



Evaluation of the flavor profiles of Yanbian-style sauced beef from differently treated raw beef samples

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ABSTRACT

In this study, we investigated the volatile flavor compounds and sensory perceptions of Yanbian-style sauced beef prepared from raw meats subjected to different treatments (hot fresh, chilled, and frozen beef). The results indicated that the treatment of raw beef significantly impacted the quality and flavor of sauced beef. Sauced chilled beef (CRSB) exhibited the highest content of fatty acids and total amino acids. A total of 48 volatile compounds were identified. Moreover, a relative odor activity value analysis identified hexanal, nonanal, heptanal, 1-octen-3-ol, and 2,3-octanedione as the characteristic flavor compounds in Yanbian-style sauced beef. The sensory evaluation demonstrated that CRSB was the most palatable and flavorful. Additionally, correlation loading plot analysis indicated strong correlations between sensory evaluation, fatty acids, amino acids, and volatile flavor compounds. These results suggest that chilled beef meat is the best raw material for the production of Yanbian-style sauced beef.

1. Introduction

Sauced meat products, which are traditional Chinese foods, utilize animal and poultry meat as the main raw materials (Zhu et al., 2021). Seasonings or spices are added to the meat following precooking, soaking, cooking, brining, and other processes to obtain the final product. Sauced meat products contain various ingredients and flavors, contributing to a complex taste profile. Notably, sauced meat products have different flavor characteristics due to differences in consumption habits, location-specific ingredients, and processing methods. Yanbian-style sauced beef is a traditional beef product popular in Jilin Province, China. Unlike other regional variations of sauced beef, Yanbian-style sauced beef incorporates vegetables, such as chili peppers, enriching its flavor and taste, which is preferred by locals. The main process steps of Yanbian-style sauced beef involve precooking, seasoning application, simmering over low heat, and finally, the addition of chili peppers.

Fresh meat, as utilized for Yanbian-style sauced beef in China, encompasses hot, chilled, and frozen meat based on market and consumption practices. Hot fresh beef refers to meat processed through the

traditional method of “early morning slaughtered and early morning marketed.” This meat undergoes slaughter without chilling treatment and can be immediately marketed following health inspection clearance (Zhou, 2008). According to traditional Chinese beliefs, consumers perceive hot fresh meat as being fresh, vibrant, and retaining its original flavor (R. Liu, Xing, Zhou, & Zhang, 2017). However, it possesses drawbacks, such as susceptibility to microbial growth and a lack of tenderness (Xiong, 2023). Chilled beef, also referred to as cooled or sour meat, undergoes pre- and post-slaughter inspection and quarantine. Subsequently, the carcass undergoes rapid cooling to ensure that the internal temperature of the muscle/raw meat reaches and remains between 0 and 4 °C for 24 h, maintaining optimal conditions for subsequent processing, circulation, and sale. Chilled meat is more tender and flavorful than hot fresh meat, owing to glycolysis and other biochemical processes occurring during the chilling process (Jeong et al., 2010). Freezing is a common method for preserving meat; at low temperatures, 80% of the water within the meat undergoes crystallization, resulting in the meat remaining frozen, thus largely inhibiting microbial growth (Archer, 2004). However, the formation of ice crystals during freezing may disrupt cell membranes in the meat, leading to the loss of juices

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upon thawing and consequent alterations in their nutritional and flavor characteristics (Archer, 2004).

The processing methods of meat products are mainly divided into thermal processing and non-thermal approaches, each contributing distinctively to flavor formation. Flavor development in meat subjected to thermal processing, such as steaming or baking, arises from interactions among precursor molecules in raw meat. These interactions involve various biochemical reactions, including pyrolysis of peptides and amino acids, degradation of ribonucleotides and sugars, Maillard reaction, Strecker degradation, lipid oxidation, and degradation of thiamine and lipids (Spanier et al., 2004). In contrast, non-thermal processes, such as wet and dry aging, predominantly entail various biochemical and structural alterations of proteins and lipids by microorganisms, enzymes, and other factors (Wojtasik-Kalinowska et al., 2023). Various types of fresh meat exhibit distinct physicochemical properties, which in turn influence the flavor and characteristics of processed meat products. Wang, Qin, Li, Xu, and Zhou (2019) investigated the textural and flavor differences of soft-boiled chicken derived from hot fresh and chilled chicken carcasses, reporting that boiled chicken from hot fresh carcasses exhibited elevated levels of 5'-inosinic acid, whereas soft-boiled chicken from chilled carcasses had a better texture. Moreover, Xiao et al. (2020) explored the effects of postmortem time (ranging from 1 h to 7 d) on the tenderness, flavor, texture, heterocyclic aromatic amines content, and sensory perception of roasted lamb, revealing higher compound concentrations at 3 days postmortem than those at other time points. Furthermore, the concentration of heterocyclic aromatic amines in roasted lamb was higher at 5 days postmortem than that at other time points. These data clearly demonstrate a significant influence of raw meat type on the characteristics of meat products.

In recent years, gas chromatography–mass spectrometry (GC–MS) and electronic noses have gained attention as methods for meat analysis, owing to their rapid, simple, and non-destructive nature. They are widely utilized for flavor detection, spoilage monitoring, meat type differentiation, and other applications (GÓrska-Horzyczak et al., 2016). The electronic tongue, a device that can simulate human taste perception, enables the detection of soluble and non-volatile compounds and is commonly used in scenarios where sensory monitoring is not feasible, such as determining adulterants in certain substances. The device offers rapid, objective, and user-friendly operation (Wang et al., 2022). Headspace solid-phase microextraction/GC–MS (HS–SPME/GC–MS) is a widely utilized technique for analyzing and detecting volatile organic compounds in food. It offers the advantages of a simple, rapid, and solvent-free extraction (Dong et al., 2019). During extraction, SPME extraction fibers are introduced into the headspace of the sampling bottle to adsorb volatile substances. Subsequently, the fibers are then inserted into the gas chromatography vaporization chamber for thermal analysis. The ensuing analysis is conducted using information from mass spectrometry libraries or standards (Zhang et al., 2023). However, there is limited information on the effects of different treatments on raw beef on the volatile flavor compounds in Yanbian-style sauced beef.

