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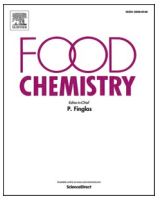

Study of the flavor dissipation mechanism of soy-sauce-marinated beef using flavor matrices

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ABSTRACT

Soy sauce-based marinade beef (SSMB) is a traditional Chinese cuisine with a unique flavor. However, pre-precooling and air-cooling tunnels are necessary industrial units in the cold chain for flavor dissipation. Sensory-directed flavor analysis was performed to identify the key aroma-active compounds in SSMB that had just completed (100 ℃), pre-cooled in the brine tanks (45 ℃), and exited the air-cooleded tunnel (10 ℃). We identified 110 aroma-active substances, of which 42 were quantified based on their high flavor dilution factors. Recombination and omission tests identified 29 odorants as the main aroma-active molecules. Additionally, the flavor matrix revealed the relationship between the aroma component expression and sensory attributes. Flavor substances derived from spices, such as eugenol, anethole, and linalool, are enriched during the pre-cooling stage. The different meat attributes of the three samples were primarily related to aldehydes generated from lipid oxidation.

1. Introduction

Soy sauce-based marinade beef (SSMB) is popular in China because of its unique aroma and taste. SSMB is produced by combining several spices with marinade sauce. After thawing and cleaning, beef is injected with marinade, mixed with brine, and in soy sauce-based marinade (composed of several spices such as clove, star anise, cinnamon, and bay leaves). Aroma is an important sensory attribute that determines beef quality and influences consumer satisfaction (Han, Zhang, Fauconnier, & Mi, 2020). The flavor developed during the SSMB formation process is quite complex, including flavor mixing of herbs and spices, the flavor formation during simmering, such as Strecker degradation, Lipid degradation, Maillard reaction and Lipid- Maillard interaction, and related reaction degree (Gong et al., 2017). Sun, Zhang, & Song (Sun, Zhang, & Song, 2020) showed that the critical aroma substances of boiled beef meatballs were hexanal, linalool, diallyl disulfide, α-pinene, eugenol, 2-ethyl hexyl acetate, 1-octene-3-ol, and anisole. In addition, 12 odorants were identified as the characteristic aroma compounds of spicy beef, including 3-methylbutanal, hexanal, pentanal, heptanal, terpinene, limonene, 4-terpinenol, linalool, octanal, (*E*)-anethole, and α-terpineol (Gong et al., 2017). However, recombination and omission experiments have not identified most odorants. The variation in odorants among cooked beef is related to the degradation of lipids rather than to the Maillard reaction, and moderately to lipid-Maillard interactions (Sohail et al., 2022). Notably, degradation of lipid substances containing 6–10 carbons, particularly aldehydes, frequently occurs in cooked meats with higher flavour dilution (FD) factors or odour activity values (OAVs)(Sohail et al., 2022). Most studies of aroma compound detection methods were focused on beef analysis (Scollan et al., 2014) and spice gravies (Liu et al., 2013), whereas the aroma of SSMB has not been widely evaluated.

In addition, SSMB processing involves two cooling stages: pre-cooling (100 °C → 45 °C) and air-cooling (45 °C → 10 °C). After pre-cooling in the brine tanks, the samples are transferred into an air-cooling tunnel. During air cooling, the central temperature of the SSMB drops from 45 °C to 10 °C within 90 min. Dissipation of aromatic compounds during processing, cooling, and cold-chain logistics is important. Flavor dissipation occurs due to covalent binding of flavor

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substances to proteins (Anantharamkrishnan & Reineccius, 2020) as well as volatilization during processing. As a result of flavor dissipation, product flavor quality can deteriorate, consumer acceptance can be reduced. Cold air sweeping in the air-cooling tunnel causes loss of the volatile flavor substances of SSMB (Qiang et al., 2022). Spices such as scallion and ginger, which contain phenolic compounds, can bind to the hydrophobic region of a protein, thus reducing the binding of nasty flavor substances like aldehyde and alcohol. This encourages the release of the nasty odor and is effective for eliminating the smell (Sun et al., 2020). The intense fragrance of spices is an important factor that enhances the flavor of beef. The aroma of anethole in star anise, the eugenol in clove and the cinnamic aldehyde in cinnamon enhance SSMB's flavor (Qiang et al., 2022). Volatility during processing affects whether consumers perceive it during consumption. We previously explored differences in aroma compounds in SSMB outside of the pot, before and after air-cooling and found that ketones, alcohols, phenolic ethers, and esters were likely lost and dissipate; these substances were mainly derived from spices (Qiang et al., 2022). Further research is required to investigate the regulation of flavor dissipation, such as the target-oriented flavor editing techniques. However, knowledge on the dissipation of key aromatic compounds in SSMB remains limited, making standard industrial production challenging. The aroma of SSMB and dissipation during the cooling process remain unclear. In this study, we (1) characterised the key aroma-active compounds in SSMB using sensory-directed flavor analysis, such as GC-O-MS, aroma extract dilution analysis (AEDA), odor activity value (OAV), and aroma recombination and omission experiment and (2) clarified the dissipation mechanism of key aroma-active compounds in SSMB during cooling. Our results provide a scientific perspective on flavor dissipation and can serve as a guide for flavor adjustments and additions during the chilling process, ensuring the high flavor quality of SSMB. This study also provides information for regulating and supplementing flavors during cooling, which may lead to improvements in the flavor of SSMB.

2. Materials and methods

2.1. Processing and sampling of SSMB

Three kinds of soy sauce marinated beef were purchased from Jiangsu Chaoyue Agricultural Development Co., Ltd (Taixing, China). The muscles (*semitendinosus*) were obtained from Brahman cattle (350–400 kg, aged 24 months). SSMB were produced on the production line including trimming, injecting marinating solution, tumbling, and stewing. The marinade fluid was made from a mixture of 12 spices (Sichuan pepper, *radix angelicae, pericarpium citri reticulatae*, star anise, cinnamon, amomum cardamomum, cumin, behenaphthalene, amomum tsao-ko, clove, pepper, bay leaves). SSMB just completed (100 °C, SSMB-100) was just finished stewing. Then, SSMB were pre-cooled to 45 °C in the brine tanks (SSMB-45). After that, SSMB were conveyed to an air-cooling tunnel. They were packed at 10 °C post the air-cooling tunnel (SSMB-10). After they were minced in 3 min, snap-frozen with liquid nitrogen, and stored at − 80 °C until analysis.

2.2. Chemicals

The investigation employed the following chemicals, all of which were acquired from Sinopharm Chemical Reagents Co., Ltd (Shanghai, China): methylene chloride, methanol, anhydrous sodium sulfate, and *n*-hexane (purity > 99 %). The subsequent authentic aroma standards were purchased from Sigma-Aldrich (Shanghai, China): hexanal (95 %), pentanal (98 %), octanal (99 %), heptanal (97 %), benzaldehyde (99.5 %), nonanal (99.5 %), (*E*)-2-decanal (97 %), cinnamyl acetaldehyde (95 %), 1-heptanol (97 %), (*E, E*)-2, 4-decadienal (96 %), (*E*)-2-undecenal (94 %), dimethyl trisulfide (95 %), 3-hydroxy-2-butanone (97 %), *p*-cresol (97 %), 3-methylthiopropanal (98 %), 2-acetylthiazole (95 %), 2-acetyl-1-pyrroline (98 %), 4-methyl-5-hydroxyethylthiazole (98 %), 1,8-cineole (98 %), ethyl maltol (97 %), methyleugenol (97 %), 2,6-dimethoxyphenol (98 %), decanoic acid (98 %), 5-methyl-4-hydroxy-3(2*H*)-furanone (97 %), and 1-octen-3-one (98 %). Meanwhile, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (97 %), linalool (97 %), 2,6-diethylpyrazine (97 %), 2-methyl-3-furanthiol (98 %), 2-acetylthiophene (98 %), anethole (98 %), anisaldehyde (97 %), α-pinene (97 %), coumarin (98 %), estragole (98 %), myristicin (97 %), γ-hexalactone (99 %), (*d*)-carvone (99 %), γ-terpinene (98 %), α-humulene (98 %), octanoic acid (98 %), and eugenol (98 %) were procured from TCI (Shanghai, China). For the count of LRIs, the *n*-alkanes ($C_7 - C_{30}$, ≥97 %) were purchased from o2si Smart Solutions (Shanghai, China). The internal standards, 2-methyl-3-heptanone (99 %) and 1,2-dichlorobenzene (99 %), were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Beijing Millennium Capital Gas Co. LTD (Beijing, China) supplied liquid nitrogen (99.999 % purity), ultrahigh-quality helium (99.9992 %), and nitrogen (99.999 % purity).

2.3. Aroma profile evaluation (APE)

APE was executed following the previous method. (Yang, Song, Wang, & Jing, 2019). Discussion among the panelists provided aroma descriptors, including spicy, caramel-like, woody, herbal, meaty, soy sauce-like. Based on the aroma of each reference at concentrations 100 times higher than their odor thresholds, they were identified as follows: γ-terpinene (woody); 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (caramel-like); estragole (herbal). The soy sauce-like aroma was indicated by 3.0 g of soy sauce in 50.0 mL of water. The spicy aroma was indicated by 10 g of star anise. The meaty characteristic referred to the aroma of 50 g boiled beef in water. The panelists were kept in the dark about the specifics of each sample by marking the sample bottles with three-digit random numbers. At each sample, each panelist evaluated in triplicate.

