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Research Article

Characterization of the Universal Flavor in Chinese Butter Hotpot by Multiple Mass Spectrometry Detection Technology

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Hotpot provides a multilevel unique flavor experience and is preferred by consumers who value various taste preferences. In the present study, results showed that alcohols, phenols, hydrocarbons, and others were the commonly predominant volatiles in all butter hotpot samples (74.43%~92.92%). However, there were merely 25 codetected compounds determined among a total of 318 aroma compounds because of the discrepant ingredients and processing technique of hotpot samples, which were sampled from several representative manufacturers. Therefore, for the first time, multiple GC-Q-TOF/MS and GC-Orbitrap-MS methods were performed to explore the more 44 potential aroma compounds of butter hotpot, in which alpha-terpinyl, acetate, nonanal, piperitone, and (E)-lignstilde could further cause the differences in flavor intensity of spices aroma, smell of grease, and roasted, charred, and nutty ($p < 0.05$). Linalool and nerol were the critical flavor precursors associated with ingredients and processing technic. Therefore, these results provide guidance for improving the butter hotpot formula and process technique.

1. Introduction

Hotpot is a traditional Chinese cuisine, and its origin can be dated back two thousand years ago. Hotpot is a kind of popular way of having a meal which is accepted by most customers for its simplification and peculiarity. The market scale of hotpot was more than 4998 billion yuan (¥) in China, in 2021, which included the northern faction characterized by tingle, spicy, and burn and the north faction represented by mutton hot pot. Haidilao, Bajiangjun, Xiaofeiyang, and Xiaolongkan are the top four brands of the southern butter hotpot, and they are particularly well known for their balanced combination of spicy taste and mouth-watering aroma. The typical butter hotpot represents a highly complex matrix that mainly includes beef butter, dried chili, scallion, ginger, garlic, rock sugar, rice wine, Pixian Doubanjiang [1], and some spices, including peppercorn, anise, and cilantro, as ingredients [2, 3]. Although the potent aroma and taste of butter hotpot mainly rely on rich ingredients, it is believed that the manufacturing technique is also responsible for the flavor extraction and development process. The manufacturing device can set the frying temperature and time; thus, the mainstream technique of butter hotpot is a combination of stir-frying and extraction. Hotpot ingredients are put into the manufacturing device; wok stirs automatic bidirectional rotation at the designed high temperatures (120°C–150°C). Subsequently, after the frying is finished, the manufacturing device stops stirring and keeps the temperature (100°C–110°C) for extracting for a certain time. The characteristic flavors produced during the manufacturing process mainly come from the ingredients, such as oil, as well as the interactions among the fatty acid, sugar, and so on [4]. Therefore, besides the ratio and order of ingredients, the set of techniques is the other key point for flavor controlling of butter hotpot and further exhibits a significant influence on customer preference.

Consumer acceptability and market competitiveness are directly affected by the odor of food [5]. 2-Acetylthiazole,

anethole, (E)-2-decenal, 1,8-cineole, (E, E)-2, 4-decadienal, nonanal, and so on significantly contributed to the aroma profile of Chinese butter hotpot seasoning and constituted the basic characteristics of the overall aroma based on the results of sensory-oriented flavor analysis and identified by aroma recombination and omission experiments [2, 6]. However, the other two research studies [3, 7] reported that linalool, (+)-limonene, 4-acetylpyrazine, toluene, and so on were recognized as the original aroma compounds of butter hotpot seasoning, although these samples were all from the same manufacturer. It can be inferred that the flavor profiles are significantly affected by its ingredients, formula, or even batch [8]. In addition, the dominant terpene and alcohol contents varied with manufacturers, among which γ-elemene, carene, α-piperene, and so on could be used as markers to distinguish the brands of butter hotpots [9]. Therefore, understanding the qualities of butter hotpot aroma at the molecular level is essential for the development of high-quality products. The identification of the complex flavor constituents in food remains a challenge of inconsistent detection methods. Among the various extraction methods, solid-phase microextraction (SPME) and solvent-assisted flavor evaporation (SAFE) show definitely different flavor profiles of hotpot [2, 7], as well as the detection techniques. The basic principle of GC-MS is that isolated compounds (through GC) are further ionized by electron impact, and the different mass-to-charge ratio molecular ions are detected by a detector. Meanwhile, the refreshing principle of GC-Q-TOF/MS is that the ionized ions are better separated by accelerators and flight tubes. The detection of the updated GC-Orbitrap-MS relies on its Orbitrap (an ion trap mass analyzer), ions oscillated around the center electrode, and two outer electrodes and are further isolated with different oscillation frequencies. GC-MS is the most used equipment in flavor science with high qualitative ability, with the inability to distinguish many isomers (especially positional isomers). By comparison, GC-Q-TOF/MS and GC-Orbitrap-MS have the advantage of working with a high resolving power and mass accuracy and are suitable for the exploration of unknown flavor compounds. Both methods have been extensively used to reveal the flavor constituents of ham [10], wines [11], and so on. Therefore, the characterization of the flavor of hotpots is affected by some factors and detection methods; meanwhile, the uniform standards for butter hotpots' sensory evaluation and awareness of key aroma compounds are still unclear so far. Thus, it is necessary to characterize the universal flavor profiles of butter hotpots and the potential sources of the typical compounds.

