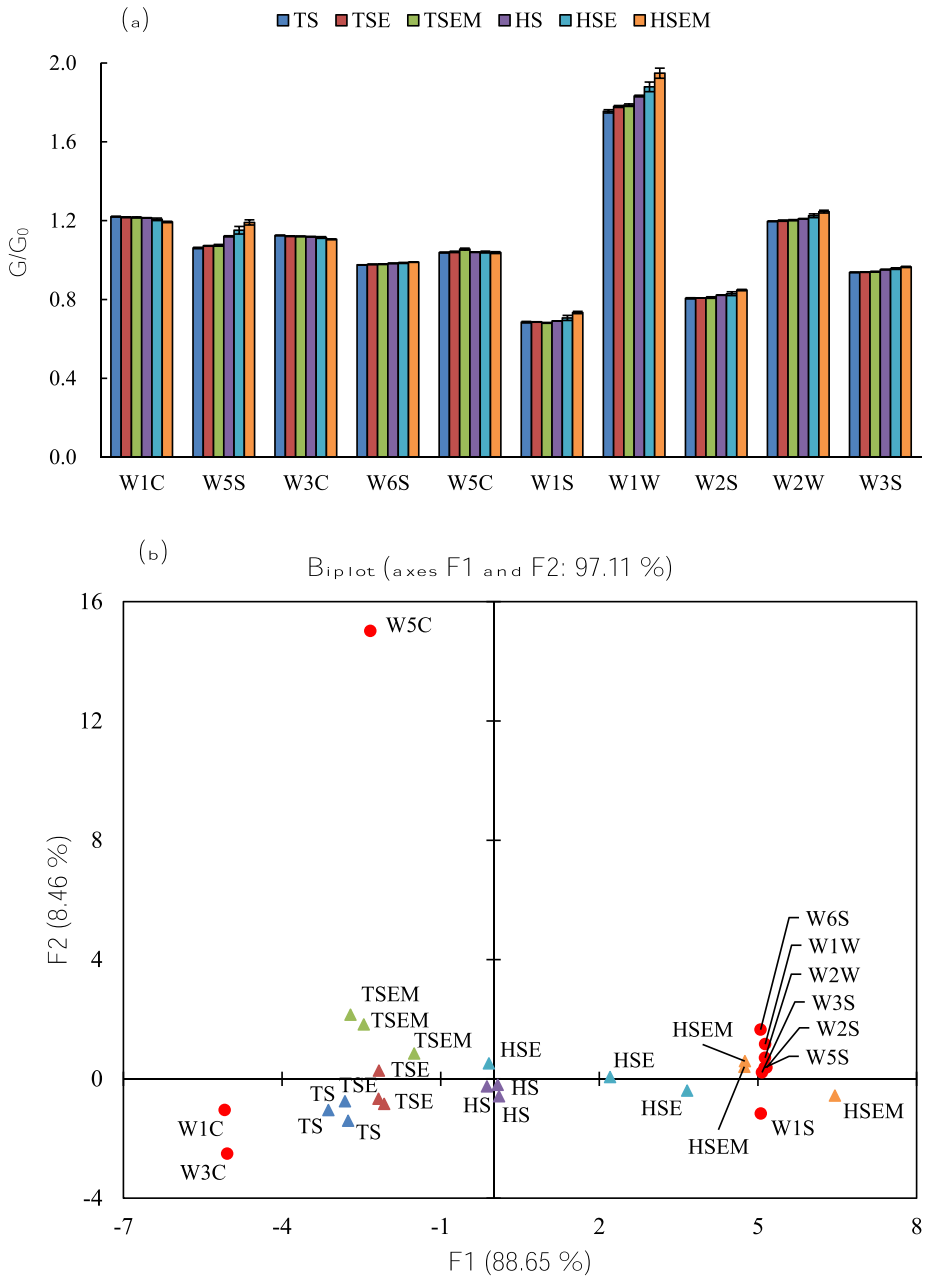

Fig. 1. Response values of ten sensors **(a)** and PCA chart **(b)** of volatile flavour compounds in stewed pork with E-nose. The sensory of 10 chemical sensors are W1C (aromatic), W5S (broad-range), W3C (aromatic), W6S (hydrogen), W5C (arom-aliph), W1S (broad-methane), W1W (sulfur-organic), W2S (broad-alcohol), W2W (sulph-chlor) and W3S (methane-aliph). TS, traditional stewing; TSE, traditional stewing with enzymatic degradation; TSEM, traditional stewing with enzymatic degradation and Maillard reaction; HS, high-temperature stewing; HSE, high-temperature stewing with enzymatic degradation; HSEM, high-temperature stewing with enzymatic degradation and Maillard reaction.