2.4. Isolation of the aroma compounds by solvent-assisted flavor evaporation (SAFE)

SSMB-100, SSMB-45, and SSMB-10 were independently crushed by an FSJ-A03D1 grinder (Bear Electric Appliance Co., LTD, Shunde, China) (5 s each time, 5 times). The temperature increase in the beef core was stopped from generating unexpected internal responses during grinding by utilizing liquid nitrogen. Beef powder (50 g), methylene chloride (200 mL), 2-methyl-3-heptanone (1 μL,1μg/μL) and 1,2-dichlorobenzene (1 μL,1μg/μL) were combined and shaken for 8 h at 120 rpm at 4 °C. Using the SAFE approach established by Engel et al. (Engel, 1999), the combined organic extracts were then subjected to high vacuum distillation (10^{-5}-10^{-6} kPa). The distillate was then distilled to around 10 mL using a Vigreux column (20 cm × 1 cm i.d.; Lianghe glass instrument business Department, Taixing, China) and dried over anhydrous sodium sulfate, proceeded by concentration to 500 μL using a mild nitrogen flow. Finally, a 10 μL syringe was used to inject an aliquot of the sample (1 μL) onto the GC column for analysis. Three times each sample was analyzed.

2.5. Isolation of the aroma compounds by solid-phase microextraction (SPME)

According to Liu (Liu et al., 2019), the SPME approach was employed to extract and quantify the flavor substances. In brief, 2.0 g of crushed material was accurately placed in a headspace vial (20 mL) after being weighed, with 1 μL of internal standard (1,2-dichlorobenzene, 0.652 μg/μL; 2-methyl-3-heptanone, 1.68 μg/μL) immediately injected. A PTFE-silicon stopper was used to seal the vial, and it was incubated in a thermostatic water bath (HH-2, Changzhou Zhibo Rui Instrument Manufacturing Co., LTD, Changzhou, China) at 55 °C for 20 min. Using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (50/30 m, 2 cm, Supelco, Bellefonte, PA, USA), the volatile aroma compounds were continuously extracted at 55 °C for 40 min. For five

minutes of thermal desorption at 250 °C, the sampler was inserted in the GC injector. Each analysis was conducted on three replicates.

2.6. Q Exactive GC -orbitrap- MS − O analysis

Aroma compounds in SSMB were identified by Q Exactive GC-Orbitrap-MS-O (Trace 1310 GC System, TSQ9000 MSD, Thermo Scientific, Bremen, Germany) equipped with a port for olfactory detection (ODP 4, GERSTEL, Demilheim, NRW, Germany). The polar DB-WAX capillary column (60 m × 0.25 mm × 0.25 μm; Agilent, Santa Clara, CA, U.S.A.) and non-polar DB-5 capillary column (60 m × 0.25 mm × 0.25 μm; Agilent, Santa Clara, CA, U.S.A.) were used for separate aroma analysis. With a final hold time of five minutes, the temperature of the column was set to rise from 40 °C (after a two-minute hold) to 230 °C at a rate of 4 °C/min. A flow rate of 1 mL/min of ultrahigh purity helium served as the carrier gas. The aroma compounds from the capillary column were separated into the mass spectrometer (MS) and ODP at a 1:1 (*v/v*) ratio. At 70 eV ionization energy, the acquisition range for the electron-impact mass spectra was 30 to 400 *m/z*.

2.7. GC × GC-O-MS analysis

To detect the aroma components of SSMB, an Agilent 8890 GC-5975B MS equipped with a cold-state modulator. During the heating and cooling processes, a solid-state modulator SSM1800 (J & X Technologies, Shanghai, China) was positioned between the two columns. The cold zone temperature was set to −51 °C, and the modulation period was changed to 4 s. The temperatures of entry hot zone and exit hot zone were 30 and 120 °C offset relative to oven temperatures. A primary polar DB-wax capillary column (30 m × 0.25 mm × 0.25 μm; Agilent, Santa Clara, CA, U.S.A.) and a mid-polar DB-17 ms column (1.85 m × 0.18 mm × 0.18 μm; Agilent, Santa Clara, CA, U.S.A.) were used as the first and second columns, respectively. The GC conditions were as follows: the carrier gas was ultrahigh-purity helium, flowing at a rate of 1 mL/min. The temperature of the oven was increased by 4 °C/min from 40 °C (held for 3 min) to 230 °C, then held at 230 °C for 5 min; both columns were housed inside the same GC oven. A sniffer port (Sniffer 9100, Brechbühler, Schlieren, Switzerland) was inserted between GC × GC and MS (Wang, Guo, Song, Meng, & Guan, 2021). Easy conversion between one-dimensional (1D) and two-dimensional (2D) modes was realized by a four port microfluidic device (Tarjan, Victoria, Australia). Besides, humid air was delivered into the pipeline of the sniffing port (Sniffer9100, Brechbiihler, Schlieren, Switzerland) to maintain the sensitivity of the nose all the time.

2.8. HS-GC-IMS analysis

The MXT-5 capillary column (15 m × 0.53 mm × 1 μm; Restek, Santa Clara, CA, U.S.A.) were used for aroma analysis. The column temperature was 60 °C, the carrier gas was N_2, and the IMS temperature was 45 °C. Using a syringe that had been preheated to 85 °C and high purity (99.99 %) nitrogen as the carrier/drift gas, 500 μL of headspace was injected. The incubation time was 15 min, the injection needle temperature was 95 °C, the incubation speed was 500 rpm. The gas flow rate program was as follows: 2 mL/min held for 2 min, 15 mL/min linearly increased over 10 min, 100 mL/min linearly increased over 20 min, and 150 mL/min linearly increased over 30 min. The data represent the means of three replicates. A number of software programs were utilized to assess IMS data, including LAV v.2.0.0 (G.A.S mbH, Dortmund, Germany) and GC-IMS Library Search. The found volatile compounds were described using retention indices and drift durations along with IMS database search software and the NIST library.

2.9. Aroma extraction dilution analysis (AEDA)

The effectiveness of the odorants in the SSMB's overall odor was evaluated using AEDA (Yang et al., 2022). Methylene chloride was used to dilute the aroma extracts ($1+1, v+v$). Q Exactive GC-Orbitrap-MS-O (Trace 1310 GC System, TSQ9000 MSD, Thermo Scientific, Bremen, Germany) was used to test each diluted solution until each smell was undetectable at a certain dilution by three competent panelists (two females and one male). The flavor dilution (FD) factor for each odorant was expressed by the highest dilution at which it could be identified. The diluted extracts were all subjected to GC-O analysis until the panelists could detect no odor at the sniffing port. The flavor dilution (FD) factor for each odorant was expressed by the highest dilution at which it could be identified. According to Chung and Cadwallader (1994), the results for perception were expressed as $\log_2$FD of the highest dilution. Wet gas (passed through 99.99 % pure air with distilled water) was given through a blank capillary column to boost the panelists' comfort and sensitivity while sniffing. Since the DB-WAX column detected more compounds than the DB-5 column, AEDA was performed on it.

2.10. Qualitative analysis

Mass spectrometry (MS) was used to identify the volatile substances. The NIST and Willy Libraries' MS peaks were compared using the Mass Spectral Search Program. The observed compounds were then confirmed with the linear retention index (LRI) (Lin et al., 2014). Under the same GC–MS detection conditions, LRIs were evaluated using the retention times of *n*-alkanes ($C_7 - C_{30}$). Furthermore, the RI value and odor description (O) were used to perform a qualitative analysis, which was confirmed by the standard compounds (STD).

2.11. Quantitative analysis

The aroma substances were quantitated using the methods reported in the literature, with a few may lead to improvements. Standard mixed solutions of 42 aroma-active compounds, including dimethyl trisulfide, 4-methyl-5-hydroxyethylthiazole, 1-octen-3-one, 2-methyl-3-furanthiol, 2,6-diethylpyrazine, eugenol, 2-acetylthiophene, linalool, 1,8-cineole, (*E, E*)-2, 4-decadienal, nonanal, 1-hexanol, anethole, octanal, 3-hydroxy-2-butanone, (*E*)-2-undecenal, ethyl maltol, 5-methyl-4-hydroxy-3(2*H*)-furanone, 3-methylthiopropanal, hexanal, 2-acetylthiazole, anisaldehyde, heptanal, 1-heptanol, α-pinene, coumarin, estragole, (*E*)-2-decenal, p-cresol, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, cinnamyl acetate, benzaldehyde, pentanal, myristicin, γ-hexalactone, (*d*)-carvone, γ-terpinene, α-humulene, octanoic acid, methyleugenol, 2,6-dimethoxyphenol, and decanoic acid was created by 1000-fold dilution of the stock solution in methylene chloride. The diluted standard was subsequently serially diluted to 7 different concentrations, and as internal standards, 1,2-dichlorobenzene (0.652 μg/μL) and 2-methyl-3heptanone (1.68 μg/μL) were added to each of them. The response ratios of the standard chemicals and the internal standard were plotted against their concentrations, respectively, to construct the standard curves that were displayed in Table 2.

2.12. Calculation of odor activity values (OAVs)

The odor-activity values (OAVs), which were calculated as ratios of the odorants concentrations divided by the corresponding orthonasal odor thresholds. This approach was used to examine the contributions of aroma-active compounds to SSMB. Each compounds perception-threshold value was determined using the methods indicated in the literature (Sohail et al., 2022). Aroma-acitve compounds with OAVs greater than 1 were defined as the key aroma substances which have a major contribution to the SSMB aroma profile.

2.13. Aroma recombination and omission experiments

The sensory panel conducted a triangle test to evaluate the differences between each omission model and the entire recombination

model. Sensory assessments were carried out at 25 ± 1 °C in an odor-free setting. The Institute of Food Science and Technology, CAAS, selected 12 healthy volunteers (six females and six men) from 30 people. All of the sensory panelists had previous expertise with food sensory evaluation. The odorless matrix was created in accordance with the preceding instructions prior to the aroma-recombination experiment. Utilizing unscented beef powder, real flavor standards with OAVs ≥ 1, and ultrapure water, recombination model 1 was created. Recombination model 2 was created by taking off one of the taste standards from recombination model 1. The aroma of recombination model 2 was different from SSMB, proving that the aroma compound it lacked was an essential aroma compound. Key aroma-active components, the odorless matrix, and ultrapure water were used to create the third iteration of the recombination model. In a triangle test, the previously described procedure was used by panelists to assess whether recombination model 3 was consistent with SSMB.