For a more comprehensive understanding of the flavors of butter hotpots, we aimed to characterize the flavor compounds in eight groups of butter hotpots by GC-MS and to further screen the more aroma compounds based on GC-Q-TOF/MS and GC-Orbitrap-MS. The analytical strategy and sensory evaluation are basically intended to be used for revealing the universal flavor profiles when this issue has not been sufficiently addressed in the butter hotpot. In addition, it is devoted to tracing the sources of the dominant flavor compounds according to the typical flavors of ingredients. This study will provide a scientific basis for improving the intensification of the butter hotpot formulas and the control of process engineering.

2. Materials and Methods

2.1. Samples Preparation. Butter hotpot flavoring sample was purchased by several local supermarkets (Chengdu SNS Biotechnology Co., Ltd., Chengdu Shu-Da-Xia Catering Management Co., Ltd., Sichuan Haidilao Catering Co. Ltd., Chengdu Xiaolongkan Food Co. Ltd., Chengdu Yangming Food Co. Ltd., and Sichuan Tianwei Food Group Co. Ltd.) and those were popular products items in China. To avoid the uneven condition of the solid-liquid mixture system of samples, the research samples were composed of three parts: the prophase, the middle, and the anaphase stage fluids. The prophase, middle, and anaphase parts of the centrifuged sample are mostly liquid oil, solid-liquid mixtures, and solid ingredients, respectively, because of the gravity effect of ingredients. The prophase, middle, and anaphase parts of the hotpot sample were well mixed into an individual sample, and then the mixing sample was sampled thrice for further analysis. Subsequently, those samples were directly transported to laboratory at 4°C and kept at −20°C before being analyzed. There were a total of 21 samples (HG-01~HG-21) analyzed and grouped into eight groups (A, B, C, D, E, F, G, and H), which were sampled from different manufacturers or the different batches of identical manufacturers.

2.2. Analysis by SPME Arrow GC-MS. Volatile compounds (VCs) were extracted by SPME Arrow fiber (1.1 mm DVB/Carbon WR/PDMS, Stableflex, Supelco, Inc., USA) and separated using an Agilent 8890–5977 B GC-MS (gas chromatography-mass spectrometry) equipped with a VF-WAX-MS capillary column (30.0 m × 0.25 mm, 0.25 μm, Agilent, Santa Clara, USA). Methyl caprylate (CAS: 11-11-5, purity 99%, Sigma-Aldrich, St. Louis, MO, USA) dissolved in methyl alcohol (purity 99.9%, GC standard, liquid Aladdin, Shanghai, China) was prepared as internal standard (IS) (final concentration, 0.0073 g/100 mL). Before the SPME Arrow process, the IS (20 μL) was added into vials manually using a syringe. Samples were pre-equilibrated at 60°C for 15 min after activating the SPME Arrow fiber and then extracted for 45 min at the same temperature. Subsequently, the extraction was directly injected into the injection port of GC-MS and desorbed at 270°C for 5 min. The detection protocol was the same as that described by Yang et al. [1] with some modifications. The analytical conditions were as follows: the temperature of the column was maintained at 40°C for 5 min, ramped to 100°C at 4°C/min, and then increased to 230°C at 6°C/min and held for 10 min. The carrier gas was helium (>99.99%) at a constant flow rate of 1 mL/min, solvent vent mode. The ion source temperature was 250°C, and the scan range of MS was m/z 30–450. The peak identification was carried out by comparing their mass spectra with the NIST2020 library database on the basis of the following criterion similarity (SI) > 800 (the highest value is 1000). The identified compounds were further

confirmed with the standard compounds available. Retention indices (RIs) were calculated with n-alkanes (C4–C30, Sigma, Aldrich Trading Co., Ltd., Shanghai, China) as the external references under identical experimental conditions.