The present study aimed to evaluate the effects of three different treatments applied to raw beef samples on the flavor characteristics of Yanbian-style sauced beef using an electronic nose, electronic tongue, and HS–SPME/GC–MS analyses. This study provides important insights into findings enhancing the selection of raw meat for meat product preparation.

2. Materials and methods

2.1. Materials

Yanbian yellow cattle rumps (derived from adult bulls of similar age, body weight, and rearing conditions) were purchased from Baoren Slaughterhouse, Yanji, Jilin Province, China. Mongolian soy sauce,

sugar, dark soy sauce, sake, monosodium glutamate, pepper powder, sesame oil, and chili peppers were purchased from Long Mart supermarket, Yanji, Jilin Province, China.

2.2. Chemicals

Boron trifluoride-methanol solution, pyrogallol acid, and sodium hydroxide were purchased from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Methanol, sodium chloride, calcium chloride, sodium sulfate, anhydrous ethanol, ligroin, phenol, and sodium citrate were obtained from Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). HCl was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The hexane used for gas chromatography was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China).

2.3. Raw beef treatment and Yanbian-style sauced beef preparation

Each cow rump was divided evenly into three 1000 g portions and then subjected to storage under different time and temperature conditions. Sauced chilled beef (CRSB), sauced hot fresh beef (HRSB), and sauced frozen beef (FRSB) samples were processed as follows. For HRSB, the meat was stored at 20 °C for 6 h immediately after slaughter. For CRSB, the beef was pre-cooled, followed by packaging and refrigeration at 4 °C for 3 d. For FRSB, the beef was refrigerated at 4 °C for 3 d before being transferred to a freezer at –18 °C for 14 d. Frozen beef was thawed in a refrigerator at 4 °C 1 d before cooking.

The preparation of sauced beef involves cutting cleaned beef into large 5 cm³ pieces (approximately eight pieces per group), followed by precooking in boiling water for 5 min. Subsequently, the meat was removed and rinsed with warm water. Each pot was then filled with 1 L of warm water, into which the pre-cooked meat and spices (240 g Mongolian soy sauce, 80 g sugar, 12 g sake, and 56 g dark soy sauce) were added. The mixture was boiled for 50 min, and chili peppers (140 g) were then added and cooked for an additional 5 min. Finally, monosodium glutamate (8 g), sesame oil (1 g), and pepper powder (2 g) were added.

2.4. Determination of fatty acids and amino acids

2.4.1. Fatty acids

The samples, each weighing 2.00 ± 0.01 g, were finely chopped and combined with 40 mL chloroform/methanol solution (2:1, v/v). Homogenization was performed using a homogenizer (F6/10, Shanghai Jingxin Industrial Development Co., Ltd., China; 145 W/60 Hz) for 60 s, followed by a 60 min waiting period, after which the mixture was filtered. To this mixture, 8.8 mL of a mixed solution containing 7.39 g/L NaCl and 0.5 g/L CaCl₂ was added. Centrifugation was conducted at 4 °C and 805g for 15 min, and the upper liquid was discarded before transferring the remaining liquid to a flat-bottomed flask. The crude fat was obtained through drying under a rotational evaporator at 40 °C.

To convert the lipids into fatty acid methyl esters (FAMES), 8 mL of 0.5 M methanol sodium hydroxide solution was added to the flask, followed by condensation and refluxing at 80 °C for 10 min. Subsequently, 8 mL of a 15% boron trifluoride-methanol solution was added, and the mixture was heated in a water bath for 2 min before being promptly cooled to room temperature. Next, 20 mL of hexane was added to the flask, shaken for 2 min, and 10 mL of saturated aqueous sodium chloride solution was then added, allowing for layering. The upper liquid was collected, and 4 g of anhydrous sodium sulfate was added. After shaking for 1 min, the sample was allowed to stand for 5 min, and the upper solution was collected into a sampling bottle.

Fatty acid content was analyzed using gas chromatography (GC) using the Thermo Trace 1300 gas chromatograph (Thermo Fisher Scientific, Waltham, MA, USA), equipped with a flame ionization detector. Helium was used as the carrier gas at a 1 mL/min flow rate with 100:1

shunt sampling. The detector injection port temperature was adjusted to 260 °C for each sample testing. For detection, the temperature was set at 280 °C, and each sample was passed through a polyethylene glycol capillary column (SP-2560, 100 m × 0.25 mm × 0.2 μm). The initial GC oven temperature was set to 170 °C, maintained at 220 °C after 30 min, and increased to 4 °C/min for 15 min. After the 15-min temperature increase, the oven temperature was set to 240 °C for an additional 20 min. Fatty acid types in the sample were identified by comparing peak retention times with standard fatty acid samples, and their contents were determined using the corresponding peak areas. The final results were expressed in mg/100 g.

2.4.2. Amino acids

Amino acids were analyzed as described by Eilertsen et al. (2012) with modifications. Briefly, approximately 1 g sample was mixed with 0.2 mL phenol and 10 mL 6 M HCl and hydrolyzed at 110 °C for 24 h. The resulting hydrolysates were dried under nitrogen and dissolved in a sodium citrate buffer (pH 2.2), filtered through a 0.22 μm membrane filter, and subjected to analysis using an amino acid autoanalyzer (L-8900, Hitachi Co., Ltd., Tokyo, Japan). Amino acid composition within the sample was determined by referencing the peak retention time of standard samples. The amino acid content was determined by calculating the corresponding peak area and is expressed in mg/100 g.

2.5. Electronic nose (E-nose) and electronic tongue (E-tongue) analysis

2.5.1. E-nose

A 5.0 g meat sample was accurately weighed, placed in a headspace vial for the E-nose, and capped. The gas was equilibrated at 40 °C for 30 min. After calibrating the E-nose detector (PEN-3; Airsense Co., Ltd., Schwerin, Germany), the prepared sample vial was placed at the appropriate position on the tray for testing. The cleaning and measurement times were 60 and 90 s, respectively. The gas and injection flow rates were set at 100 mL/min. Each set of samples was assessed three times in parallel, and signal values were recorded at the 90th s. The results were analyzed using the Winmuster software (version 1.6.2., copyright Airsense Analytics GmbH). Details of the substances sensitive to the E-nose sensor are presented in Table S1.