2.14. Statistical analysis

Data on flavor characteristics of SSMB samples was compiled based on the actual production situation and sensory evaluation results. Likewise, a data set of compounds was constructed using AEDA, and OAV data. Pearson correlation coefficient was used to visualize the associations between expression of aroma compounds and sensory characteristics. R software was utilized to perform the multivariate analysis. Microsoft Excel 2016 was used to create the radar chart and bar diagram. The IBM SPSS Statistics 22 program was used to execute one-way ANOVA and Duncan's multiple-range tests. At $P \leq 0.05$, data were considered statistically significant. Results are expressed as the means ± standard deviations.

3. Results and discussion

3.1. Aroma profile evaluation (APE)

To gain an overview of the aroma differences across three SSMB samples at different cooling stages, aroma profiles were analysed. In the brine tanks, pre-cooling stages (100℃→45℃) were conducted. SSMB were soak in marinade fluid at this period. During air cooling tunnel stages (45℃→10℃), the SSMB itself was cooled without marinade fluid. The spider map (Fig.1) shows that the aroma profiles of SSMB-100 and SSMB-10 were comparable. However, SSMB-45 showed higher scores for spicy, herbal, and meaty aromas, whereas the intensity of the woody

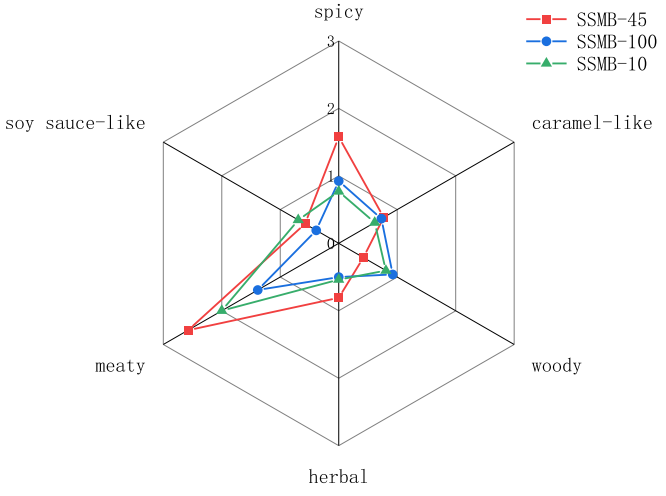


Fig. 1. Aroma profiles of soy sauce marinated beef (SSMB-100, just completed), pre-cooled to 45 °C in the brine tanks (SSMB-45, after pre-cooling) and coming out of the air-cooling tunnel (SSMB-10, after air-cooling tunnel).

aroma was lower in SSMB-45. The scores for caramel-like odor were almost identical. among the three groups. After pre-cooling in brine tanks, marine fluid absorption enhanceds the solubility of myofibrillar proteins, thus improving flavor (Song, Huang, Li, Han, & Zhang, 2021). Notably, pre-cooling intensified the meaty odor. But SSMB-100 and SSMB-10 showed similar attribute score. Flavor dissipation is severe during air cooling tunnel stages, which results in the loss of herbal and spicy attributes (Qiang et al., 2022).

3.2. Identification and expression evaluation of aroma compounds in SSMB

The three SSMB samples contained 110 aroma-active compounds, which can be divided into nine chemical classes: heterocyclic compounds, aldehydes, ketones, terpenoids, benzene-containing compounds, alcohols, esters, acids, and ethers (Table 1). We identified 109 aroma-active substances in SSMB-45 but only 63 were identified in SSMB-100, 66 of which were aroma-active in SSMB-10.

Among all these three samples, Heterocyclicc compounds are dominant. Furthermore, the Heterocyclic group contained the largest number of aroma-active substances, with FD factors of >32. These substances play a crucial role in the flavor of SSMB. Two aminoketones condense to produce nitrogen-containing Heterocyclic molecules. Pyrazines are the most abundant aromatic nitrogen-containing heterocyclicc compounds. Pyrazines, which are created during the Maillard reaction, are a group of organic compounds that contribute to the characteristic flavor of beef (Sohail et al., 2022). In this study, seven odorants, namely 2-methylpyrazine (nutty), 2,6-dimethylpyrazine (meaty, nutty), 2,3-dimethylpyrazine (nutty), 5-methyl-2-ethylpyrazine (grassy), 6-methyl-2-ethylpyrazine (roast), 2,6-diethylpyrazine (nutty), and 2-methyl-6-vinylpyrazine (hazelnut, roast), showed higher FD factors in SSMB-45 but lower FD factors in SSMB-100. In addition, during processing of SSMB, to remove off-flavors and enhance freshness, spices were soaked in white wine or cooking wine. Pyrazines are important compounds that affect the aroma of Chinese liquors (Fan, Xu, & Zhang, 2007) and may be one sources of pyrazine compounds in samples. Heterocyclic compounds containing oxygen can be produced from reducing sugars. For example, the cyclisations of 3-deoxyosone can result in the production of furfural. In SSMB-100, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone exhibited the highest FD factor of 131072. However, the FD factor decreased to 256 during cooling. 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone, which is also present in soy sauce, wines, soy paste, and beer (Chen et al., 2023), is formed via the Maillard reaction. Although this substance reached an FD factor of only 256 in SSMB-45, it was still released during pre-cooling but was not enriched. 2,5-Dimethyl-4-hydroxy-3(2*H*)furanone is the most prominent contributor to the characteristic caramel-like flavor of stewed beef and juice (Guth, 1994) and strongly contributes to the overall flavor profile of beef, along with other aroma compounds formed during the cooking process. In thermally cooked meat, the Maillard reaction and lipid-Maillard interaction produce odor-active chemicals contained sulfur. They are frequently created when reducing sugars, such as glucose and ribose, combine with glutathione or cysteine (Zhao et al., 2019). Notably, 4-methyl-5-hydroxyethylthiazole, which not identified using gas chromatography-mass spectrometry (GC-MS,) was observed using the GC × GC-olfactory (O)-MS and Q Exactive GC-Orbitrap-MS-O system. Consequently, combining numerous instruments to evaluate aroma-active component is necessary to provide details on the volatile profiles of samples. 2-Methyl-3-furanthiol, generated via Strecker degradation of methionine, has been reported mostly in cooked meat (Sohail et al., 2022). Additionally, 2-furfurylthiol and 2-methyl-3-furanthiol can derived from furfural and 5methyl-4-hydoxy-3(2*H*)-furanone with H_2S via Strecker degradation of cysteine, respectively (Sohail et al., 2022).

The second-most abundant component was aldehydes. Anisaldehyde (caramel-like) had the highest FD factor of 131072, followed by

Table 1
Aroma-Active Compounds in soy sauce marinated beef during pre-cooling and air-cooling tunnel.