2.3. Sensory Evaluation. According to the method described by Yu et al. [6] and Yang et al. [1], with some modifications, 8 panelist evaluators (4 males and 4 females, 20 to 40 ages) regularly engaged in the sensory analysis were selected from Chengdu SNS Biotechnology Co., Ltd., and Sichuan Academy of Agricultural Sciences. Among them, 6 panelists had the professional skills of previously participating in sensory evaluation tests for seasoning condiments, such as hotpot, soybean sauce, chili paste, and beef tallow with more than two years of experience. The other 2 panelists have a keen sense of smell. They were trained twice a week in a month according to the process described by Gao et al. [12] before formal sensory evaluation. The training included the basics of sensory analysis, identification of sensory properties, and the establishment and use of the scales.

One sample of each group (A~H) was selected for sensory evaluation. 20.0 g of each sample was served in a standard white disk covered, coded with a 3-digit random code, and eight samples were randomly selected for evaluation by the sensory panelists. Those solidified samples were digested in liquids before evaluation. Assessors were required to evaluate each sample with a short break at room temperature and standard white light. Sample evaluation was performed in duplicate. The panelists were asked to describe the samples in four aspects: flavor, taste, appearance, and coordination. To visualize the sensory outcomes, descriptors were converted to scores from one to nine, and the results were plotted in a spider web diagram.

2.4. Analysis by SPME Arrow GC-Q-TOF/MS. Deep analysis by GC-Q-TOF/MS (Gas Chromatography Time-of-Flight Mass Spectrometry) was carried out on a selected typical sample which covered most types and contents of the volatiles based on GC-MS analysis. Volatiles of HG-12 (Table S1) were extracted using the same SPME Arrow fiber, the process of which was according to the method described in 2.2. Then, the adsorbed volatiles were desorption at 260°C for 3 min and were analyzed by an Agilent 8890−7250 GC-Q-TOF/MS system equipped with a DB-Heavy WAX column (60 m × 0.25 mm × 0.50 μm, Agilent, Santa Clara, USA) according to the method described by Xu et al. [13]. The initial temperature of the GC oven was at 37°C for 3 min, then the temperature increased to 100°C at a 6°C/min rate, and finally increased to 260°C at a rate of 10°C/min. Helium (99.99% purity) was used as carrier gas and flowed at 1 mL/min, and the split ratio was 5 : 1. The ionization energy (EI) was EI-70 eV, and low-energy was 12 eV, the ionization temperature was 200°C, and the quantity scanning range was from 20 to 550 amu. Each compound was compared with the reference spectra (the NIST libraries), and the retention times for n-alkanes (C4–C30) under the same condition were used to check volatiles.

2.5. Analysis by SPME GC-Orbitrap-MS. Similarly, the same sample (HG-12) with a 2.3 was further determined by GC-Orbitrap-MS. A GC-Orbitrap-MS system (Q Exactive GC, Thermo Scientific, Bremen, Germany) consisting of a TriPlus RSH autosampler was used. The helium carrier gas (99.99% purity) flowed at 1.2 mL/min. SPME fiber (DVB/CAR/PDMS, Supelco, Inc., Bellefonte, PA, USA) was used to gather volatiles, as the same method as in 2.2. Then, the absorbing volatiles were desorbed at 270°C for 3 min, and the split ratio was 20 : 1. GC separation was performed on a 60 m × 0.25 mm × 0.25 μm TG-WAXMS column (Thermo Scientific) using the same temperature program of 2.2. EI was performed at 70 eV with the source temperature set at 250°C. Full scan MS acquisition was done in profile mode using an m/z range of 30–450. The actual resolution of MS was 60000. Similarly, the qualitative of volatiles was performed by the NIST database and RIs (C4–C30).

2.6. Statistical Analysis. GC-MS data were tabled in the form of average values ± standard deviation using Microsoft Excel 2021 (Microsoft Co., USA). The statistically significant differences among data were analyzed by SPSS 16.0 statistical software (IBM Inc., Chicago, IL, USA) using analysis of variance (ANOVA) at $p = 0.05$ level. Principal components analysis (PCA) and partial squares discriminant analysis (PLS-DA) were performed using SIMCA-P (Umetrics, Sweden) to visualize the difference in volatiles between samples.