2.5.2. E-tongue

For the E-tongue analysis, 10 mg of crushed meat samples were weighed into a centrifuge tube, 90 mL of deionized water was added, and the mixture was homogenized for 1 min. The mixture was then centrifuged at 4 °C and 2236g for 10 min and filtered through three layers of gauze. The resulting filtrate was transferred to a designated cup for analysis using the E-tongue (SA-402B, Insent Co. Seattle, WA, USA). The sensor array comprises six taste sensors, namely AEE (umami), CTO (salty), COO (bitter), CAO (sour), AE1 (astringent), and GL1 (sweetness), along with two reference electrodes. The test procedure involved three cleaning cycles (90s, 120 s, 120 s), a self-check (30s), a sample test (30s), two additional cleaning cycles (3 s, 3 s), and an aftertaste test (30s).

2.6. Volatile flavor compounds

2.6.1. Volatile compounds

Volatile flavor compounds were extracted using headspace solid-phase microextraction. Each sample (2.0 g) was accurately weighed in a special headspace, followed by equilibration at 20 °C for 15 min. The sample was extracted using a 65 μm PDMS/DVB fiber head in a sand bath at 60 °C for 30 min. The fiber was then rapidly inserted into the GC inlet and thermally resolved in a non-split mode for 2.5 min, followed by GC-MS analysis.

The GC conditions were as follows: column: DB-5MS, 30.0 m × 0.25 mm × 0.25 μm; inlet temperature: 250 °C; carrier gas: helium; column flow rate: 1.0 mL/min; purge flow: 3.0 mL/min; splitting ratio: 50:1. The

ramp-up procedure was as follows: 40 °C for 10 min, followed by ramp to 200 °C at a ramp rate of 5 °C/min, and subsequently to 280 °C at a ramp rate of 20 °C/min to 280 °C, with a 5-min hold. The MS conditions were as follows: ion source temperature 200 °C, interface temperature 280 °C, solvent delay time 2 min, electron energy 70 eV, and scan mass range m/z 40–550. Tentative identification was based on mass spectra matching in the standard NIST08 library and retention indices (RI) reported in the literature.

2.6.2. Relative odor activity value

The relative odor activity value (ROAV) was used to analyze the contribution of each volatile compound to flavor. Based on the method described by Liu et al. (S. Liu, Zhang, Harlina, Zhou, & Peng, 2020), the ROAV of the volatile flavor compound with the greatest flavor contribution was set as $ROAV_{stan} = 100$, and that for other volatile compounds was calculated as follows:

$$ROAV_n \approx 100 \times \frac{C_{n\%}}{C_{stan\%}} \times \frac{T_{stan}}{T_n},$$

where $C_{n\%}$ and T_n are the relative content and thresholds of the other volatile compounds, respectively. $C_{stan\%}$ and T_{stan} are the relative content and threshold, respectively, of the volatile compound contributing the most to flavor.

2.7. Sensory evaluation

Sensory evaluation of the sauced beef was conducted by 20 trained evaluators (10 men and 10 women), and three sensory evaluations were conducted, with a consistent composition of the evaluation team for each evaluation. Prior to the sensory evaluation, all the evaluators participated in three 3-h training sessions. These sessions included exposure to numerous samples, comprehensive discussion and description of specific indicators of sensory evaluation, and formulation of descriptors according to the characteristics, finally reaching a consensus on the description of the sensory evaluation criteria, including flavor, juiciness, taste, muscle tissue condition, and color appearance. The sensory evaluation utilized a 20-score anchor, the specific details of which are listed in Table S2.

During the evaluation, evaluators scored the samples according to the established sensory evaluation criteria. The evaluation environment was quiet, odor-free, well-ventilated, and at an adequate distance from the sample preparation area to prevent any potential influence from odors. Each evaluator received a cup of water, a rating sheet, and facial tissues. The evaluators were required to gargle between evaluations of different samples, and no communication was allowed during the evaluation period. The rating sheet only included the scoring criteria, sample scores, and dates, without any personal information of the evaluators. All participants provided informed consent prior to this study, ensuring the protection of the rights and privacy of each participant.

2.8. Statistical analysis

All experiments were conducted in triplicate using identical formulas, methods, and techniques across different cattle specimens. Storage conditions (three different times and temperatures) and the process of sauced beef preparation were considered fixed effects, while the cattle were considered random effects. Each experiment was analyzed in triplicate, and the data are expressed as mean ± standard deviation. Statistical analyses were performed using a one-way analysis of variance (ANOVA) using SPSS software (version 22.0; IBM Inc., Armonk, NY, USA). Significant differences were determined using Duncan's multiple-range test. The level of significance was set at $P < 0.05$. Graphs were generated using Origin software (version 2023B; Originlab Co., Northampton, MA, USA). Correlations between sensory

evaluation, fatty acids, amino acids, and volatile flavor compounds were analyzed using Unscrambler X software (version 10.4, CAMO Software, Oslo, Norway).

3. Results and discussion

3.1. Fatty acids and amino acids

Fatty acids are important precursors of volatile flavor compounds, and their relative content and composition are closely related to the characteristic flavor profiles. The fatty acid composition and content of Yanbian-style sauced beef prepared using differently treated raw beef samples are shown in Table 1. As shown in Table 1, 16 fatty acids, including 7 saturated fatty acids (SFAs) and 9 unsaturated fatty acids (UFAs), were detected. Among the treatments, the highest total fatty acid content was observed in CRSB, whereas the lowest was observed in FRSB. Holman, Coombs, Morris, Bailes, and Hopkins (2018) reported higher SFA levels but lower UFA levels in frozen beef samples than in their unfrozen counterparts. In our study, the lower fatty acid content observed in FRSB may be attributed to acid oxidation during freezing. This effect may be exacerbated by ice crystal formation during freezing and subsequent muscle cell rupture during thawing (Feng et al., 2022), as some fatty acids within cells undergo oxidation under enzymatic action and leak out during the thawing process, resulting in a decrease in their content (He et al., 2021). These findings are consistent with the study by Al-Dalali, Li, and Xu (2022b), reporting that during decompartmentalization occurring in the processes of meat freezing, thawing, and cooking, different redox enzymes, such as cytochromes, facilitate the oxidation of SFAs and monounsaturated fatty acids (MUFAs) into flavor-active compounds, such as aldehydes and alcohols, resulting in a reduction in the fatty acid content.