in stewed pork samples, which were divided into umami amino acids (UAAs; Asp and Glu), sweet amino acids (SAAs; Ser, Gly, Thr and Ala), bitter amino acids (BAAs; His, Arg, Val, Met, Ile, Leu and Phe) and other amino acids (Pro, Tyr and Lys). Among them, the total content of SAAs (112.9–148.2 mg/100 g) was highest in all stewed pork samples, indicating that the SAAs were predominant in the stewed pork samples. The result of the total content of SAAs above in this study is consistent with those reported in stewed pork rib broth (Hou, Liu, Xu, Zhou, & Li, 2018). For these SAAs, the highest concentration of Thr was found, followed by Ala, Gly and Ser. The UAAs, SAAs and BAAs showed higher values in high-temperature stewed pork (HS, HSE and HSEM) than those in traditional stewed pork (TS, TSE and TSEM), respectively. This may be because the heat treatments applied during meat processing could generate a major release of free amino acids (Díaz, Fernandez, Fernando, Hoz, & Ordoez, 1997).

The total free amino acids (TFAAs) were presented higher contents in HSE samples than those in HS samples, and HSEM samples had the lowest contents of TFAAs. It indicated that the flavorzyme hydrolysed proteins to produce more FAAs (Xu et al., 2018), while the decrease in FAAs after adding xylose was related to the formation of volatile compounds by Maillard reaction (Song et al., 2019). No significant difference of TFAAs has been found in TS, TSE and TSEM samples. Among FAAs, the content of Asp in HSEM samples was highest, HS and HSE samples had the highest content of Glu, which could give stewed pork a strong umami taste. The contents of SAAs (Ser, Gly, Thr and Ala) in HSEM samples were less than that of the other two samples (HS and HSE). HS and HSE samples showed a higher content of BAAs, except for His and Arg, than the other stewed pork samples. The bitterness produced BAAs could be masked by sweet and umami substances such as Asp, Glu, Ser, Gly, Thr and Ala. In order to determine the contribution of free amino acids to the taste of stewed pork, the taste threshold was introduced (Table 3). The taste activity values (TAVs) of each amino

Table 3
Free amino acid contents (mg/100 g) and taste threshold of stewed pork with different processing methods.

Free amino acids	Traditional stewed pork			High-temperature stewed pork			*p* value	Taste threshold (mg/100 g)
	TS	TSE	TSEM	HS	HSE	HSEM		
Asp	2.4 ± 0.1^{c}	2.8 ± 0.1^{bc}	3.3 ± 0.4^{ab}	2.5 ± 0.5^{c}	2.8 ± 0.3^{bc}	3.7 ± 0.3^{a}	0.002	100
Glu	13.9 ± 0.5^{a}	11.5 ± 0.8^{b}	13.0 ± 0.2^{ab}	15.1 ± 1.0^{a}	14.4 ± 0.3^{a}	11.0 ± 2.4^{b}	0.004	30
ΣUAA	16.3 ± 0.4^{ab}	14.2 ± 0.8^{b}	16.3 ± 0.2^{ab}	17.6 ± 1.0^{a}	17.2 ± 0.5^{a}	14.7 ± 2.7^{b}	0.030	
Ser	5.3 ± 0.2^{b}	2.9 ± 0.3^{c}	6.1 ± 0.3^{b}	8.3 ± 0.9^{a}	8.1 ± 0.3^{a}	2.6 ± 0.1^{c}	0.000	150
Gly	7.1 ± 0.5^{bc}	6.2 ± 0.3^{c}	7.5 ± 0.2^{b}	9.5 ± 0.6^{a}	7.6 ± 0.1^{b}	6.3 ± 1.0^{c}	0.000	130
Thr	96.0 ± 3.5^{bc}	113.6 ± 7.9^{a}	88.5 ± 6.3^{c}	115.9 ± 8.1^{a}	106.3 ± 1.8^{ab}	97.2 ± 2.7^{bc}	0.000	260
Ala	5.6 ± 0.5^{c}	8.0 ± 0.7^{d}	9.7 ± 0.1^{c}	14.6 ± 1.5^{a}	12.1 ± 1.3^{b}	6.8 ± 0.6^{de}	0.000	60
ΣSAA	114.1 ± 4.7^{c}	130.7 ± 8.7^{b}	111.7 ± 6.2^{c}	148.2 ± 11.2^{a}	134.0 ± 3.1^{b}	112.9 ± 4.2^{c}	0.000	
His	18.0 ± 0.7^{c}	16.9 ± 0.1^{d}	17.7 ± 0.1^{c}	18.4 ± 0.2^{bc}	18.8 ± 0.0^{b}	23.1 ± 0.6^{a}	0.000	20
Arg	27.2 ± 1.2^{a}	18.1 ± 1.1^{d}	24.8 ± 1.4^{bc}	26.5 ± 1.8^{ab}	23.5 ± 0.3^{c}	23.1 ± 0.6^{c}	0.000	50
Val	4.8 ± 0.1^{cd}	5.3 ± 0.2^{bc}	5.5 ± 0.3^{b}	7.1 ± 0.6^{a}	7.5 ± 0.2^{a}	4.6 ± 0.1^{d}	0.000	40
Met	11.5 ± 0.1^{a}	10.9 ± 0.1^{b}	11.9 ± 0.1^{a}	12.1 ± 0.7^{a}	11.8 ± 0.0^{a}	10.5 ± 0.0^{b}	0.000	190
Ile	8.1 ± 0.2^{c}	7.8 ± 0.3^{c}	9.3 ± 0.4^{b}	10.4 ± 0.5^{a}	10.6 ± 0.1^{a}	7.7 ± 0.1^{c}	0.000	90
Leu	4.4 ± 0.3^{b}	1.1 ± 0.1^{c}	N.D.	5.7 ± 0.6^{a}	5.2 ± 0.2^{a}	0.1 ± 0.0^{d}	0.000	30
Phe	21.1 ± 0.6^{bc}	20.6 ± 0.6^{c}	21.5 ± 0.3^{ab}	21.5 ± 0.5^{ab}	22.0 ± 0.1^{a}	20.8 ± 0.0^{bc}	0.011	90
ΣBAA	95.1 ± 1.5^{b}	80.6 ± 1.4^{d}	90.7 ± 2.4^{c}	101.8 ± 3.8^{a}	99.5 ± 0.8^{a}	89.9 ± 1.4^{c}	0.000	
Pro	5.7 ± 0.1^{c}	5.7 ± 0.2^{c}	5.8 ± 0.2^{c}	7.3 ± 0.2^{b}	8.5 ± 0.1^{a}	5.7 ± 0.2^{c}	0.000	300
Tyr	5.8 ± 0.0^{e}	15.7 ± 1.1^{bc}	17.2 ± 1.3^{b}	12.3 ± 1.0^{d}	41.2 ± 2.8^{a}	13.5 ± 0.6^{cd}	0.000	/
Lys	0.5 ± 0.2^{d}	0.1 ± 0.0^{d}	1.3 ± 0.4^{c}	3.4 ± 0.5^{a}	2.4 ± 0.2^{b}	0.2 ± 0.0^{d}	0.000	50
Total	237.5 ± 6.8^{b}	247.0 ± 10.5^{b}	243.1 ± 10.2^{b}	290.7 ± 16.5^{a}	302.8 ± 6.5^{a}	236.8 ± 3.5^{b}	0.000	