3. Results and Discussion

3.1. Identification of Volatiles in Butter Hotpot by SPME-Arrow-GC-MS. Samples were clustered into eight groups because of their manufacturers and categories. SPME-Arrow-GC-MS was used to analyze volatile flavor compounds in samples at different manufacturers, as well as the different categories of identical manufacturers. The total contents ranged from 369.39 ± 38.96 mg/kg to 2739.32 ± 439.97 mg/kg, and a total of 570 volatiles were identified, including 48 alcohols, 85 esters, 19 phenols, 57 aldehydes, 67 ketones, 33 acids, 102 hydrocarbons, and 159 other compounds in terms of their chemical structure. Generally speaking, alcohols, phenols, hydrocarbons, and others were the predominant volatiles in all samples, with the total of those accounting for 74.43%~92.92%. From the stacked graph (Figure 1(a)), it can be found intuitively that the types of volatiles in samples have changed significantly as the different manufacturers and separate categories. For example, the B group (HG-01, HG-02, and HG-03) was clustered into a single cluster as the extremely low esters. In particular, the content of linalyl acetate in the B group was significantly lower than that in the other groups, in which precursors linalool and acetic acid had correspondingly low levels. Among them, linalool mostly originated from ingredients such as *Capsicum*, and *Prickly Ash* [14–16]. It was worth noting that the content of eucalyptol in the B group was significantly higher than that in the other groups, which originated from some individual plants and with strong antibacterial activity against food-

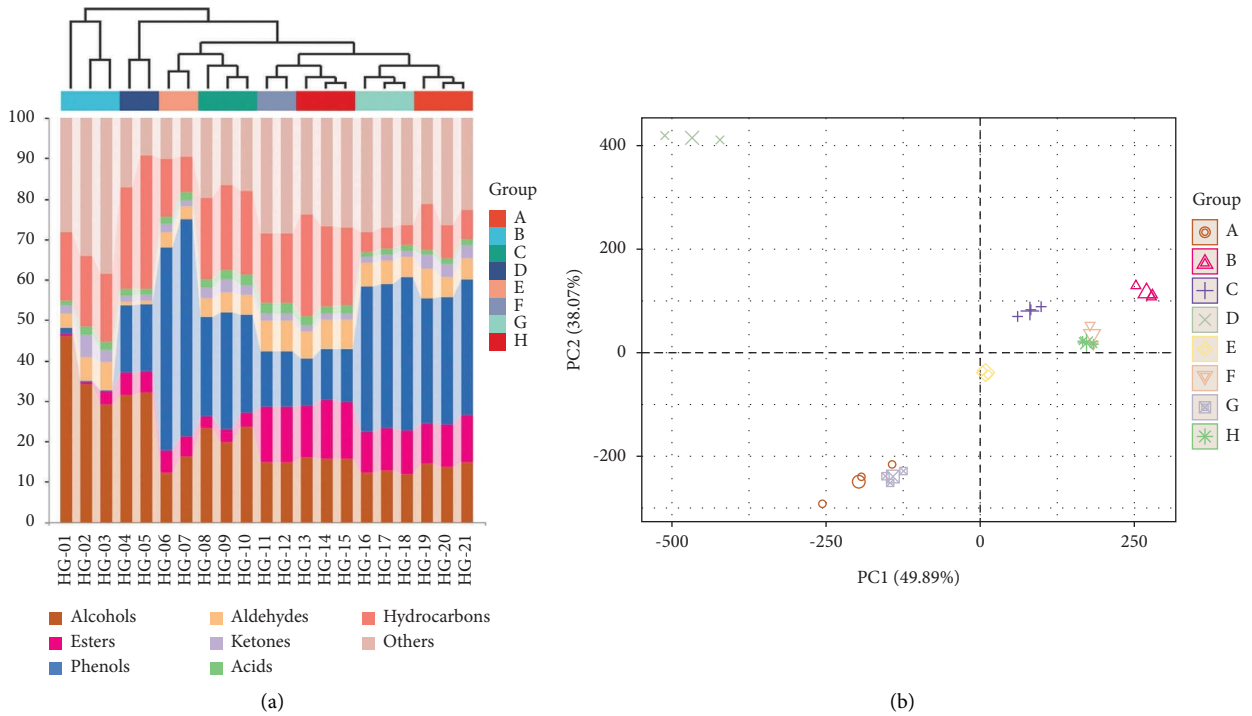

Figure 1: (a) Relative content of classified volatiles and cluster analysis. (b) PCA plot of groups based on volatile compounds. Volatile compounds are classified into eight classifications in terms of their chemical structure.