In the present study, the highest SFA content was noted in CRSB, reaching 48.79 mg/100 g, whereas the lowest was observed in FRSB, reaching 35.87 mg/100 g. Palmitic acid (C16:0) and stearic acid (C18:0) were the main components of SFAs in sauced beef, consistent with the findings by Shi et al. (2020). Notably, the difference in C16:0 and C18:0 content in each group exhibited the same trend as the difference in SFA content. Among SFAs, myristic acid (C14:0) is crucial for increasing cholesterol, whereas C16:0 tends to lower serum cholesterol levels (Sundram, Hayes, & Siru, 1994). The C16:0 content in all groups was

Table 1

Fatty acid composition and content of Yanbian-style sauced beef prepared from differently treated raw beef samples.

	Fatty acid content (mg/100 g)		
	HRSB	CRSB	FRSB
C4:0	1.52 ± 0.17 ^a	1.50 ± 0.04 ^a	1.47 ± 0.06 ^a
C14:0	1.82 ± 0.19 ^b	2.49 ± 0.40 ^a	1.75 ± 0.16 ^b
C14:1	0.47 ± 0.07 ^a	0.63 ± 0.14 ^a	0.66 ± 0.03 ^a
C15:0	0.45 ± 0.14 ^a	0.53 ± 0.03 ^a	0.46 ± 0.10 ^a
C16:0	20.44 ± 1.03 ^b	26.06 ± 1.10 ^a	19.75 ± 1.60 ^b
C16:1	2.18 ± 0.17 ^b	3.31 ± 0.48 ^a	2.61 ± 0.15 ^b
C17:0	0.67 ± 0.17 ^b	1.13 ± 0.31 ^a	0.59 ± 0.15 ^b
C17:1	0.76 ± 0.17 ^a	0.77 ± 0.23 ^a	ND
C18:0	12.50 ± 0.57 ^a	14.34 ± 1.39 ^a	10.46 ± 0.31 ^b
C18:1n9t	1.17 ± 0.11 ^b	1.70 ± 0.09 ^a	0.62 ± 0.08 ^c
C18:1n9c	23.24 ± 0.87 ^b	32.65 ± 1.15 ^a	18.96 ± 0.51 ^c
C18:2n6c	10.51 ± 0.61 ^a	10.33 ± 1.15 ^a	7.56 ± 0.40 ^b
C18:3n3	1.62 ± 0.10 ^a	1.85 ± 0.20 ^a	1.70 ± 0.33 ^a
C20:3n6	1.15 ± 0.08 ^{bc}	1.50 ± 0.07 ^a	1.15 ± 0.19 ^{bc}
C20:4n6	5.21 ± 0.11 ^a	4.86 ± 0.40 ^a	3.77 ± 0.64 ^b
C24:0	1.28 ± 0.16 ^b	2.74 ± 0.93 ^a	1.58 ± 0.17 ^b
∑SFA	37.81 ± 1.59 ^b	48.79 ± 0.05 ^a	35.87 ± 0.16 ^c
∑MUFA	27.02 ± 18.48 ^b	38.49 ± 1.15 ^a	22.85 ± 0.55 ^c
∑PUFA	18.81 ± 0.50 ^a	18.88 ± 0.74 ^a	14.18 ± 1.34 ^b

HRSB: sauced hot fresh beef; CRSB: sauced chilled beef; FRSB: sauced frozen beef. Results are expressed as mean ± standard deviation. Superscript letters (a–c) depict significant differences within the same row ($P < 0.05$).

significantly higher than that of C14:0 ($P < 0.05$), indicating the superior nutritional profile of Yanbian-style sauced beef.

MUFAs can reduce the content of saturated fats and cholesterol in the body, effectively preventing cardiovascular diseases, such as coronary heart disease (Mente, de Koning, Shannon, & Anand, 2009; Skeaff & Miller, 2009). As shown in Table 1, oleic acid (C18:1n9c) was detected as the primary MUFA in all groups. Oleic acid can significantly reduce the contents of low-density lipoproteins and triglycerides, prevent atherosclerosis, and promote the absorption of other fatty acids (Minguet et al., 2022). In CRSB, the C18:1n9c content was 32.65 mg/100 g, significantly higher than that in other groups ($P < 0.05$).

Polyunsaturated fatty acids (PUFAs) play important roles in stabilizing cell membrane functions, regulating gene expression, maintaining cytokine and lipoprotein homeostasis, and promoting growth and development (Lopez-Huertas, 2010; Scollan et al., 2014). In our study, relatively high levels of linoleic acid (C18:2n6c) and arachidonic acid (C20:4n6), of which linoleic acid is an essential fatty acid, were detected in all groups. The difference in the PUFA content exhibited the same trend as the difference in the C18:2n6c content, with CRSB exhibiting significantly higher PUFA levels than the other two groups ($P < 0.05$).

Amino acids play a pivotal role in contributing to the taste and flavor of meat and are produced through the generation of volatile compounds via the Maillard reaction and Strecker degradation (Spanier et al., 2004). The amino acid content in the Yanbian-style sauced beef prepared from differently treated raw beef samples is presented in Table 2. Meat products with higher concentrations of amino acids provide more substrates for the Maillard reaction, thereby enhancing the flavor (C. C. Cao et al., 2017). Among the samples, CRSB had the highest total amino acid content of 457.77 mg/100 g, whereas the lowest content of 383.71 mg/100 g was observed in FRSB. The differences in total umami, sweet, and bitter amino acid levels observed among the groups followed the same pattern as those in total amino acid levels.

Table 2

The amino acid composition and content of Yanbian-style sauced beef processed from differently treated raw beef samples.