Note: Each value is expressed as mean ± SD. $^{a–e}$ Different letters in the same row indicate that there is significant difference ($P < 0.05$, along the lines). UAA: Umami amino acid, SAA: Sweet amino acid, BAA: Bitter amino acid. N.D., not detectable. TS, traditional stewing; TSE, traditional stewing with enzymatic degradation; TSEM, traditional stewing with enzymatic degradation and Maillard reaction; HS, high-temperature stewing; HSE, high-temperature stewing with enzymatic degradation; HSEM, high-temperature stewing with enzymatic degradation and Maillard reaction.

acid were calculated (Liu, Xia, Wang, & Chen, 2019) by taste threshold. It can be seen that the TAVs of free amino acids in stewed pork were <1 to less contributed taste, however, the umami taste of stewed pork might increase by the synergistic interaction between FAAs and nucleotides.

3.2.2. 5′-nucleotide and EUC analysis of stewed pork

As can be seen in Table 4, 5′-nucleotides (AMP, GMP and IMP) of traditional stewed pork (TS, TSE and TSEM) exhibited higher contents compared with that in high-temperature stewed pork (HS, HSE and HSEM). This might be one possible reason that the higher temperature in the steaming step of stewed pork could accelerate the degradation of nucleotides (Jayasena et al., 2015). Whether traditional stewed pork (TS, TSE and TSEM) or high-temperature stewed pork (HS, HSE and HSEM) with the addition of flavorzyme and flavour precursors (D-xylose, L-cysteine and thiamine) showed an increasing trend in the contents of AMP, GMP and IMP, indicating enzymatic hydrolysis and Maillard reaction were prone to promote the formation of flavour nucleotides. Similarly, the total contents of 5′-nucleotides also had significantly increased in traditional stewing groups (TS, TSE and TSEM) or high-temperature stewing groups (HS, HSE and HSEM).