borne pathogens [17]. Therefore, it can be inferred that the volatiles difference was mainly related to the specific ingredients. In the other cluster, the D group (HG-04 and HG-05) was classified into a single subcluster due to the higher alcohols, especially the linalool-endowed floral aroma. In addition, the E group (HG-06 and HG-07) showed the highest phenols, in which ethyl maltol was usually used as a fixative agent derived from exogenous addition. Significantly, the internal volatile composition of the C group was roughly the same because the samples were collected from identical manufacturer with discrepancies in categories, as well as the F, H, G, and A groups. The higher esters in F, H, G, and A groups owed to linalyl acetate, which with a floral and fruity aroma. Furthermore, the other class was highlighted in all samples as the predominant anethole and estragole originating from spice materials. PCA analysis was performed to reveal detailed information on the differences in volatiles between groups. As shown in Figure 1(b), the PCA bi-plot explained 87.96% of the total variance in the first two dimensions. Groups were clearly separated in the PCA plot, indicating that the butter hotpot flavor was significantly varied by manufacturers and categories. It was consistent with what Sun et al. [2] reported; hotpots collected from different companies showed a decentering relation in the statistical analysis loading plot.

3.2. Origin of Predominant Volatiles. To screen the potential aroma compounds varying with ingredients and their processing technique, 317 aroma compounds are visualized in Figure 2(a). 25 compounds were codetected in all groups, accounting for 49.71%–88.84% (Figure 2(b)), which indicated that the backbone of the butter hotpot flavor was certain and similar because that the ingredients formula was roughly same. In addition, there were 53–84 aroma compounds specifically identified in each group, presuming that the ingredients' proportions and their processing technic were different. In general, anethole, ethyl maltol, linalool, linalyl acetate, eucalyptol, eugenol, and estragole were predominant, which was consistent with the previous report [3]. Among them, anethole, eugenol, and estragole originated from aniseed (*Illicium verum*), clove (*Syringa oblata Lindl.*), and pepper (*Piper nigrum* L.), respectively (data not shown), the odor-active of which was verified by aroma recombination and omission tests [2]. Similarly, eucalyptol was relatively high, especially in the A, D, and G groups, indicating that the raw dry matter was more than in other groups. It was worth noting that (E)-2-decenal, (E)-2-octenal, (E, E)-2, 4-decadienal, (E)-2-nonenal, and nonanal were highlighted in the F and H group, which were associated with the quality of beef tallow [18]. For example, nonanal with a typical and strong fatty odor is regarded as the key odorant whatever the beef tallow origin (data not shown). In addition, ethyl maltol was a mixture of commercial preparations [19], which is entirely from the exogenous additive.

PLS-DA is a multivariate statistical analysis method with supervised pattern recognition. PLS-DA was performed to separate the sample groups based on the dominant aroma compounds (relative content >1%) after data normalization. As shown in Figure 3(a), the D group was separately located on the fourth quadrant with a higher content of linalool, D-limonene, octanoic acid, and cis-allocimene. The A and G groups were