Amino acid species	Amino acid content (mg/100 g)		
	HRSB	CRSB	FRSB
Asp	39.54 ± 1.22 ^b	46.81 ± 0.31 ^a	39.26 ± 1.04 ^b
Glu	73.05 ± 1.53 ^b	83.98 ± 1.70 ^a	72.17 ± 0.88 ^b
∑UAAs	112.59 ± 2.69 ^b	130.79 ± 1.39	111.43 ± 1.92 ^b
*Thr	18.73 ± 0.69 ^b	21.43 ± 1.04 ^a	18.09 ± 0.19 ^b
Ser	18.81 ± 0.51 ^b	21.13 ± 0.23 ^a	17.91 ± 0.65 ^b
Gly	20.23 ± 1.31 ^{ab}	22.19 ± 0.43 ^a	18.67 ± 1.61 ^b
Ala	26.10 ± 1.02 ^b	28.94 ± 0.36 ^a	24.96 ± 1.40 ^b
Pro	20.05 ± 7.95 ^a	22.55 ± 8.90 ^a	20.87 ± 7.23 ^a
∑SAAs	103.93 ± 9.70 ^a	116.24 ± 10.16 ^a	100.51 ± 10.70 ^a
*Val	12.61 ± 2.85 ^a	14.70 ± 1.90 ^a	11.97 ± 1.82 ^a
*Iso	11.71 ± 1.04 ^a	14.08 ± 2.01 ^a	11.37 ± 0.94 ^a
*Leu	32.49 ± 0.41 ^b	37.92 ± 1.28 ^a	29.81 ± 1.16 ^c
Tyr	15.22 ± 2.05 ^a	17.52 ± 3.15 ^a	13.79 ± 0.98 ^a
*Phe	18.30 ± 1.49 ^{ab}	21.07 ± 2.24 ^a	17.38 ± 0.86 ^b
*Lys	35.79 ± 2.94 ^b	41.60 ± 1.88 ^a	34.75 ± 3.32 ^b
His	13.01 ± 1.24 ^a	15.06 ± 2.13 ^a	12.19 ± 2.22 ^a
Arg	29.49 ± 2.72 ^a	34.23 ± 4.30 ^a	28.55 ± 1.85 ^a
∑BAAs	168.64 ± 8.23 ^b	196.18 ± 6.81 ^a	159.80 ± 7.98 ^b
Cys	1.56 ± 0.22 ^a	1.78 ± 0.25 ^a	1.54 ± 0.12 ^a
*Met	10.72 ± 0.31 ^b	12.79 ± 0.29 ^a	10.44 ± 0.35 ^b
∑TAAs	397.44 ± 9.08 ^b	457.77 ± 10.60 ^a	383.71 ± 15.85 ^b
∑EAAs	140.37 ± 3.30 ^b	163.58 ± 8.15 ^a	133.80 ± 7.12 ^b
∑NEAAs	257.08 ± 11.38 ^b	294.18 ± 7.57 ^a	249.90 ± 15.76 ^b
EAAs/TAAs (%)	35.35 ± 1.53 ^a	35.73 ± 1.74 ^a	34.93 ± 1.38 ^a
EAAs/NEAAs (%)	54.76 ± 3.60 ^a	55.71 ± 4.13 ^a	53.74 ± 3.21 ^a

Results are expressed as mean ± standard deviation. Superscript letters (a–c) depict significant differences within the same row ($P < 0.05$).

UAAs: umami amino acids; SAAs: sweet amino acids; BAAs: bitter amino acids; TAAs: Total amino acids; EAAs: Total essential amino acids; NEAAs: Total non-essential amino acids.

* Indicates essential amino acids.

The major amino acids in the Yanbian-style sauced beef were Glu, Asp, Lys, Leu, Arg, and Ala. Glu was the most abundant amino acid, accounting for approximately 19% of the total amino acids. It is the major amino acid contributing to the intensity of the umami taste (Yang et al., 2020). Overall, these results are consistent with prior research; variations in amino acid levels can be attributed to differences in the methods used to prepare the sauced beef (Gatellier, Kondjoyan, Portanguen, & Santé-Lhoutellier, 2010; Wang et al., 2019) and the addition of soy sauce (Kamal et al., 2016).

Cystine and methionine, two sulfur-containing amino acids, are considered non-flavor-presenting amino acids; however, they are important contributors to the flavor formation of cooked meat via the Maillard reaction (Mottram, 1998). The methionine content was the highest in CRSB at 12.79 mg/100 g, whereas the cystine content did not differ significantly among the groups ($P > 0.05$).

The essential amino acids (EAAs) of the Yanbian-style sauced beef prepared from differently treated raw beef samples differed significantly ($P < 0.05$), with the highest and lowest contents of 163.58 mg/100 g and 130.75 mg/100 g observed in CRSB and FRBSB, respectively. The variations in non-essential amino acid content in the three groups correlated with those observed in EAA content, with the highest non-essential amino acid content (294.18 mg/100 g) observed in CRSB.

The ratio of EAAs to total amino acids (EAAs/TAAs) across the samples ranged from 33.99% to 35.78%, which was close to the FAO/WHO recommended value of 40%, suggesting the presence of high-quality protein (Vinayashree & Vasu, 2021). Although CRSB exhibited the highest EAAs/TAAs ratio of 35.55%, there was no significant difference among the groups ($P > 0.05$).

3.2. E-nose and E-tongue

The E-nose is primarily used to identify gaseous molecules using odor sensors. The differences in the E-nose signal response data for Yanbian-style sauced beef processed from differently treated raw beef samples are shown in Fig. 1A. Overall, the abundance of odor compounds was higher in the CRSB group than in the other two groups; sensors R2, R6, R7, R8, and R9 exhibited different degrees of response. This result underscores the significant influence of raw beef treatment on the odor profile of Yanbian-style sauced beef, with nitrogen oxides, sulfides, alkanes, alcohols, aldehydes, ketones, and aromatic compounds being the main components causing these odor differences. However, the specific volatile flavor substances remain unknown and require further investigation.

The principal component analysis loading plot of the E-nose response data demonstrated that PC1 and PC2 contributed 90.1% and 7.2% of the total variance, respectively, indicating that the principal components

could reflect all the characteristics of volatile odors in Yanbian-style sauced beef from different raw beef samples (Fig. 1B). The differences among the samples were mainly in PC1, and the data points of the three groups were distinct, indicating complete separation by the E-nose and that each group exhibited its distinct aroma region.

The E-tongue analysis effectively delineated differences in taste among the groups. The differences in the E-tongue response signal data of the different groups of Yanbian-style sauced beef are presented in Fig. 2. Although the response intensity of taste was essentially the same in all groups, there were minor differences among the three sensors for saltiness, astringency, and sourness. Notably, the response intensity of the saltiness sensor was the highest.