IMP was the most predominant flavour-contributing 5′-nucleotide in stewed pork and is known to impart a pleasant taste (Yue, Zhang, Jin, Deng, & Zhao, 2016). The interaction of IMP with some sweet amino acids like Ser, Gly and Ala has been shown to contribute to intensifying umami taste (Kawai, Okiyama, & Ueda, 2002). According to the taste threshold of nucleotides, the TAVs of IMP were much greater than 1 to provide more umami taste. As for AMP and GMP, the contents of HS samples were the lowest and those of TSEM samples were the highest. Although the TAVs of AMP and GMP in the pork samples were lower than 1, GMP is a stronger flavour enhancer contributing to a meaty flavour (Yue et al., 2016) and the synergistic interaction between IMP and AMP in eliciting umami taste should be considered (Fuke & Ueda, 1996). Due to the synergistic effect of flavour nucleotides and MSG-like components (Glu and Asp), it might greatly increase the umami taste in marinated chicken (Li et al., 2016). Therefore, EUC values were suggested to evaluate the umami taste of stewed pork. TSE, TSEM, HSE and HSEM samples had the higher level of EUC values (Table 4). Overall, the stewed pork with enzymatic hydrolysis or Maillard reaction had a more umami taste than other stewed pork samples.

3.2.3. Fatty acid composition of stewed pork

Fatty acid composition in meat is important for consumers due to its major contributions to meat flavour (Aaslyng & Meinert, 2017). As shown in Table 5, it was observed that 9 fatty acids were presented in stewed pork with different processing methods. The main fatty acids detected in stewed pork samples were C16:0 (palmitic acid), C18:0

Table 4
Nucleotide contents (mg/100 g), taste threshold and EUC of stewed pork with different processing methods.

Nucleotides	Traditional stewed pork			High-temperature stewed pork			*p* value	Taste threshold (mg/100 g)
	TS	TSE	TSEM	HS	HSE	HSEM		
5′-AMP	20.2 ± 0.3^{c}	25.5 ± 2.1^{a}	18.8 ± 0.4^{c}	19.3 ± 0.4^{c}	22.7 ± 1.2^{b}	27.0 ± 0.3^{a}	0.000	50
5′-GMP	2.6 ± 0.0^{b}	2.7 ± 0.0^{ab}	2.9 ± 0.0^{a}	1.6 ± 0.0^{d}	2.0 ± 0.2^{c}	1.9 ± 0.2^{c}	0.000	12.5
5′-IMP	107.6 ± 1.2^{d}	116.1 ± 3.4^{c}	149.4 ± 2.1^{a}	77.7 ± 1.1^{e}	116.1 ± 2.7^{c}	126.9 ± 1.1^{b}	0.000	25
[a]Flavor 5′-nucleotide	130.4 ± 1.0^{d}	144.3 ± 5.5^{c}	171.1 ± 2.5^{a}	98.6 ± 1.5^{e}	140.8 ± 3.1^{c}	155.7 ± 1.1^{b}	0.000	
[b]EUC (mg MSG/100 g)	1.9 ± 0.0^{bc}	2.2 ± 0.0^{ab}	2.3 ± 0.1^{a}	1.6 ± 0.1^{c}	2.2 ± 0.1^{ab}	1.9 ± 0.4^{bc}	0.006	

Note: Each value is expressed as mean ± SD. Means with different superscripts in the same row indicate significant difference ($P < 0.05$).

[a] Flavor nucleotides = 5′-IMP+5′-GMP+5′-AMP.

[b] The equivalent umami concentration (EUC, g monosodium glutamate (MSG) per 100 g) represents the concentration of MSG equivalent to the umami intensity given by the mixture of MSG and the 5′-nucleotide. TS, traditional stewing; TSE, traditional stewing with enzymatic degradation; TSEM, traditional stewing with enzymatic degradation and Maillard reaction; HS, high-temperature stewing; HSE, high-temperature stewing with enzymatic degradation; HSEM, high-temperature stewing with enzymatic degradation and Maillard reaction.

Table 5
Concentrations (mg/kg) of fatty acids in stewed pork with different processing methods.