no	aroma-active compounds	RI		odor perception	FD			identification method	extraction method
		DB-WAX	DB-5		SSMB-100	SSMB-45	SSMB-10		
1	methanethiol	< 700	< 700	cabbage, foot	2	8	8	O/STD	SPME/SAFE
2	2-methylbutanal	910		chocolate, malty	4	2	4	MS/RI/O/STD	SPME
3	3-methylbutanal	913	< 700	chocolate, malty	4	2	4	MS/RI/O/STD	SPME
4	pentanal	975		green	16	512		MS/RI/O/STD	SPME
5	*α*-pinene	1022	933	pine	32	2048	1024	MS/RI/O/STD	SPME/SAFE
6	2-methyl-3-buten-2-ol	1031		herbal		4	64	MS/RI/O/STD	SAFE
7	acetic acid butyl ester	1071	813	pungent, sweet		256	256	MS/RI/O/STD	SAFE
8	hexanal	1082	799	grassy, green		1024	2	MS/RI/O/STD	SPME/SAFE
9	*β*-pinene	1109		pine		32		MS/RI/O/STD	SPME/SAFE
10	sabinene	1122	973	pine, polish	16	8	16	MS/RI/O/STD	SAFE
11	3-carene	1150	1009	pungent, herb		8		MS/RI/O/STD	SPME/SAFE
12	heptanal	1186	901	floral, citrus	8	4096	256	MS/RI/O/STD	SPME/SAFE
13	limonene	1201		citrus, fragant		128		MS/RI/O/STD	SPME/SAFE
14	3-methylbutanol	1204	731	cheese		16		MS/RI/O/STD	SPME/SAFE
15	1,8-cineole	1209	1032	camphor, cool, mint	256	512	512	MS/RI/O/STD	SPME/SAFE
16	ethyl hexanoate	1221		anise, herbal		16		MS/RI/O/STD	SAFE
17	2-pentylfuran	1233	990	green bean, butter		16		MS/RI/O/STD	SPME/SAFE
18	*γ*-terpinene	1248	1058	bitter		512		MS/RI/O/STD	SPME/SAFE
19	2-methylpyrazine	1266	825	nutty	32	256		MS/RI/O/STD	SPME/SAFE
20	*p*-cymene	1273	1024	wood		32		MS/RI/O/STD	SAFE
21	3-hydroxy-2-butanone	1286	708	cream-like	64	2048	128	MS/RI/O/STD	SPME/SAFE
22	octanal	1291	1003	green, citrus, lemon	256	1024	512	MS/RI/O/STD	SPME/SAFE
23	3-methyl-1-pentanol	1292		pungent		256		MS/RI/O/STD	SAFE
24	cyclohexanone	1295		sweet, wine-like	64	64	32	MS/RI/O/STD	SAFE
25	2,6-dimethylpyrazine	1320		meaty, nutty		128		MS/RI/O/STD	SPME/SAFE
26	ethylpyrazine	1335		roast	16	8	512	MS/RI/O/STD	SAFE
27	6-methyl-5-hepten-2-one	1336	984	fruity	4	8	8	MS/RI/O/STD	SPME/SAFE
28	1-hexanol	1344		woody	256	512	512	MS/RI/O/STD	SPME
29	2-acetyl-1-pyrroline	1350	858	popcorn, beef	1024	512	16	RI/O	SPME
30	2,3-dimethylpyrazine	1351	918	nutty	256	16	256	MS/RI/O/STD	SAFE
31	dimethyl trisulfide	1354	975	alliaceous	2048	4096	4096	MS/RI/O/STD	SPME/SAFE
32	5-methyl-2-ethylpyrazine	1392	998	grassy	64	256	128	MS/RI/O/STD	SPME/SAFE
33	6-methyl-2-ethylpyrazine	1386		roasted potato	32	32	64	MS/RI/O/STD	SAFE
34	nonanal	1395	1104	citrus, fatty	64	512	64	MS/RI/O/STD	SPME/SAFE
35	1-octen-3-one	1405		mushroom	1024	2048	4096	RI/O/STD	SAFE
36	(*E*)-2-octenal	1435		nut, fat	2	4	2	MS/RI/O/STD	SAFE
37	1-octen-3-ol	1441	980	mushroom		64		MS/RI/O/STD	SPME
38	2,6-diethylpyrazine	1445		nutty	32	1024	1024	MS/RI/O/STD	SAFE
39	1-heptanol	1446		floral	4	512	32	MS/RI/O/STD	SPME
40	3-methylthiopropanal	1449	911	roasted potatoes		1024		MS/RI/O/STD	SAFE
41	5-methyl-2,3-diethylpyrazine	1452		earthy	2	4	2	MS/RI/O/STD	SPME/SAFE
42	furfural	1466	830	sweet	32	256		MS/RI/O/STD	SPME/SAFE
43	2-methyl-3-furanthiol	1471		meaty	4	2048	32	RI/O/STD	SAFE
44	2-methyl-6-vinylpyrazine	1491	1017	hazelnut, roast		2	128	MS/RI/O/STD	SPME/SAFE
45	copaene	1500	1348	wood, spice		256		MS/RI/O/STD	SPME
46	2-acetylfuran	1507		butter, meaty	32	32	64	MS/RI/O/STD	SPME/SAFE
47	2-methyl-4-(1-methylethyl)-thiazole	1514		meat, roast, sulfur	4	256	32	MS/RI/O/STD	SAFE
48	*d*-camphor	1525		pine, spice		256		MS/RI/O/STD	SAFE
49	benzaldehyde	1530		fruity, sweet	8	2048	4	MS/RI/O/STD	SPME/SAFE
50	linalool	1541	1099	floral	1024	4096	2048	MS/RI/O/STD	SPME/SAFE
51	linalyl acetate	1555		fruit, resinous, sweet		64		MS/RI/O/STD	SPME/SAFE
52	2-pentylpyridine	1570	1171	popcorn		256		MS/RI/O/STD	SAFE
53	5-methylfurfural	1577	960	caramel, sweet		128		MS/RI/O/STD	SPME/SAFE
54	isophorone	1601	1121	spice		256		MS/RI/O/STD	SAFE
55	4-terpineol	1604	1181	pine	32	64		MS/RI/O/STD	SPME/SAFE
56	2-acetylpyridine	1608		nutty		64		MS/RI/O/STD	SAFE
57	*γ*-butyrolactone	1636		caramel		32		MS/RI/O/STD	SPME
58	(*E*)-2-decenal	1648	1262	hay	128	1024	512	MS/RI/O/STD	SAFE
59	benzeneacetaldehyde	1649	1043	berry, floral, flower		256		MS/RI/O/STD	SPME/SAFE
60	2-acetylthiazole	1652		rice	2	512	4	MS/RI/O/STD	SPME/SAFE
61	2-furanmethanol	1657	852	burnt, caramel, cooked, honey	4	4		MS/RI/O/STD	SPME/SAFE
62	3-methyl-butanoic acid	1665		cheese	4	4		MS/RI/O/STD	SAFE
63	estragole	1675	1198	anise, herb, licorice	16	512	32	MS/RI/O/STD	SPME/SAFE
64	*α*-humulene	1681	1457	spice, wood		64	512	MS/RI/O/STD	SPME/SAFE
65	*γ*-hexalactone	1695		coconut		2048		MS/RI/O/STD	SPME/SAFE
66	5-methyl-2-thiophenecarbaldehyde	1735	1120	broth, sulfur		8		MS/RI/O/STD	SPME/SAFE
67	piperitone	1736		fresh, mint		256		MS/RI/O/STD	SPME/SAFE
68	(*d*)-carvone	1742		caraway		512		MS/RI/O/STD	SPME/SAFE

(*continued on next page*)

Table 1 (*continued*)

no	aroma-active compounds	RI		odor perception	FD			identification method	extraction method
		DB-WAX	DB-5		SSMB-100	SSMB-45	SSMB-10		
69	(*E*)-2-undecenal	1756	1363	resin, soap	2	256	32	MS/RI/O/STD	SAFE
70	2-acetylthiophene	1755		sulfurous, nutty	1024	1024	2048	MS/RI/O/STD	SAFE
71	1-(4,5-dihydro-2-thiazolyl)-ethanone	1775		burnt, caramel, popcorn, roast		256		MS/RI/O/STD	SPME/SAFE
72	4-ethyl-benzaldehyde	1784		bitter almond		32		MS/RI/O/STD	SPME/SAFE
73	benzenepropanal	1785	1161	green, fruity		256		MS/RI/O/STD	SAFE
74	(*E, E*)-2,4-decadienal	1791		fatty	64	4096	1024	MS/RI/O/STD	SAFE
75	anethole	1798	1286	anise, licorice		512	128	MS/RI/O/STD	SAFE
76	cyclotene	1828	1023	licorice, nutty	2	32		MS/RI/O/STD	SPME/SAFE
77	(*E*)-anethole	1832		anise	4	16		MS/RI/O/STD	SPME/SAFE
78	calamenene	1842		clove, floral, herb, spice		8		MS/RI/O/STD	SAFE
79	o-guaiacol	1860	1084	bacon, cream, medicine, phenol, smoke	16	16	64	MS/RI/O/STD	SAFE
80	benzenemethanol	1878	1034	fruity, sweet	64	256	8	MS/RI/O/STD	SPME/SAFE
81	phenethyl alcohol	1916	1112	corn flakes, floral		128		MS/RI/O/STD	SAFE
82	2-thiophenemethanol	1944	1030	chicken soup, metal	8	32	32	MS/RI/O/STD	SAFE
83	benzothiazole	1964		ceramal	4	128	32	MS/RI/O/STD	SPME/SAFE
84	2-acetylpyrrole	1970	1063	hazelnut, licorice, nut	8	128	32	MS/RI/O/STD	SPME/SAFE
85	phenol	2004		medicine, phenol, sharp, smoke, spice	256	32	256	MS/RI/O/STD	SAFE
86	methyleugenol	2012	1398	clove, floral, spice, warm	131,072	256	32	MS/RI/O/STD	SAFE
87	ethyl maltol	2020	1193	caramel	131,072	131,072	131,072	MS/RI/O/STD	SPME/SAFE
88	2,5-dimethyl 4-hydroxy- −3(2*H*)-furanone	2027	1066	caramel, honey, sweet	131,072	256	256	MS/RI/O/STD	SAFE
89	anisaldehyde	2033	1255	caramel, mint, sweet	131,072	131,072	131,072	MS/RI/O/STD	SPME/SAFE
90	(*E*)-cinnamaldehyde	2048		cinnamon, honey,		128		MS/RI/O/STD	SPME
91	octanoic acid	2063		fatty, fruity		1024	16,384	MS/RI/O/STD	SPME/SAFE
92	5-acetyldihydro-2(3*H*)-furanone	2066		wine			32	MS/RI/O/STD	SAFE
93	p-cresol	2079		dry tarry, medicine		128	8192	MS/RI/O/STD	SPME/SAFE
94	methyl 2-methoxybenzoate	2096		herb, sharp	128	256	16	MS/RI/O/STD	SAFE
95	5-methyl-4-hydroxy-3(2*H*)-furanone	2112	1042	sweet, caramel	512	2048	4	MS/RI/O/STD	SPME/SAFE
96	hexadecanal	2135	1714	burnt		128	32	MS/RI/O/STD	SPME/SAFE
97	2-phenoxyethanol	2144		pleasant		16	16	MS/RI/O/STD	SAFE
98	cinnamyl acetate	2152	1443	floral		8192		MS/RI/O/STD	SAFE
99	eugenol	2166	1351	clove, smoke, spice	32	1024	4	MS/RI/O/STD	SPME/SAFE
100	2-methoxy-4-vinylphenol	2195		clove, cooked	128	128	128	MS/RI/O/STD	SAFE
101	2,6-dimethoxyphenol	2222		clove, medicine		2048		MS/RI/O/STD	SAFE
102	myristicin	2266		nutmeg, spice, warm	512	256	2048	MS/RI/O/STD	SAFE
103	hydroxydihydromaltol	2268	1146	bitter, earth, herb, roast	4	256	32	MS/RI/O/STD	SAFE
104	decanoic acid	2273	1366	fatty, rancid	8	8192	128	MS/RI/O/STD	SAFE
105	4-methoxybenzyl alcohol	2280	1284	anise, flower, herb, sweet	128	32	64	MS/RI/O/STD	SAFE
106	4-methyl-5-hydroxyethylthiazole	2307	1274	nutty		512		MS/RI/O/STD	SAFE
107	chavicol	2333		medicine		16		MS/RI/O/STD	SAFE
108	*trans*-isoeugenol	2350	1447	cinnamon, clove, flower		16	2	MS/RI/O/STD	SPME
109	indole	2447		floral		16		MS/RI/O/STD	SAFE
110	coumarin	2464	1435	sweet, hay	128	2048	512	MS/RI/O/STD	SAFE