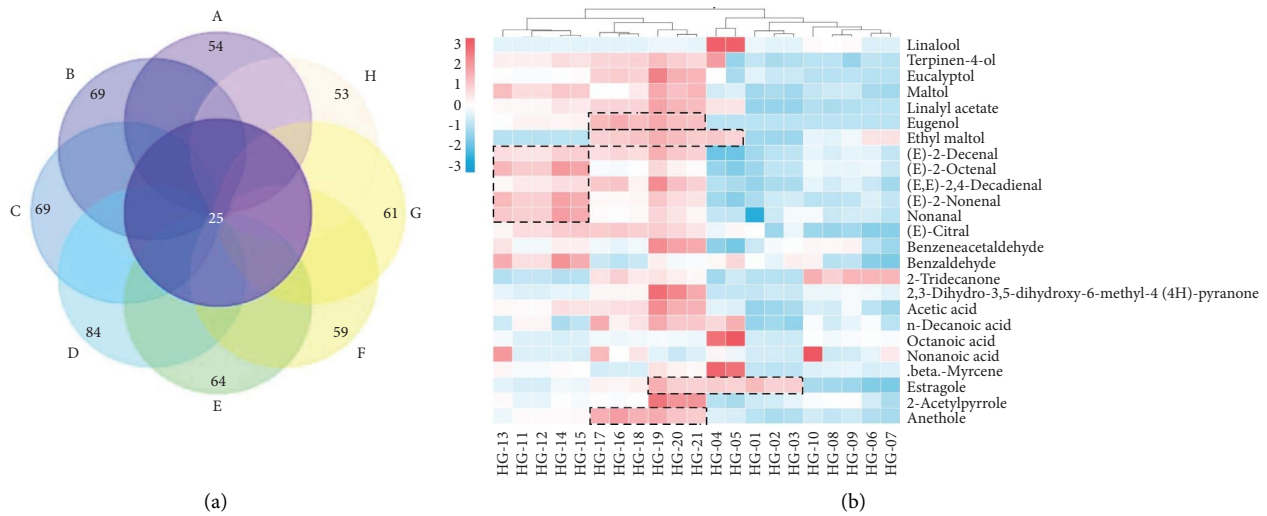

FIGURE 2: (a) Venn diagram of the aroma compounds. (b) Heatmap analysis of the codetected aroma compounds. Venn diagram is used to visualize the similarities and differences of the varieties of aroma compounds which are the aroma parts of volatile compounds. The codetected aroma compounds mean the compounds detected in all samples.

surrounded by most aroma compounds, especially neral, maltol, 4-terpinenyl acetate, and so on. In addition, the aroma composition and intensity of the B, C, E, F, and H groups were roughly the same. The results of R^2 (0.0347) and Q^2 (−0.154) ensured that the model was not overfitting. Therefore, variable importance in projection (VIP) values were calculated from the PLSR model and used to recognize differential aroma compounds. Among these 49 aroma compounds, 11 had VIP values higher than 1, suggesting that they contribute significantly to the butter hotpot aroma profiles. As illustrated in Table S2, most differential compounds originated from ingredients; in particular, D-limonene, sabinene, beta-phellandrene, and beta-myrcene were the primary components of spiceries. In addition, ethanol was merely identified in four groups, which were associated with the ingredient of fermented glutinous rice [13]. In fact, besides the ingredients, the composition and content of aroma compounds definitively varied with manufacturing technique. Compounds mentioned in Figure 3(b) were detectable and aroma-contributing in samples. The synthesis of most compounds involved in multiple paths, in which linalool and nerol were the key intermediate components referred to many synthetic paths. In addition, besides the origin of materials, (E)-2-nonenal, maltol, and eugenol were the island models in the pathway; as is to say, there were unique precursors detected in samples. Interestingly, (E)-2-octenal, (E, E)-2, 4-decadienal, and (E)-2-decenal shared precursors and commonly endowed fat, meat, and green fragrant. Therefore, the overall aroma profiles were roughly the same caused by the technique process, with discrepancies in strength, although some precursors and their content originated from materials were not the same.

3.3. Sensory Evaluation. Sensory descriptive analysis was used to realize the special flavor and taste of butter hotpot. The detailed evaluation criteria were according to the sensory wheel and descriptors reported by Yu et al. [6]. Some descriptors were proposed by panelists (Table S3), and there were three dominant flavor attributes of hotpot, which were descriptors of the chili-like aroma, fatty/meaty aroma, and soy sauce-like aroma. Therefore, the quantitative descriptive analysis was conducted using the mentioned colour and lustre, chili-like, fatty and meaty aroma, soy sauce-like, spicy and numb taste, and salty and umami taste descriptors as a guide. As for the aroma profile (Figure 4), the sensory profiles of those samples were roughly the same, with discrepancy in intensity. Among them, the turbidity and pleasing colour showed a big difference ($p < 0.01$) in various samples. This might be due to the ratio of crude fat, foods with a high starch content, pepper with a high water content, and so on. In addition, the highest sensory evaluation score of the chili-like aroma was recorded in the sample HG-04, with the relatively low soy sauce-like score. It was associated with the ratio of capsicum/capsicum oleoresin [20] rather than broad bean paste [6]. Similarly, HG-11 with the highest scores of fatty and meaty aromas varied with the quality of beef tallow [18]. It was clear that the umami taste of butter hotpot largely depended on monosodium glutamate, chicken bouillon, yeast extract, amino acids, and so on, and the salty taste is provided by salt. Therefore, the formula of samples was to a great extent the intensity of its salty and umami taste ($p < 0.01$). By the way, the butter hotpot is noted for its spicy and numb taste; thus, the scores of those samples were roughly the same. Furthermore, as for the overall coordination scores, samples except HG-04, HG-11, and HG-19 have undifferentiated scores, indicating that these samples had greater flavor acceptance. More importantly, the overly projected flavor probably caused the unfriendly coordination.