3.3. Volatile flavor compounds

Non-volatile water-soluble precursors and lipids form volatile compounds through various reactions, including thermal degradation and lipid oxidation (Gardner & Legako, 2018). The composition of volatile flavor compounds in the Yanbian-style sauced beef prepared from differently treated raw beef samples is shown in Table 3. A total of 48 volatile flavor compounds, namely 11 aldehydes, 7 alcohols, 2 ethers, 3 acids, 1 ketone, and 24 hydrocarbons, were isolated and identified from the sauced beef samples. The compounds resulting from lipid oxidation and fatty acid degradation mainly include aldehydes, alcohols, ketones,

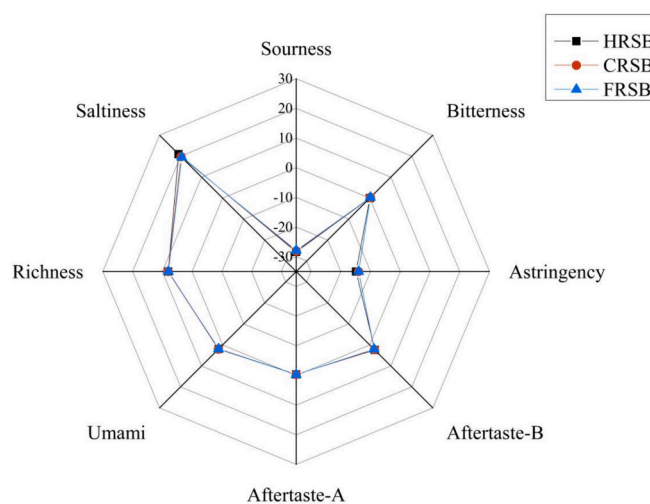


Fig. 2. Radar diagram of the electronic tongue response data of Yanbian-style sauced beef processed from differently treated raw beef samples.

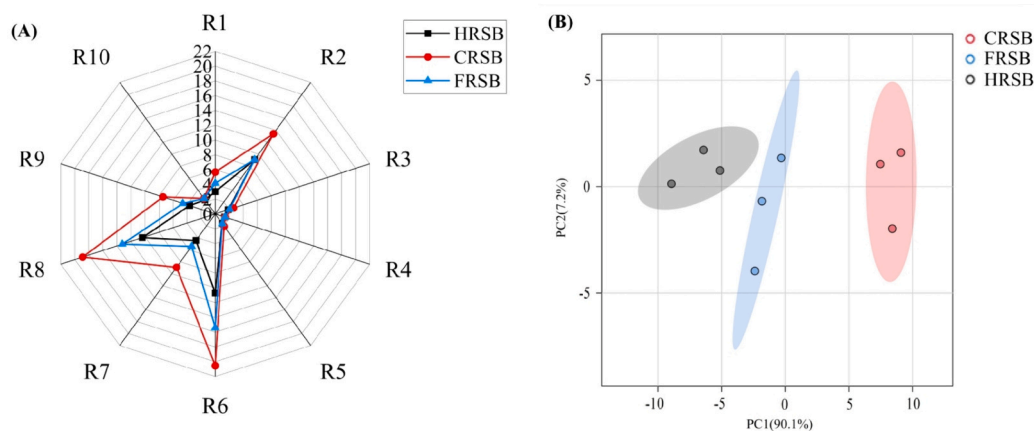


Fig. 1. Radar diagram of the electronic nose (E-nose) response data (A) and principal component analysis loading plot (B) of the E-nose response data of Yanbian-style sauced beef processed with differently treated raw beef samples.

Table 3

The volatile flavor composition of Yanbian-style sauced beef prepared from differently treated raw beef samples.

Substance name	CAS	RI	Relative peak area (%)		
			Hot fresh	Chilled	Frozen
<i>Aldehydes</i>					
Hexanal	66-25-1	806	21.87 ± 18.39 ^a	4.20 ± 3.61 ^c	12.86 ± 8.69 ^b
Nonanal	124-19-6	1104	5.96 ± 1.20 ^a	3.93 ± 0.22 ^a	5.91 ± 1.38 ^a
Heptanal	111-71-7	905	1.70 ± 0.04 ^a	0.76 ± 0.18 ^b	1.24 ± 0.46 ^{ab}
Benzaldehyde	100-52-7	982	ND	1.01 ± 0.23 ^a	0.90 ± 0.27 ^a
Octanal	124-13-0	1005	2.10 ± 0.33 ^b	ND	4.01 ± 0.91 ^a
Tetradecanal	124-25-4	1601	1.84 ± 0.30 ^a	2.47 ± 1.50 ^a	2.08 ± 1.47 ^a
Decanal	112-31-2	1204	ND	0.49 ± 0.04 ^a	0.53 ± 0.09 ^a
5-Methylfuranal	620-02-0	963	ND	0.90 ± 0.15 ^a	1.01 ± 0.19 ^a
Stearaldehyde	638-66-4	1999	ND	0.87 ± 0.79	ND
Tridecanal	10,486-19-8	1502	ND	ND	0.63 ± 0.07
Dodecanal	112-54-9	1402	ND	ND	0.78 ± 0.69
<i>Alcohols</i>					
1-Octen-3-ol;	4312-99-6	943	8.68 ± 0.79 ^a	0.48 ± 0.01 ^b	0.62 ± 0.24 ^b
2-Ethyl-1-hexanol	104-76-7	995	0.91 ± 0.06	ND	ND
Linalool	78-70-6	1101	ND	1.03 ± 0.15 ^a	0.45 ± 0.02 ^b
2-Propyl-1-heptanol	10,042-59-8	1194	ND	ND	0.55 ± 0.24
Dihydrolinalool	18,479-51-1	1490	ND	ND	1.95 ± 0.23
<i>Ethers</i>					
Diallyl disulfide	2179-57-9	1117	ND	ND	0.58 ± 0.15
Vinyl isopropyl ether	926-65-8	52	ND	ND	1.34 ± 0.89
<i>Acids</i>					
L(+)-Lactic acid	79-33-4	838	1.62 ± 0.76	ND	ND
Palmitic acid	57-10-3	1968	ND	1.46 ± 0.68	ND
Nonanoic acid	122-05-0	1235	ND	ND	0.41 ± 0.03
<i>Ketones</i>					
2,3-Octanedione	585-25-1	1088	2.67 ± 0.98 ^a	0.82 ± 0.23 ^b	1.60 ± 0.95 ^{ab}
<i>Esters</i>					
Diisobutyl phthalate	84-69-5	1908	ND	ND	0.58 ± 0.03
Ptalic acid, butyl octyl ester	84-78-6	2434	ND	ND	0.96 ± 0.41
<i>Alkanes</i>					
Hexadecane	544-76-3	1612	1.20 ± 0.48 ^a	0.60 ± 0.15 ^b	0.60 ± 0.20 ^b
3-Methylundecane	1002-43-3	1150	ND	1.06 ± 0.04 ^a	1.12 ± 0.45 ^a
2,2,4,6,6-Pentamethylheptane	13,475-82-6	981	2.57 ± 0.55 ^b	9.70 ± 2.17 ^a	10.63 ± 1.63 ^a
n-Dodecane	112-40-3	1214	3.98 ± 0.81 ^a	2.21 ± 0.81 ^a	ND
2,6-Dimethylundecane	17,301-23-4	1185	ND	0.53 ± 0.14 ^a	0.55 ± 0.10 ^a
2-Bromododecane	13,187-99-0	1446	ND	0.54 ± 0.11 ^a	0.61 ± 0.17 ^a
(D)-limonene	5989-27-5	1018	ND	9.26 ± 2.41 ^a	4.31 ± 0.19 ^b