benzaldehyde (fruity, caramel-like), with an FD value of 2048. Hexanal, heptanal, and benzaldehyde are the main aldehydes components of beef (Sun et al., 2020). Anisaldehyde is derived from several natural sources, including anise, fennel, star anise, cinnamon, and cloves. Benzaldehyde is found in plants such as cinnamon and laurel leaves and is responsible for their characteristic aromas. Benzenepropanal confers the characteristic aroma of cinnamon (Liu et al., 2013). Because spices are present in marinade, soaking meat in a marinade during the pre-cooling stage enhances the flavor of SSMB. Therefore, FD factors were high in SSMB-45 but low in SSMB-100. Hexanal has also been used to assess the degree of lipid oxidation and food quality (Al-Dalali, Li, & Xu, 2022). The hexanal content increased during the pre-cooling stage but was reduced in the cooling tunnel. During the cooling stage, nonanal and benzaldehyde exhibit erratic increases and decreases, respectively. During the pre-cooling in the brine tanks, analysis of the hexanal content revealed that SSMB was highly oxidizeds. Lipids degrade into both unsaturated and saturated fatty acids during heating. As a result, hydroperoxides are formed, which react to generate aldehydes (Wilson & Katz, 1972). According to previous studies, heating polyunsaturated fatty acids between 85 °C and 100 °C can produce heptanal, hexanal, nonanal, octanal, and (*E, E*)-2,4-decadienal (Christlbauer & Schieberle, 2009; Wilson & Katz, 1972). The products of lipid degradation with structures containing 6–10 carbons, particularly saturated and unsaturated aldehydes (e.g., octanal and 1-octen-3-one), are frequently present in cooked meats at high FD factors or OAVs (Du, Zhen, Wang, Cheng, & Xie, 2020). Hexanal can be produced via thermal degradation of n-6 polyunsaturated fatty acids at 37 °C, making hexanal a suitable indicator of lipid peroxidation (Frankel, Hu, & Tapper, 1989). Octanal, heptanal, nonanal, and (*E, E*)-2,4-decadienal are saturated and unsaturated aldehydes that can be produced through oxidation of n-3 unsaturated fatty acids, such as linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid (Elmore, Mottram, Enser, & Wood, 1999). As the chain length of the molecule increased, the degree of aldehyde dissipation also increased.

These three samples contained 14 aroma-active terpenoids. Terpenoid odorants are present in many plant samples. Ten odorants, namely α-pinene (pine-like), β-pinene (pine-like), limonene (herbal), 1,8-cineole (herbal, minty), *p*-cymene (woody), γ-terpinene (bitter), copaene (woody), linalool (floral), 4-terpineol (spicy), and α-humulene (spicy, woody), showed FD factors > 32. Seven aroma-active phenylpropenes were identified using GC-O. Methyleugenol exhibited the highest FD factor of 131072 in SSMB-100. These aroma-active compounds contribute to the woody or herbal aroma in the comprehensive profile.

Table 2
Concentrations, Odor Thresholds, and Odor Activity Values (OAVs) of Key Aroma-Active Compounds in SSMB-100, SSMB-45 and SSMB-10.

no	aroma-active compounds	liner equation	R^2	concentration (μg/kg)									odor thresholdin water (μg/kg)	OAVs		
				100			45			10				100	45	10
1	dimethyl trisulfide	y = 1.0389x − 0.0579	0.9982	3790	±	17	5930	±	47	3640	±	91	0.1[a]	37,900	59,300	36,400
2	4-methyl-5-hydroxyethylthiazole	y = 4.0174x − 0.2946	0.9958	4600	±	253	7410	±	72	4630	±	80	0.3[b]	15,333	24,700	15,433
3	1-octen-3-one	y = 0.2387x − 0.0153	0.9971	36.1	±	2.1	78.2	±	0.8	79.6	±	0.6	0.003[a]	12,033	26,067	26,533
4	2-methyl-3-furanthiol	y = 1.2894x − 0.0663	0.9984	71.3	±	1.1	81.4	±	0.4	75.9	±	1.8	0.007[a]	10,186	11,629	10,843
5	2,6-diethylpyrazine	y = 0.1698x − 0.0054	0.9998	30.1	±	4.6	69.3	±	3.1	64.2	±	0.2	0.006[b]	5017	11,550	10,700
6	eugenol	y = 0.8287x − 0.0585	0.9982	2880	±	209	2970	±	24	2880	±	50	0.71[b]	4056	4183	4056
7	2-acetylthiophene	y = 0.2974x − 0.0175	0.9982	152	±	5	155	±	1	356	±	2	0.08[a]	1900	1938	4450
8	linalool	y = 0.4797x − 0.0293	0.9979	202	±	31	621	±	4	584	±	1	0.22[b]	918	2823	2655
9	1,8-cineole	y = 0.2691x + 0.0031	0.9996	1450	±	5	2890	±	1	2820	±	2	1.1[b]	1318	2627	2564
10	(*E, E*)-2, 4-decadienal	y = 0.9274x − 0.0594	0.9971	42.6	±	3.1	86.1	±	0.0	82.6	±	1.1	0.077[a]	553	1118	1073
11	nonanal	y = 0.1253x − 0.0011	0.9998	1150	±	50	1610	±	11	1110	±	13	1.1[a]	1045	1464	1009
12	1-hexanol	y = 1.0971x − 0.0571	0.9983	1380	±	109	2100	±	45	1330	±	31	5.6[b]	246	375	238
13	anethole	y = 0.015x − 0.0011	0.9889	1530	±	56	4870	±	31	1430	±	89	21.8[b]	70	223	66
14	octanal	y = 0.0547x + 0.0026	0.9992	79.4	±	9.2	108	±	4.8	96.1	±	3.1	0.587[a]	135	184	164
15	3-hydroxy-2-butanone	y = 0.137x − 0.0112	0.9975	1870	±	3	2560	±	5	1820	±	3	14[a]	134	183	130
16	(*E*)-2-undecenal	y = 0.1725x − 0.0019	0.9974		–		199	±	3	186	±	1	1.4[a]	–	142	133
17	ethyl maltol	y = 0.1732x − 0.0139	0.9967	224	±	18	287	±	38	262	±	36	2[b]	112	144	131
18	5-methyl-4-hydroxy-3(2*H*)-furanone	y = 0.0048x − 0.0003	0.9975	71.4	±	0.2	121	±	0.7	67.5	±	1.3	1.6 [b]	44.6	75.6	42.2
19	3-methylthiopropanal	y = 0.2941x − 0.0157	0.9984	17.5	±	0.5	44	±	0.4	47.4	±	0.0	0.45[b]	39	98	105
20	hexanal	y = 0.1045x − 0.0063	0.9982	89.5	±	3.7	155	±	0.3	154	±	1.5	5[a]	18	31	31
21	2-acetylthiazole	y = 0.8151x + 0.0176	0.9994	75.6	±	2.6	83	±	0.2	80.2	±	0.4	3[a]	25	28	27
22	anisaldehyde	y = 1.9497x − 0.1063	0.9983	343	±	4	381	±	1	331	±	1	27[b]	13	14	12
23	heptanal	y = 0.0788x − 0.0016	0.9992	47.2	±	1.8	48.2	±	0.8	48.1	±	0.8	2.8[a]	17	17	17
24	1-heptanol	y = 1.0886x + 0.0124	0.9997	53.3	±	2.5	89.9	±	7.2	86	±	7.9	5.8[a]	9	16	15
25	*α*-pinene	y = 0.7186x − 0.04	0.9982	79.4	±	4.9	175	±	1.1	171	±	1.5	14[b]	6	13	12
26	coumarin	y = 1.3487x − 0.096	0.9962	383	±	21	410	±	3	371	±	5	34[b]	11	12	11
27	estragole	y = 1.1643x − 0.0625	0.9982	63.1	±	2.9	77.4	±	1.5	61.5	±	7.4	6[b]	11	13	10
28	(*E*)-2-decenal	y = 0.2874x − 0.0198	0.9971	84.6	±	3.9	200	±	0.0	85.2	±	0.6	17[a]	5	12	5
29	*p*-cresol	y = 1.192x − 0.0653	0.9979		–		10.9	±	1.2	73.8	±	2.6	3.9[b]	–	3	19
30	2,5-dimethyl-4-hydroxy-3(2*H*)-furanone	y = 1.0628x − 0.0703	0.9971	67.9	±	1.5	68.6	±	4.3	49.7	±	7.6	22.3[b]	–	3	2
31	cinnamyl acetate	y = 1.6432x − 0.1144	0.9965		–		240	±	1		–		150[b]	–	2	–
32	benzaldehyde	y = 1.6496x − 0.0916	0.9982	150	±	2.9	386	±	15.4	90.9	±	3.2	750[a]	< 1	< 1	< 1
33	pentanal	y = 0.0937x − 0.0064	0.9975	7.78	±	3.2	37.6	±	3.1		–		12[a]	1	3	–

(*continued on next page*)

Table 2 (*continued*)

no	aroma-active compounds	liner equation	R^2	concentration (μg/kg)									odor thresholdin water (μg/kg)	OAVs		
				100			45			10				100	45	10
34	myristicin	y = 0.7426x − 0.0444	0.998	21	±	0.2	12.6	±	0.5	27.5	±	1.1	25[b]	1	1	1
35	γ-hexalactone	y = 0.9903x − 0.0573	0.9981		–		128	±	0		–		260[a]	–	< 1	–
36	(*d*)-carvone	y = 0.3673x + 0.0057	0.9996		–		53	±	1		–		160[b]	–	< 1	–
37	γ-terpinene	y = 0.4991x − 0.0273	0.9982		–		285	±	3		–		1000[b]	–	< 1	–
38	α-humulene	y = 0.269x − 0.015	0.9983		–		142	±	5	156	±	3	160[b]	–	1	1
39	octanoic acid	y = 0.0547x + 0.0026	0.9992		–		524	±	1	377	±	6	3000[b]	–	< 1	< 1
40	methyleugenol	y = 1.1918x − 0.0616	0.9982	91.8	±	6.5	83	±	0.6	38.4	±	2.2	820[b]	< 1	< 1	< 1
41	2,6-dimethoxyphenol	y = 1.3654x − 0.0817	0.9979		–		265	±	17		–		1850[b]	–	< 1	–
42	decanoic acid	y = 0.1891x − 0.013	0.999	108	±	22	253	±	8	211	±	6	10,000[b]	< 1	< 1	< 1

[a] Odor detection threshold in water copied from Aroma compounds identified in cooked meat: A review;
[b] Odor detection threshold in water copied from Gemert, L. J. V. Odour Thresholds: Compilations of Flavor Threshold Values in Air, Water and Other Media, Second enlarged and revised edition.