Fatty acids	Traditional stewed pork			High-temperature stewed pork			*p* value
	TS	TSE	TSEM	HS	HSE	HSEM	
C14:0	85.1 ± 0.6^{a}	82.5 ± 6.3^{ab}	80.9 ± 1.6^{ab}	84.0 ± 2.8^{ab}	82.9 ± 5.8^{ab}	74.2 ± 9.5^{b}	0.241
C16:0	787.9 ± 14.7^{a}	746.4 ± 43.4^{ab}	699.2 ± 16.7^{bc}	685.3 ± 37.7^{cd}	638.7 ± 30.2^{d}	579.4 ± 20.3^{e}	0.000
C18:0	798.1 ± 14.6^{a}	774.9 ± 6.1^{a}	675.7 ± 9.6^{b}	667.9 ± 4.7^{b}	640.2 ± 33.8^{b}	558.3 ± 28.8^{c}	0.000
C16:1	262.8 ± 2.8^{a}	260.2 ± 7.4^{a}	245.1 ± 10.3^{b}	295.5 ± 6.6^{b}	228.2 ± 20.4^{c}	211.0 ± 6.1^{d}	0.000
C17:1	804.7 ± 32.6^{bc}	735.7 ± 13^{b}	676.6 ± 4.7^{b}	818.9 ± 18.4^{a}	690.1 ± 7.4^{cd}	545.9 ± 7.4^{d}	0.000
C18:1	889.1 ± 55.3^{c}	832.8 ± 27.5^{b}	754.7 ± 33.8^{a}	1234.0 ± 162.2^{a}	952.2 ± 52.9^{c}	940.6 ± 61.7^{d}	0.000
C18:2	2664.4 ± 146.4^{c}	2990.3 ± 37.3^{bc}	3058.8 ± 10.7^{bc}	2870.4 ± 28.7^{a}	2450.5 ± 18.8^{b}	2126.8 ± 70.3^{b}	0.000
C20:4	1348.6 ± 108.1^{bc}	1407.2 ± 59.7^{bc}	1547.9 ± 58.7^{bc}	1391.1 ± 33.5^{a}	1250.0 ± 33.1^{b}	1074.6 ± 72.4^{c}	0.000
C20:5	117.2 ± 5.3^{a}	122.5 ± 6.8^{ab}	132.2 ± 5.8^{a}	119.1 ± 3.7^{b}	110.0 ± 6.2^{d}	106.2 ± 2.0^{e}	0.001
SFA	1671.0 ± 18.7^{bc}	1603.8 ± 34.3^{b}	1455.8 ± 10.5^{a}	1437.2 ± 39.6^{b}	1361.8 ± 58.2^{b}	1211.9 ± 48.5^{d}	0.000
MUFA	1956.5 ± 60.9^{b}	1828.7 ± 36.1^{bc}	1676.4 ± 47.5^{d}	2348.4 ± 148.6^{a}	1870.5 ± 80.5^{cd}	1697.5 ± 59.7^{cd}	0.000
PUFA	4130.1 ± 245.3^{b}	4520.0 ± 30.2^{b}	4739.0 ± 44.0^{a}	4380.6 ± 6.1^{b}	3810.5 ± 9.9^{c}	3307.6 ± 88.6^{e}	0.000
Total	7757.7 ± 225.6^{b}	7952.5 ± 100.5^{ab}	7871.2 ± 83.7^{b}	8166.3 ± 110.3^{a}	7042.8 ± 31.8^{c}	6217.1 ± 168.3^{d}	0.000

Note: Each value is expressed as mean ± SD. Means with different superscripts in the same row indicate significant difference ($P < 0.05$). SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. TS, traditional stewing; TSE, traditional stewing with enzymatic degradation; TSEM, traditional stewing with enzymatic degradation and Maillard reaction; HS, high-temperature stewing; HSE, high-temperature stewing with enzymatic degradation; HSEM, high-temperature stewing with enzymatic degradation and Maillard reaction.

(stearic acid), C17:1 (ginkgolic acid), C18:1 (oleic acid), C18:2 (linoleic acid) and C20:4 (arachidonic acid), which were approximately 94% of the total fatty acids. Compared with traditional stewed pork (TS, TSE and TSEM), the single fatty acid or total saturated fatty acids (SFAs), total monounsaturated fatty acids (MUFAs) and total polyunsaturated fatty acids (PUFAs) changed significantly ($P \leq 0.001$) in high-temperature stewed pork (HS, HSE and HSEM), however, C14:0 (myristic acid) was not markedly different in all stewed pork samples. For traditional stewed pork, SFA and MUFA of TSEM samples had significantly lower ($P \leq 0.05$) than those of TS and TSE samples. On the contrary, PUFA increased significantly ($P \leq 0.05$) in TSEM samples which was probably because the higher level of Maillard reaction products (Hwang, Kim, Woo, Lee, & Jeong, 2011) inhibit autoxidation of PUFA during processing. The concentrations of SFA, MUFA, PUFA in high-temperature stewed pork (HS, HSE and HSEM) decreased significantly ($P < 0.05$), when flavormyze, D-xylose, L-cysteine and thiamine was added. It may be due to the thermally induced reactions between fatty acid oxidation products, enzymatic hydrolysis products and Maillard reaction precursors, which could form a large number of volatile compounds. From the above results, it can be concluded that the stewed pork combined with both enzymatic hydrolysis and Maillard reaction increased the possibility of fatty acid oxidation.