3.4. Deeply Characterizing the Volatiles by GC-Q-TOF/MS and GC-Orbitrap-MS Overall Profiles Compared to GC-MS Results. To further reveal the flavor characteristics of butter hotpot, GC-Q-TOF/MS and GC-Orbitrap-MS were used to

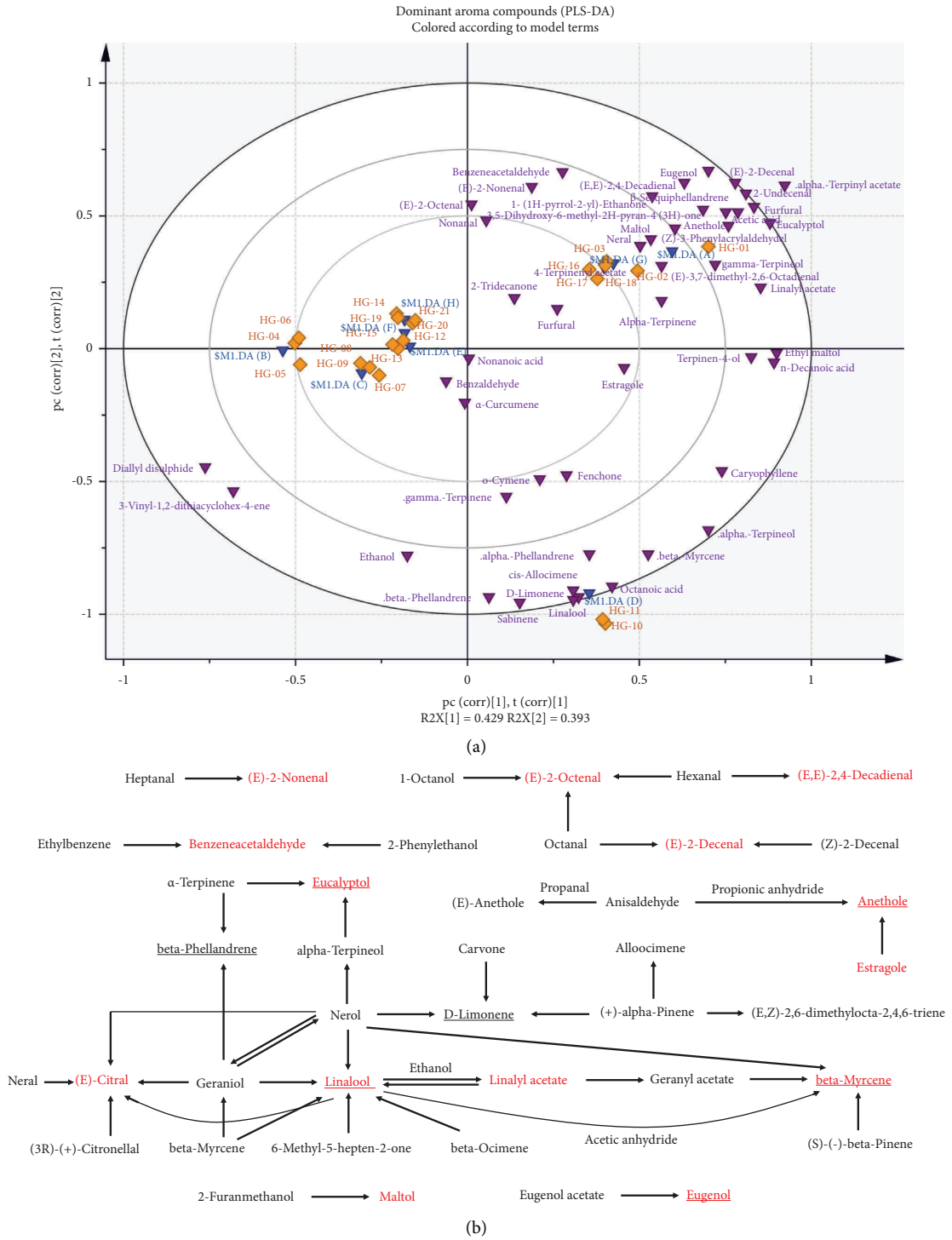

Figure 3: (a) PLS-DA plot of groups based on dominant aroma compounds (relative content >1%). (b) Potential synthetic route of the dominant and differential aroma compounds. The red labels represent the codetected and dominant compounds, and the underlined labels represent the differential compounds with VIP > 1.