α-Pinene and linalool key odorants of cooked beef balls (Sun et al., 2020). α-Pinene, β-pinene, α-humulene are found in star anise; limonene is present in *Pericarpium citri reticulatae*; 1,8-cineole, γ-terpinene, 4-terpineol, and copaene can be found in fennel; and *p*-cymene, linalool is present in clove and thyme (Gao et al., 2008; Liu et al., 2013; Zhao et al, 2007). Ten terpenoids showed the highest FD factors in SSMB-45, suggesting that pre-cooling of soy sauce enhanced the impact of spices on meat flavor. After sample processing in the cooling tunnel, seven terpenoids were no longer detected, i.e., α-pinene, 3-carene, β-pinene, limonene, ethyl hexanoate, γ-terpinene, *p*-cymene, copaene, and 4-terpineol. This result indicates that the aroma-active compounds were lost to the atmosphere in the cold air-cooling tunnel.

Ten aroma-active ketones were found in SSMB. 3-Hydroxy-2-butanone (cream-like) and coumarin (caramel-like) had the highest FD in SSMB-45 (2048) but lower FD in SSMB-100 (64). 3-Hydroxy-2-butanone is a characteristic flavor substance found in beef that is generated during Maillard reaction and Lipid-Maillard interaction (Hu et al., 2020). Coumarin is present in both cinnamon and cardamon extracts. Eight of the ten alcohols had FD factors > 32. Six phenols were detected, including 2-methoxy-4-vinylphenol (clove), which displayed a stable and constant FD factor (128). Therefore, 2-methoxy-4-vinylphenol may bind firmly to meat proteins. Overall, five aroma-active esters were detected and showed FD factors > 32, namely estragole (anise), anethole (anise), methyleugenol (clove), eugenol (clove), and myristicin (nutmeg). The three samples yielded seven aroma-active benzene-containing compounds. These benzene-containing compounds are derived from different herbs (Qiang et al., 2022). Five benzene-containing compounds showed the largest FD factors in SSMB-45, suggesting that pre-cooling of soy sauce enhances the effect of spices on meat flavor. After sample processing in the cooling tunnel, the impact of these species dissipated. For example, the FD value of eugenol was enriched in the pre-cooling stages but decreased in the air cooling tunnel stages. However, the FD value of methyleugenol kept decreasing during the whole cooling stages. When methyl molecule was added to the substance, its ability to bind proteins is altered. Three aroma-active ketones were found in the SSMB, namely acetoin, 1-octen-3-one and coumarin. Octanoic acid (a fatty acid) in SSMB-10 showed the highest FD factor 16384. These newly formed acid compounds may cause sweaty and rotten odors, resulting in a poor sensory score (Xu et al., 2022). Two aroma-active maltols were detected using GC-O. Among the three samples, ethyl maltol exhibited the highest FD factor (131,072); this compound was previously detected in soy sauce (Wanakhachornkrai, 2003), and may enhance the caramel-like flavor. The presence of hydroxydihydromaltol enriched the flavor of SSMB. Dimethyl trisulfide (alliaceous, FD 4096) is generated from Maillard reaction and lipid-Maillard interaction (Yu, 1995). Dimethyl trisulfide had the highest FD in SSMB-45 (4096) and SSMB-10 (4096) but lower FD in SSMB-100 (2048). According to the study of Anantharamkrishnan and Reineccius (2020), dimethyl trisulfide is covalently bound to β-lactoglobulin at different pH, temperature, and water activity levels. Through binding of aroma-active compounds and proteins, dynamic changes in temperature and water activity levels would affect flavor dissipation during cooling.

3.3. Quantitative analysis of aroma-active compounds and calculation of OAVs

Using external standard curve measurement, 42 aroma-active compounds with FD factors ranging from 512 to 131072 were quantified (Table 2). The contribution of the aroma compounds was determined using the OAV. Compounds with OAVs > 1 are thought to contribute to the overall aroma profile and are defined as key aroma-active substances (Greger & Schieberle, 2007). Table 2 shows the 29 aroma compounds with OAVs > 1. The most abundant compound was dimethyl trisulfide, followed by 4-methyl-5-hydroxyethylthiazole, eugenol, 1-octen-3-one, anethole, linalool, 1,8-cineole, 3-hydroxy-2-butanone, (*E, E*)-2, 4-decadienal, nonanal, dodecanal, ethyl maltol, octanal, (*E*)-2-undecenal, 1-hexanol, hexanal, 2-acetylthiazole, anisaldehyde, heptanal, 1-heptanol, α-pinene, coumarin, and estragole. (*E*)-2-Decenal, *p*-cresol, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, cinnamyl acetate, benzaldehyde, pentanal, and myristicin. Some compounds had OAV < 1. The results of aroma extract dilution analysis clearly overstated these components because any GC-O method volatilizes the full amount of a given odorant but does not consider the influence of the matrix (Franitza, Granvogl, & Schieberle, 2016). Although methyleugenol showed a high FD factor, its OAV was < 1 because the odor threshold was extremely high.

The OAV of key odorants from the Maillard reaction, such as dimethyl trisulfide and 4-methyl-5-hydroxyethylthiazole, increased gradually after pre-cooling. The result was in accordance with-methionine sulfoxide reacts with glucose to produce dimethyl trisulfide (Liu et al., 2019). Most byproducts the Maillard reaction may be

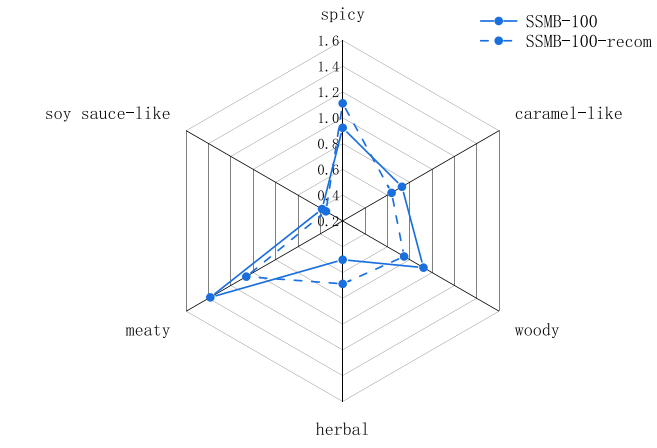
responsible for the strong, meaty flavor of SSMB (Sohail et al., 2022). High contents of dimethyl trisulfide in SSMB have a direct relationship with the Maillard reaction. Therefore, improving the Maillard process may improve the flavor of SSMB. The OAV of methylglyoxal, which can be condensed with cysteine to produce 2-acetylthiazole (Sohail et al., 2022), was also stable in the three samples. This may be because of their differential binding to myofibrillar proteins. The OAV of 3-methylthiopropanal increased gradually during cooling. This compound is synthesizeds from methionine via Strecker degradation (Sohail et al., 2022). During the precooling stage, the core temperature was appropriate for the Maillard reaction. α-Dicarbonyls generated through Amadori rearrangement readily react with amino acids to produce aroma-active aldehydes. Nonanal, octanal, and 2-undecenal are mostly derived from oleic acid (Sohail et al., 2022). The OAV showed an increasing trend for octanal, nonanal, and (*E*)-2-undecenal (Table 2). The OAV of octanal in SSMB-45 was 50 % compared with higher than that in SSMB-100. (*E*)-2-Undecenal was not detected in SSMB-100. The results showed that oleic acid oxidation continued during pre-cooling. Hexanal and (*E, E*)-2,4-decadienal are primarily derived from the oxidation of linoleic acid (Du et al., 2019). There was a dramatic increase in the OAV after pre-cooling, and OAV remained stable in the air-cooled tunnel. Those aldehydes were mostly generated via lipid degradation during cooling. Lipid-Maillard interactions can also add to the overall fragrance profile of SSMB by interacting with numerous types of intermediate products from diverse processes.

In meat marinades, spices contribute to the flavor of meat. Many spices are added during the processing of marinated beef; their main components include terpenoids and phenolic ether compounds. Because of the low odor threshold of some of these compounds, the OAV was typically high, which greatly contributed to the overall flavor of the marinated beef. The clove-like and woody flavors of eugenol can provide depth and complexity to flavor the of SSMB. Many species, such as cloves, cinnamon, and Sichuan peppercorn, contain eugenol (Qiang et al., 2022). Anethole is an organic compound with a caramel-like aroma and is found in fennel, star anise, caraway, cinnamon, and licorice (Gao et al., 2008). Some species such as fennel seeds, cinnamon, and bay leaves also contain linalool. 1,8-Cineole is present in several spices, including cardamom, bay leaves, and thyme (Zhao et al., 2007). These flavor substances derived from spices are enriched in the marination fluid during the pre-cooling stage.