3.2.4. *Taste composition analysis of stewed pork by E-tongue*

PCA was applied to provide an overview of taste compounds of stewed pork samples using E-tongue data. As shown in Fig. 2, the first

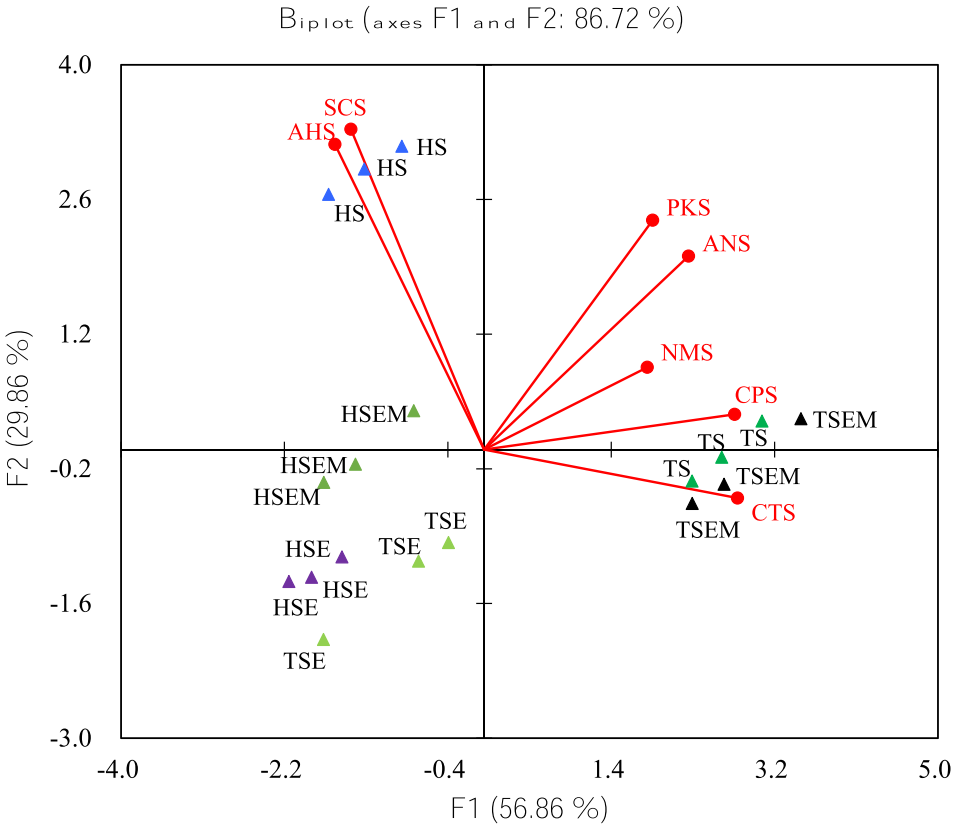

Fig. 2. PCA score plot of E-tongue data for stewed pork with different processing methods. TS, traditional stewing; TSE, traditional stewing with enzymatic degradation; TSEM, traditional stewing with enzymatic degradation and Maillard reaction; HS, high-temperature stewing; HSE, high-temperature stewing with enzymatic degradation; HSEM, high-temperature stewing with enzymatic degradation and Maillard reaction. Taste sensor: AHS (to detect sour taste), SCS (to detect bitterness), PKS (to detect complex taste), ANS (to detect sweetness), NMS (to detect umami taste), CPS (to detect complex taste), CTS (to detect salty taste).

two PCs explained 59.86% and 29.86% of the data variance, respectively. The total contribution rate was over 85%, which showed that most of the information can reflect the overall taste characteristics of the stewed pork samples. Six different stewed pork samples were clearly divided into three groups (HS; TS and TSEM; HSEM, HSE and TSE). The sample dots of HS, HSEM, HSE and TSE were located on the positive side of PC1, whereas TS and TSEM samples were on the opposite side. This indicated that the taste characteristics of TS and TSEM samples were significantly different from those of HS, HSEM, HSE and TSE samples. The sample points of HSEM, HSE and TSE were clustered together, and TS and TSEM samples were close to each other, which meant that there was a similar taste profile. The sample dots of HS were distributed separately in the upper left side, which was described as sourness and bitterness because of their short distance to sensor AHS and SCS. TS and TSEM samples were responsible for sensor CTS (to detect salty substances) and sensor PKS, ANS and NMS were lowly relevant to the stewed pork samples.

4. Conclusions

In this present study, the types and contents of volatile compounds in high-temperature stewed pork (HS, HSE and HSEM) were higher significantly ($P < 0.05$) than those of traditional stewed pork (TS, TSE and TSEM) in particular of aldehydes. For high-temperature stewed pork, sample HSEM showed the most abundant flavor compounds such as aldehydes (52.6%), alcohols (13.8%) and heterocyclic compounds (5.4%). Most of odor-active compounds in HSEM samples had the highest OAVs, which could contribute more typical aroma to stewed pork. All stewed pork samples were clearly divided into two groups, including traditional stewed samples (TS, TSE and TSEM) and high-temperature stewed samples (HS, HSE and HSEM), which indicated that the volatile composition of two groups of pork samples was significantly different. HS, HSE and HSEM samples had the higher contents of UAAs, SAAs and BAAs than those of TS, TSE and TSEM samples, because the high temperature promoted the major release of FAAs. The contents of 5′-nucleotides (AMP, GMP and IMP) in high-temperature stewed pork (HS, HSE and HSEM) showed a lower level due to heat-induced decomposition of nucleotides. The contents of fatty acids in stewed pork samples decreased significantly ($P < 0.05$) when flavormyze, xylose, cysteine and thiamine were added. It can be concluded that high-temperature stewed pork (HS, HSE and HSEM) improve the taste and odor characteristic, of which HSEM was particularly prominent in the formation of odor compounds.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