Table 3 (continued)

Substance name	CAS	RI	Relative peak area (%)		
			Hot fresh	Chilled	Frozen
Tridecane	629-50-5	1313	ND	0.50 ± 0.01 ^a	0.48 ± 0.02 ^a
Tetradecane	629-59-4	1413	ND	0.55 ± 0.06	ND
Isocaryophyllene	118-65-0	1425	1.21 ± 0.19 ^b	ND	4.67 ± 1.25 ^a
Heptane	142-82-5	717	3.64 ± 2.16 ^a	ND	1.33 ± 0.48 ^b
3-Methyldecane	6418-41-3	1349	ND	ND	0.66 ± 0.17
3,5,5-Trimethyl-1-hexene	4316-65-8	757	ND	0.83 ± 0.14	ND
3-Methylene-undecane	1002-43-3	1150	ND	0.31 ± 0.01 ^a	0.78 ± 0.59 ^a
2-Methyldecane	6975-98-0	1051	ND	ND	0.70 ± 0.13
3,4,5,6-Tetramethyloctane	62,185-21-1	958	ND	0.47 ± 0.05	ND
Terpinolene	586-62-9	1084	ND	0.67 ± 0.08	ND
δ-Elementene	20,307-84-0	1329	ND	0.70 ± 0.11	ND
β-Pinene	127-91-3	943	ND	1.87 ± 0.47	ND
n-Pentadecane	629-62-9	1512	ND	ND	0.55 ± 0.14
α-Humulene	6753-98-6	1453	ND	0.66 ± 0.11	ND
3,7-Dimethyl-1,3,6-octatriene	13,877-91-3	1040	ND	11.53 ± 3.19	ND
(+)-α-pinene	80-56-8	948	ND	0.59 ± 0.15	ND
3-Isopropenyl-5,5-dimethyl cyclopentene	-	1068	ND	ND	4.26 ± 0.71

Results are expressed as mean ± standard deviation.

Superscript letters (a–c): significant differences within the same row (P < 0.05). ND: the substance was not detected.

–: not detected.

RI: retention index.

hydrocarbons, and furans. In contrast, those produced through the Maillard reaction mainly include sulfur- and nitrogen-containing compounds (Ba, Park, Dashmaa, & Hwang, 2014). In the present study, individual electronic nose response values did not correspond to the content of volatile flavor compounds, owing to variances in detection thresholds for each substance, requiring further analysis in conjunction with ROAV. A total of 14, 30, and 34 volatile flavor compounds were detected in HRSB, CRSB, and FRSB, respectively, indicating substantial differences in the volatile flavor compounds among the groups.

The relative aldehyde content was higher in HRSB, the relative alkane content was higher and more diverse in CRSB, and the relative alcohol content was higher in FRSB. Aldehydes are the main degradation products of lipid oxidation, and some are also partially produced via Maillard or Strecker reactions. Given their low threshold content, aldehydes are abundant in sauced beef and serve as important aroma compounds (Insausti, Beriain, Lizaso, Carr, & Purroy, 2008). Among them, aldehydes with high content include hexanal, nonanal, heptanal, octanal, and tetradecanal, which have grassy, beefy, fatty, and citrus aromas, respectively. In the present study, while the relative aldehyde content was higher in HRSB than in CRSB or FRSB, FRSB contained the most diverse array of aldehydes.

Alcohols are primarily derived from the heat oxidation of PUFAs (Zhang, Qin, Lin, Shen, & Saleh, 2015) and may be oxidized to aldehydes or participate in esterification reactions, contributing to variations in both the content and type of alcohols observed among the groups in the study. Although 7 alcohols were detected in the sauced beef samples, alcohols are usually considered to contribute less to aroma owing to

their high threshold values. Moreover, 1-often-3-ol, an important aromatic component of meat products (Zhou, 2008), was detected in all three groups in this study.

Ketones and hydrocarbons mostly originate from the oxidative decomposition of fats, branched alkanes may arise from the oxidation of branched fatty acids, and esters may be formed through the esterification reaction of alcohols and acids. Although ketones, acids, and esters were detected in sauced beef samples, their contribution to the aroma is deemed negligible owing to their high threshold and relatively low content. Notably, under the same analytical conditions, we observed significant differences in the types and relative content of volatile flavor compounds among the groups, indicating the significant influence of raw beef treatments on the production of flavor compounds in Yanbian-style sauced beef.

Human olfactory sensitivity to different compounds varies, and the lowest concentration at which a substance can be perceived is usually referred to as the detection threshold. At low concentrations, compounds with a low detection threshold are more likely to be perceived, whereas at high concentrations, compounds with higher thresholds are easier to detect or smell. To objectively evaluate aroma, the OAV index, which is the ratio between the concentration of individual compounds in a sample and their threshold concentrations (odor threshold value), is used to combine the compound concentration and detection threshold (D. Liu, Zhou, & Xu, 2008). However, given the complexity of samples often containing numerous volatile compounds, absolute quantification is challenging. Thus, the ROAV is commonly used to measure the contribution of different volatile compounds to flavor perception (S. Liu et al., 2020).