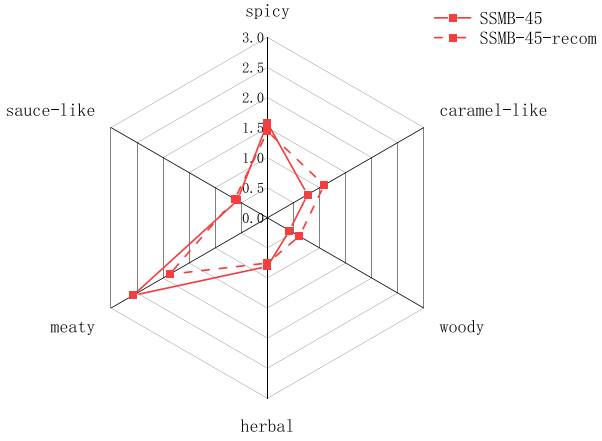
3.4. Aroma recombination and omission experiment

An aroma recombination experiment was performed to verify the key aroma-active compounds in SSMB. An odorless matrix was created using the method described in section 2.13, with 75 % ultrapure water and 29 aromatic substances with OAVs > 1. Using a triangle test for sensory evaluation, 12 trained sensory panellists assessed the comparability of each recombination model. The results are shown in Fig. 2. The three recombinates had characteristic intense aromas similar to those of the original SSMB samples, demonstrating that all key aroma-active compounds had been successfully identified.

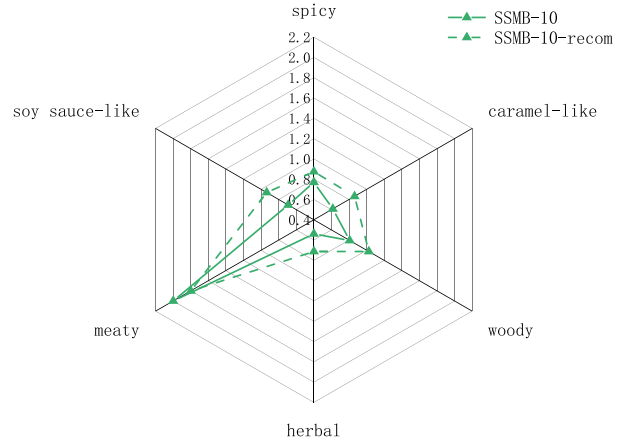
An omission experiment was conducted using 29 models, each with one component removed to examine the role of each component in overall sensory perception. The results of the triangular tests for each component are listed in Table 3. All assessors detected the omission of eugenol, 4-methyl-5-hydroxyethylthiazole, dimethyl trisulfide, 1-octen-3-one, anethole, linalool, 1,8-cineole, 3-hydroxy-2-butanone, (*E, E*)-2,4-decadienal, 3-methylthiopropanal, 2-methyl-3-furanthiol, nonanal, ethyl maltol, hexanal, and 5-methyl-4-hydroxy-3(2*H*)-furanone, indicating the importance of these compounds in the aroma of SSMB. Furthermore, the absence of some odorants affected the aroma perception of SSMB, including 3-methylthiopropanal, 2,6-diethylpyrazine, 2-acetylthiazole, anisaldehyde, heptanal, 1-heptanol, and α-pinene in SSMB-100; 2,6-diethylpyrazine, 2-acetylthiophene, anisaldehyde, coumarin, estragole, benzaldehyde, pentanal, octanal, α-pinene, 3-methylthiopropanal, and (*E*)-2-undecenal in SSMB-45; octanal, (*E*)-2-undecenal, 2-acetylthiophene, hexanal, α-pinene, anisaldehyde, coumarin, estragole, 2-acetyl-1-pyrroline, and 3-methylthiopropanal in SSMB-10. According to the SSMB-45 results, some aldehydes generated from lipid oxidation, such as pentanal, octanal, and (*E*)-2-undecenal, played critical roles in the omission experiment after pre-cooling. The results were in accordance with Chen et al., 2023. These three aldehydes showed negatively correlation with meaty attribute. Through lipid



(A)



(B)



(C)

Fig. 2. Aroma profiles of SSMB-100 (A, solid line), SSMB-45 (B, solid line) and SSMB-10 (C, solid line) and SSMB-100- recombinate (A, broken line), SSMB-45-recombinate (B, broken line) and SSMB-10- recombinate (C, broken line).

Table 3
Triangle Test Results of Each Key Aroma-Active Compound (OAV > 1) by Omission Experiments in SSMB-100, SSMB-45 and SSMB-10.

Key aroma-active compounds	n (100)	significant	n (45)	significant	n (10)	significant
eugenol	12	***	12	***	12	***
4-methyl-5-thiazoleethanol	12	***	12	***	12	***
dimethyl trisulfide	12	***	12	***	12	***
1-octen-3-one	12	***	11	***	12	***
anethole	11	***	11	***	12	***
linalool	11	***	11	***	12	***
1,8-cineole	11	***	11	***	11	***
3-hydroxy-2-butanone	11	***	11	***	11	***
hexanal	11	***	10	***	9	**
nonanal	10	***	10	***	11	***
dodecanal	10	***	4		10	***
furaneol	10	***	10	***	10	***
ethyl maltol	9	**	10	***	10	***
octanal	9	**	8	*	9	**
(*E*)-2-undecenal	9	**	8	*	9	**
1-hexanol	9	**	6		9	**
(*E, E*)-2,4-decadienal	9	**	11	***	11	***
2-acetylthiazole	9	**	6		7	
anisaldehyde	8	*	9	**	9	**
heptanal	8	*	6		6	
1-heptanol	8	*	4		6	
α-pinene	8	*	8	*	9	**
coumarin	7		9	**	9	**
estragole	6		9	**	8	*
(*E*)-2-decenal	6		–	–	4	
2-acetyl-1-pyrroline	6		7		8	*
cinnamyl acetate	4		–	–	8	*
benzaldehyde	3		9	**	7	
pentanal	–	–	9	**	3	
p-cresol	1		2	0	3	
myristicin	–	–	–	–	2	

oxidation, aldehydes with grassy and nutty odors are also produced and are responsible for the higher meaty attribute in SSMB. However, the number of key aroma-active compounds decreased after air cooling, suggesting severe flavor dissipation. The flavor balance of the recombinants changed after certain components were removed. For example, after eliminationng eugenol, anethole, 1,8-cineole, and coumarin, the herbal attribute was decreased; removal of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone decreased the caramel-like attribute, and removal of 1-octen-3-one and 3-hydroxy-2-butanone significantly weakened the grassy smell. Overall, the omission experiments established 29 odorants as key aroma-active compounds in SSMB.

3.5. Correlation analysis of aroma compounds and aroma profile of SSMB using a flavor matrix

To clearly define the respective contributions of fragrance compounds to flavor attributes of SSMB, the Pearson correlation coefficient between the OAVs of the aroma compounds and APE scores was determined. Most aldehydes showed an inverse relationship with meaty, as shown in Fig. 3. Particularly, hexanal was negatively correlated with meaty, herbal, and caramel-like aromas, whereas it was positively correlated with the woody aroma. Nonanal and pentanal were negatively correlated with a soy sauce-like aroma. The various flavor properties of the SSMB are influenced by the number of aldehydes. Notably, 2,5-dimethyl, 4-hydroxy-3(2*H*)-furanone, and methyleugenol were positively correlated with the caramel-like aroma and appeared to contribute to the caramel-like quality. Overall, the interactions of several aroma components generated the flavor characteristics of SSMB.

3.6. Flavor dissipation of aroma-active compounds during chilling processing

The volatile compounds in SSMB-100, SSMB-45, and SSMB-10 were similar, although the contributions of each aroma-active substance in the three samples differed significantly. Eugenol, 4-methyl-5-hydroxyethylthiazole, dimethyl trisulfide, 1-octen-3-one, anethole, linalool, and 1,8-cineole significantly affected all three SSMB samples. The aroma components in SSMB are derived from three sources: lipid degradation, the Maillard reaction, lipid-Maillard interactions, with thiamine as precursors. The Maillard reaction occurs when carbohydrates and amino acids with free amino groups are heated. Meanwhile, unsaturated lipids are degraded. More than half of the volatile compounds in cooked meat are produced by lipid oxidation and degradation. There are many lipid degradation products that have distinct flavors, but they can also interact with Maillard reaction products to create a cascade of flavor compounds. A number of compounds have been identified in the aroma of beef, although not all of them have an effect on it (Gong et al., 2017). Compounds in meat aromas are determined by their precursors, such as fatty acid composition and reaction conditions, such as temperature and time.

Generally, these volatile compounds are responsible for beef meaty attribute. Thermal oxidizations during pre-cooling in tanks contributes to the desirable beef flavor, which were impacted by hexanal, nonanal, octanal, and (*E, E*)-2,4-decadienal generated from lipid oxidation (Sohail et al., 2022). The breakdown of linoleic acid's 9-hydroperoxides can lead to the formation of (*E, E*)-2,4-decadienal. After pre-cooling, its concentration and significance increased in SSMB-45. The key aroma-active aldehydes generated from lipid oxidation generally increased after pre-cooling, although some volatile aldehydes dissipated, such as (*E*)-2-undecenal. The odor-active compounds of lipid degradation were pentanal and benzaldehyde, which were difficult to detect in SSMB-10. The flavor compounds are primarily produced by phospholipids; in a comparison between meat and defatted meat without triglycerides, the meat flavor was not different, whereas in a comparison between meat and defatted meat without both triglycerides and phospholipids, significant differences were observed (Sohail et al., 2022). 1-Heptanol, which has an herbal and green flavor, is a unique key aroma-active compound in SSMB-100. Dimethyl trisulfide, 2-methyl-3-furanthiol, and 3-methylthiopropanal are key odorants in the Maillard reaction (Sohail et al., 2022). According to the omission outcomes, dimethyl