Informed consent

Informed consent was obtained from all individual participants included in the study.

CRediT authorship contribution statement

Dong Han: Formal analysis, Writing – original draft. **Chun-Hui Zhang:** Funding acquisition, Methodology. **Marie-Laure Fauconnier:** Writing – review & editing. **Wei Jia:** Investigation. **Jing-Fan Wang:** Methodology, Investigation. **Fei-Fei Hu:** Methodology, Investigation. **Dao-Wen Xie:** Investigation.

Declaration of competing interest

Dong Han declares that he has no conflict of interest. Chun-Hui Zhang declares that he has no conflict of interest. Marie-Laure Fauconnier, Wei Jia, Jing-Fan Wang, Fei-Fei Hu and Dao-Wen Xie declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.111229.

References

Aaslyng, M. D., & Meinert, L. (2017). Meat flavour in pork and beef - from animal to meal. *Meat Science, 132*, 112–117.
Aparicio, R., Morales, M. T., & Alonso, M. V. (1996). Relationship between volatile compounds and sensory attributes of olive oils by the sensory wheel. *Journal of the American Oil Chemists' Society, 73*(10), 1253–1264.
Benet, I., Guàrdia, M. D., Ibañez, C., Solà, J., Arnau, J., & Roura, E. (2015). Analysis of SPME or SBSE extracted volatile compounds from cooked cured pork ham differing in intramuscular fat profiles. *LWT-Food Science and Technology, 60*(1), 393–399.
Dashdorj, D., Amna, T., & Hwang, I. (2015). Influence of specific taste-active components on meat flavor as affected by intrinsic and extrinsic factors: An overview. *European Food Research and Technology, 241*(2), 157–171.
Díaz, O., Fernandez, M., Fernando, G. D. G. D., Hoz, L. D. L., & Ordoez, J. A. (1997). Proteolysis in dry fermented sausages: The effect of selected exogenous proteases. *Meat Science, 46*(1), 115–128.
Dong, X. B., Li, X., Zhang, C. H., Wang, J. Z., Tang, C. H., Sun, H. M., et al. (2014). Development of a novel method for hot-pressure extraction of protein from chicken bone and the effect of enzymatic hydrolysis on the extracts. *Food Chemistry, 157*, 339–346.
Folch, J., Lees, M., & Stanley, G. S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry, 226*(1), 497–509.
Fuke, S., & Ueda, Y. (1996). Interactions between umami and other flavor characteristics. *Trends in Food Science & Technology, 7*(12), 407–411.
Han, D., Mi, S., Zhang, C. H., Li, J., Song, H. L., Fauconnier, M. L., et al. (2019). Characterization and discrimination of Chinese marinated pork hocks by volatile compound profiling using solid phase microextraction gas chromatography-mass spectrometry/olfactometry, electronic nose and chemometrics. *Molecules, 24*(7), 1385.
Han, D., Zhang, C. H., Fauconnier, M. L., & Mi, S. (2019). Characterization and differentiation of boiled pork from Tibetan, Sanmenxia and Duroc×(Landrace×Yorkshire) pigs by volatiles profiling and chemometrics analysis. *Food Research International, 130*, Article 108910.
Hofmann, T., & Schieberle, P. (1995). Evaluation of the key odorants in a thermally treated solution of ribose and cysteine by aroma extract dilution techniques. *Journal of Agricultural and Food Chemistry, 43*(8), 2187–2194.
Hou, M. M., Liu, D. M., Xu, X. L., Zhou, G. H., & Li, C. B. (2018). Effect of postmortem aging time on flavor profile of stewed pork rib broth. *International Journal of Food Properties, 21*(1), 1449–1462.
Hwang, I. G., Kim, H. Y., Woo, K. S., Lee, J., & Jeong, H. S. (2011). Biological activities of Maillard reaction products (MRPs) in a sugar–amino acid model system. *Food Chemistry, 126*(1), 221–227.
Jayasena, D. D., Ahn, D. U., Nam, K. C., & Jo, C. (2013). Flavour chemistry of chicken meat: A review. *Asian-Australasian Journal of Animal Sciences, 26*(5), 732–742.
Jayasena, D. D., Jung, S., Kim, H. J., Yong, H. I., Nam, K. C., & Jo, C. (2015). Taste-active compound levels in Korean native chicken meat: The effects of bird age and the cooking process. *Poultry Science, 94*(8), 1964–1972.
Kawai, M., Okiyama, A., & Ueda, Y. (2002). Taste enhancements between various amino acids and IMP. *Chemical Senses, 27*(8), 739–745.
Kosowska, M., Majcher, M. A., & Fortuna, T. (2017). Volatile compounds in meat and meat products. *Food Science and Technology, 37*(1), 1–7.
Li, H., Li, X., Zhang, C. H., Wang, J. Z., Tang, C. H., & Chen, L. L. (2016). Flavor compounds and sensory profiles of a novel Chinese marinated chicken. *Journal of the Science of Food and Agriculture, 96*(5), 1618–1626.
Liu, D. Y., Li, S. J., Wang, N., Deng, Y. J., Sha, L., Gai, S. M., et al. (2017). Evolution of taste compounds of Dezhou-braised chicken during cooking evaluated by chemical analysis and an electronic tongue system. *Journal of Food Science, 82*(5), 1076–1082.
Liu, T. T., Xia, N., Wang, Q. Z., & Chen, D. W. (2019). Identification of the non-volatile taste-active components in crab sauce. *Foods, 8*(8).
Liu, Y., Xu, X. L., & Zhou, G. H. (2007). Changes in taste compounds of duck during processing. *Food Chemistry, 102*(1), 22–26.