In the present study, CRSB exhibited the highest ROAV, indicative of the strongest odor. In general, compounds with ROAV ≥ 1 are considered key flavor compounds in a sample. In the HRSB, CRSB, and FRSB groups, 6, 11, and 11 volatile flavor compounds were detected, respectively, 5 of which, namely hexanal, nonanal, heptanal, 1-octen-3-ol, and 2,3-octanedione, were present in all three groups. These five substances are likely the characteristic volatile flavor compounds of Yanbian-style sauced beef. Sun, Zhang, and Song (2021) identified 8 key aroma compounds in boiled beef meatballs, including hexanal, linalool, and diallyl disulfide, α -Pinene, eugenol, 2-ethylhexyl acetate, 1-octene-3-ol, and anisole. Gong et al. (2017) identified 12 key aroma compounds in spicy stewed beef, namely 3-methylbutanal, hexanal, pentanal, heptanal, terpinene, limonene, 4-terpineol, linalool, octanal, (*E*)-anethole, and α -terpineol. Similar results were obtained in our study, particularly in the CRSB group, where substances such as (D)-limonene, (+)- α -pinene, linalool, and terpinolene were detected.

Notably, as the storage time of the raw beef increased, the fruit and vegetable flavors in the sauced beef increased. Nonanal, heptanal, and linalool have a citrus-like aroma, 1-Octen-3-ol has a mushroom-like aroma, and (D)-limonene has a carrot-like aroma, and the levels of these compounds were higher in the CRSB and FRSB groups than in the HRSB group. Furthermore, the lipidaceous composition of FRSB was more pronounced; the ROAVs of substances such as octanal and tridecanal were higher in the FRSB group than in the other two groups. This result may be attributed to fat oxidation resulting from prolonged storage. Notably, Al-Dalali et al. (2022b) identified hexanal, octanal, phenylacetaldehyde, 2-ethyl-1-hexanol, and 1-heptanol as biomarkers for frozen storage in raw beef samples, using partial least squares discriminant analysis.

3.4. Sensory evaluation

Fig. S1 illustrates the sensory evaluation results of HRSB, CRSB, and FRSB samples. CRSB exhibited the highest scores for taste and flavor, with significant differences observed ($P < 0.05$). potentially attributable to the total amino acid and volatile flavor compound content. HRSB exhibited a higher score for juiciness than that of the CRSB and FRSB groups. FRSB had a significantly lower score of muscle tissue condition

than that of the HRSB and CRSB groups. This result is likely attributed to the oxidation of proteins and mechanical damage occurring during frozen storage (R. Cao et al., 2023). No significant differences were observed in the color scores among the three groups ($P > 0.05$), indicating that different treatments of raw beef can influence the sensory attributes and overall fondness of the final product.

For Yanbian-style sauced beef, the raw materials contain seasonings with flavor or flavor precursors such as soy sauce and sugar, which can affect the overall flavor and contribute to the taste profile. In this study, we observed no significant differences in the results of the *E*-tongue analysis among the three groups, as seasonings played a dominant role in influencing the taste. However, for other analyses, seasoning did not significantly contribute to variation or had minimal impact, given the consistent addition of the same amount and type of seasoning and the use of identical production methods. In future studies, we will assess the effect of hot fresh, chilled, and frozen meat on the flavor characteristics of meat products without the incorporation of seasonings. Moreover, we will incorporate raw meat indicators into a comprehensive analysis.

Following data normalization, we used partial least squares discriminant analysis (PLS-DA) to reveal the correlations between sensory evaluation, fatty acids, amino acids, and volatile flavor compounds (Compounds in Table 4) in the Yanbian-style sauced beef prepared using differently treated raw beef samples (Fig. 3). Factor-1 explained 83% of the total variance, and factor-2 explained 12% of the total variance, indicating that the top two factors clearly determined the correlations among the variables. Most compounds were positively correlated with sensory properties. Among them, (D)-limonene was identified as having the strongest correlation with flavor properties. (D)-Limonene is a monocyclic monoterpene commonly used as a flavor and fragrance

Table 4

ROAVs and contribution of key odorant compounds to Yanbian-style sauced beef.

Odorant	Threshold value ($\mu\text{g}/\text{kg}$)	ROAV			Odor description
		HRSB	CRSB	FRSB	
Hexanal	2.4	100	100	100	green, grassy
Nonanal	2.8	23.359	80.204	39.391	citrus-like, soapy
Heptanal	2.8	6.663	15.510	8.265	citrus-like, fatty
Tetradecanal	67	0.301	2.107	0.579	fatty wax
1-Octen-3-ol	1.5	5.780	18.286	7.714	mushroom-like
2,3-octanedione	12	2.442	3.905	2.488	buttery
Benzaldehyde	41.7	ND	1.384	0.403	cherry, nutty
Octanal	0.8	28.807	ND	93.546	fatty
Decanal	5	ND	5.600	1.978	fatty
(D)-limonene	13	ND	40.703	6.187	carrot-like
3,7-dimethyl-1,3,6-octatriene	55	ND	11.979	ND	terpene-like
Linalool	24	ND	2.452	0.350	citrus-like,
Diallyl disulfide	1.3	ND	ND	8.326	garlic-like
Tridecanal	8	ND	ND	1.470	tallow-like, beef-like
Dodecanal	14	ND	ND	1.040	soapy
β -Pinene	180	ND	0.594	ND	terpene-like
(+)- α -pinene	58	ND	0.581	ND	needle-like, resin-like
Terpinolene	41	ND	0.934	ND	honey
α -Humulene	160.5	ND	0.236	ND	balsamic
Heptane	330	0.121	ND	ND	vinegar-like
2-Ethyl-1-hexanol	40	0.250	ND	ND	citrus-like
					ethereal, fruity

The threshold values of the compounds in water and odor descriptions were retrieved from Vcf Home (vcf-online.nl) and Leibniz-LSB (leibniz-lsb.de). ND indicates that the substance was not detected.

HRSB: sauced hot fresh beef; CRSB: sauced chilled beef; FRSB: sauced frozen beef; ROAV: relative odor activity value.

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