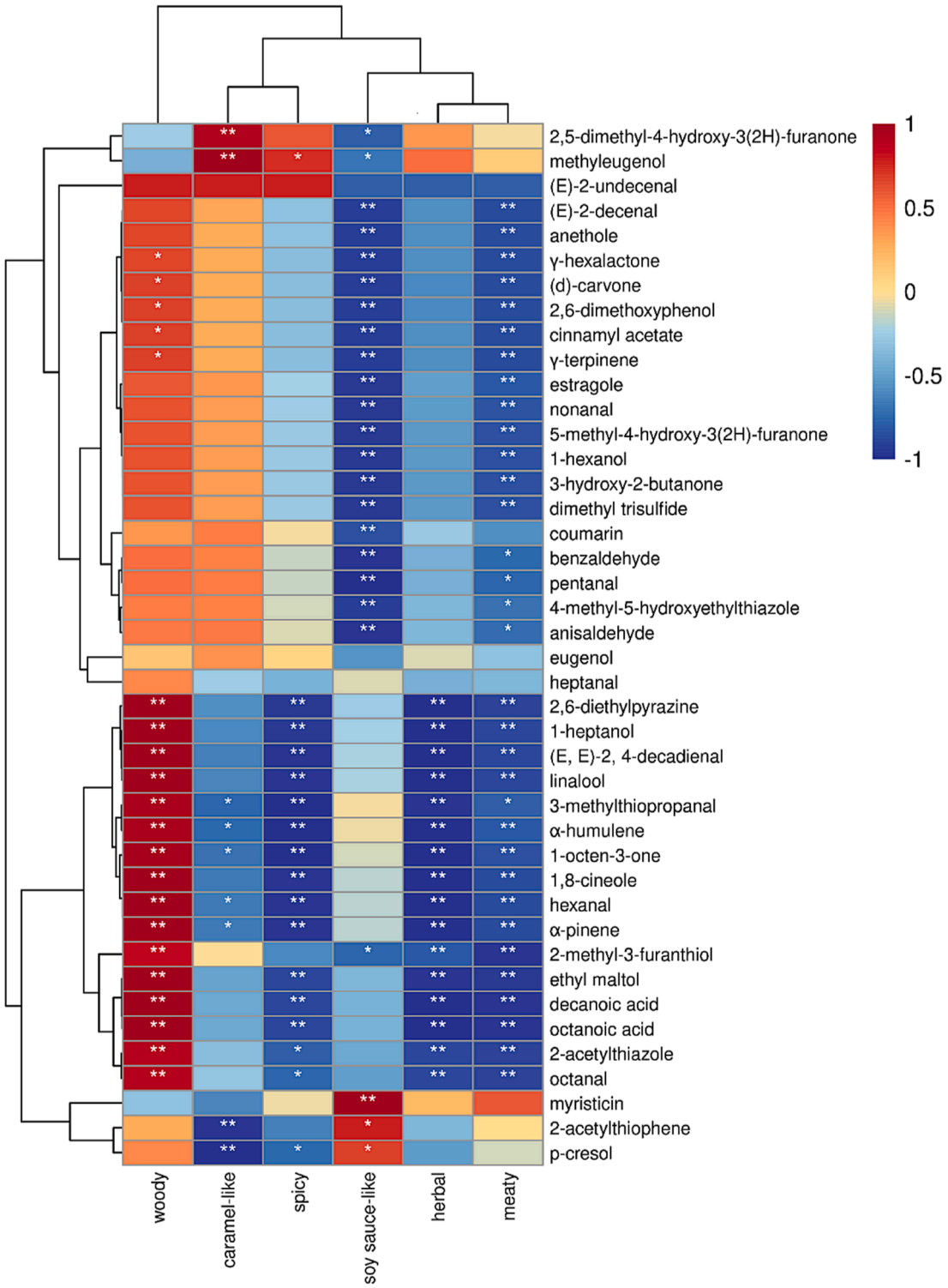


Fig. 3. The relationship between aroma compounds and flavor characteristics based on sensory evaluation. ("*", significant ($p \leq 0.05$); "**", highly significant ($p \leq 0.01$)).

sulphide, a species-specific component of beef, had a high OAV. During heating, methionine sulfoxide and glucose combine to generate dimethyl sulfoxide, which is degraded during pre-cooling in brine tanks. 5-Methyl-4-hydoxy-3(2*H*)-furanone can be converted into 2-methyl-3-furanthiol through Strecker degradation of cysteine (Sohail et al., 2022). In contrast, 2-methyl-3-furanthiol has a relatively low odor threshold and strongly contributed to the meaty aroma. Ribose/cysteine forms 3-methylthiopropanal from thiamine (Sohail et al., 2022). 3-Methylthiopropanal can be generated by Strecker degradation of methionine (Sohail et al., 2022). 2-Acetylthiazole was only identified as a key aroma-active compound in SSMB-100, whereas 2-acetyl-1-pyrroline was only identified in SSMB-10. Both are characterized by a popcorn aroma. Thus, although the caramel-like attribute scores of the three APE samples were similar, their key aroma-active compounds differed. Sulphur-containing compounds contribute to the unique aroma of meat products. Among the most important amino acids for the meaty

flavor of Maillard compounds are those containing sulphur, especially Cys (Hou et al., 2017; Sohail et al., 2022). For example, Gly and Val promote the formation of pyrazines, while Leu and Ile promote furfural formation (Hou et al., 2017). Proteolytic breakdown increases the content of peptides and free amino acids during aging. Our recombination experiments showed that sulphur-containing compounds such as 4-methyl-5-hydroxyethylthiazole and dimethyl trisulfide are key aroma-active compounds in SSMB. Sulphur-containing substances play crucial roles in meaty and caramel-like attributes. The thermal reactions of sugars or peptides with cysteine are linked to sulphur-containing compounds (Hou et al., 2017). The reaction between homocysteine thiolactone and glucose results in the production of dimethyl trisulfide (Yu, 1995). The Maillard reaction between methionine and glucose is involved in the production of 3-methylthiopropanal. During pre-cooling, the temperature in the brine tank was still sufficient to provide suitable conditions for the Maillard reaction. Many spices and herbs in SSMB marinating fluid have spiciness and herbal attributes that can be transferred to the SSMB. Compared with those of SSMB-100, the variety and content of flavors in SSMB-45 were more comprehensive and enriched during pre-cooling. Marinating fluids contain NaCl and spices, which enhance protein solubility and hydration. Therefore, the water-binding capacity of SSMB was improved and marinate fluid absorption enhancesd the comprehensive flavor profile of SSMB.

In addition to the characteristics of proteins and aromas, external or environmental factors, including temperature, oxidizings state, pH, and ionic strength, influence protein-flavor interactions (Shen, Zhao, & Sun, 2019; Sohail et al., 2022). Desorption was increased by the direct effect of the rising temperature on taste, which enhanced the activity coefficient. Moreover, the tendency for natural proteins to aggregate when heated reduces their protein-flavor-binding ability, which is an indirect effect (Shen et al., 2019). As a result, competition caused by conformational adjustments of proteins and the activity of flavor compounds after heat treatment determines whether flavor compounds are retained or released. Low temperatures are typically beneficial for the binding of flavor compounds to myofibrillar proteins (Y. Xu et al., 2020). In our study, higher temperatures (stewing) increasesd the number of binding sites by exposing interior hydrophobic sites. Although higher temperatures promote the binding of aromatic substances to proteins, lower temperatures require longer processing times (precooling in marinating fluid). During preparation and storage, cooled beef may undergo oxidation reactions. Proteins can be oxidised by various reactive oxygen species, which can change their functional properties, such as their ability to bind flavors (Shen et al., 2019). At high oxidation levels, the hydrophobic association was weaker and covalent interaction was stronger (Zhang, Kang, Zhang, & Lorenzo, 2021). Strong interactions eventually form between the flavoring and protein in covalent reactions, which normally occurs slowly but continue throughout the shelf life (Anantharamkrishnan & Reineccius, 2020). Volatilisation is another important method for SSMB flavor dissipation. After processing in the air-cooling tunnel, the SSMB showed severe flavor loss. Interestingly, the $\log_2$FD of some aroma compounds increased after the air-cooling tunnel, such as *p*-cresol, 1-octen-3-one, myristicin, 2-acetylthiophene, octanoic acid, and α-humulene. This may be because of steric hindrance; during cooling, buried binding sites are more accessible to small flavor compounds than to large molecules. Before pea protein gels are formed, addition of aldehyde flavors may cause additional protein denaturation, resulting in the creation of stronger gels. Additionally, steric hindrance of the gel network or mass transfer from the gel to the gas phase may impact flavor release behaviour (Shen et al., 2019).

4. Conclusion

Sensory-directed flavor analysis revealed 29 major aroma-active compounds in SSMB, including eugenol, 4-methyl-5-hydroxyethylthiazole, dimethyl trisulfide, 1-octen-3-one, anethole, linalool, 1,8-cineole, 3-hydroxy-2-butanone, (*E, E*)-2,4-decadienal, hexanal, nonanal, dodecanal, and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone as the most important key odorants for overall aroma. Despite their similar compositions, the three samples exhibited distinct aroma profiles and key aroma-active compound compositions. During the pre-cooling stage, these flavor substances, such as eugenol and anethole, are enhanced by soaking in the marinated fluid. Through the air-cooling tunnel process, flavor is severely dissipated. The aldehydes created by lipid oxidation were partially responsible for the differences in meaty attribute characteristics between the three SSMB samples. Although they exhibited similar caramel-like attributes, their compositions differed because of the Maillard reaction. Further studies are needed to evaluate the dynamic kinetic changes in key aroma-active compounds throughout the cooling process of SSMB. Various control techniques for flavor dissipation, including editing techniques, need to be investigated further. Correlations between protein structure and key aroma-active flavor compounds were investigated to clarify the flavor dissipation mechanism during the cooling process.

CRediT authorship contribution statement

Jingfan Wang: Investigation, Methodology, Writing – original draft. **Ping Yang:** Methodology. **Junmei Liu:** Methodology, Conceptualization, Writing – review & editing. **Weifang Yang:** . **Yu Qiang:** Methodology, Conceptualization. **Wei Jia:** Conceptualization, Methodology. **Dong Han:** Conceptualization, Writing – review & editing. **Chunhui Zhang:** Resources, Writing – review & editing, Funding acquisition. **Giorgia Purcaro:** Methodology, Writing – review & editing. **Marie-Laure Fauconnier:** Investigation, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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