Lorenzo, J. M., Carballo, J., & Franco, D. (2013). Effect of the inclusion of chestnut in the finishing diet on volatile compounds of dry-cured ham from Celta pig breed. *Journal of Integrative Agriculture, 12*(11), 2002–2012.

Maughan, C., & Martini, S. (2012). Identification and quantification of flavor attributes present in chicken, lamb, pork, beef, and Turkey. *Journal of Food Science, 77*(1–3), S115–S121.

Pham, A. J., Schilling, M. W., Mikel, W. B., Williams, J. B., Martin, J. M., & Coggins, P. C. (2008). Relationships between sensory descriptors, consumer acceptability and volatile flavor compounds of American dry-cured ham. *Meat Science, 80*(3), 728–737.

Qi, J., Liu, D. Y., Zhou, G. H., & Xu, X. L. (2017). Characteristic flavor of traditional soup made by stewing Chinese yellow-feather chickens. *Journal of Food Science, 82*(7–9), 2031–2040.

Song, S., Fan, L., Xu, X., Xu, R., Jia, Q., & Feng, T. (2019). Aroma patterns characterization of braised pork obtained from a novel ingredient by sensory-guided analysis and gas-chromatography-olfactometry. *Foods, 8*(3), 87.

Sun, H. M., Wang, J. Z., Zhang, C. H., Li, X., Xu, X., Dong, X. B., et al. (2015). Changes of flavor compounds of hydrolyzed chicken bone extracts during Maillard reaction. *Journal of Food Science, 79*(12), C2415–C2426.

Tian, X., Wang, J., & Cui, S. (2013). Analysis of pork adulteration in minced mutton using electronic nose of metal oxide sensors. *Journal of Food Engineering, 119*(4), 744–749.

Xu, X., You, M., Song, H., Gong, L., & Pan, W. (2018). Investigation of umami and kokumi taste-active components in bovine bone marrow extract produced during enzymatic hydrolysis and Maillard reaction. *International Journal of Food Science and Technology, 53*(11), 2465–2481.

Yang, Y., Sun, Y., Pan, D., Wang, Y., & Cao, J. (2018). Effects of high pressure treatment on lipolysis-oxidation and volatiles of marinated pork meat in soy sauce. *Meat Science, 145*, 186–194.

Yang, W. J., Yu, J., Pei, F., Mariga, A. M., & Hu, Q. (2016). Effect of hot air drying on volatile compounds of Flammulina velutipes detected by HS-SPME-GC-MS and electronic nose. *Food Chemistry, 196*(1), 860–866.

Yue, J., Zhang, Y., Jin, Y., Deng, Y., & Zhao, Y. (2016). Impact of high hydrostatic pressure on non-volatile and volatile compounds of squid muscles. *Food Chemistry, 194*, 12–19.

Zhao, J., Wang, M., Xie, J. C., Zhao, M. Y., Hou, L., Liang, J. J., et al. (2017). Volatile flavor constituents in the pork broth of black-pig. *Food Chemistry, 226*, 51–60.

Zou, Y., Kang, D., Liu, R., Qi, J., Zhou, G., & Zhang, W. (2018). Effects of ultrasonic assisted cooking on the chemical profiles of taste and flavor of spiced beef. *Ultrasonics Sonochemistry, 46*, 36–45.