explore many more aroma compounds of sample HG-11 (Figure S1), thus avoiding the inaccuracy caused by low-resolution GC-MS. A total of 377 VCs were identified by the GC-Q-TOF/MS, of which 234 compounds possess aroma contribution (Table S4). Meanwhile, 138 aroma compounds were detected by GC-Orbitrap-MS among the total 277 VCs (Table S4). Among the dominant aroma compounds (relative content >1%), eucalyptol, linalool, (E)-cinnamaldehyde, benzeneacetaldehyde, (E, E)-2, 4-decadienal, (E)-2-decenal, sabinene, anethole, eugenol, ethyl maltol, acetic acid, linalyl acetate, and estragole were simultaneously characterized by those three methods, those were roughly present in the core

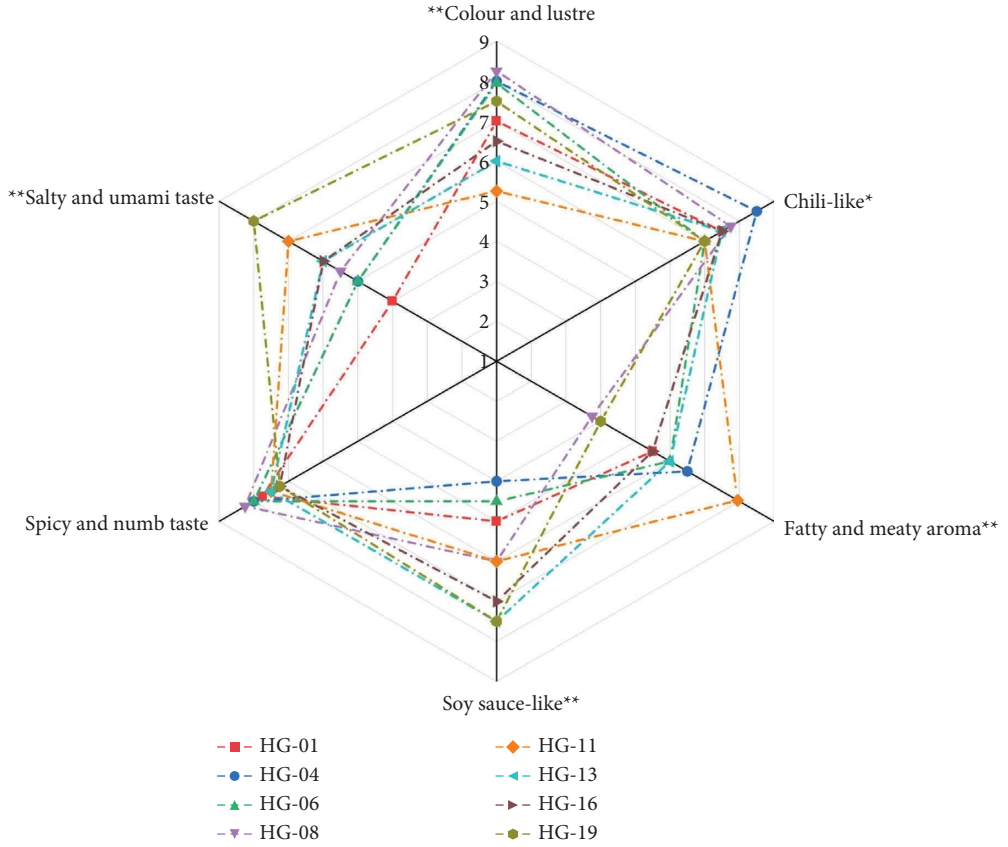

Figure 4: Sensory profiles of butter hotpot with the ratings given by the trained panel. The statistic difference is calculated by statistical significance ($^{**}p < 0.01$ and $^{*}p < 0.05$).

flavor of butter hotpot [3]. Compared to the GC-MS results, the coverage rate of the codetected compounds was 68.38% (GC-Q-TOF/MS) and 38.41% (GC-Orbitrap-MS), respectively. In compounds, 7 esters, 6 aldehydes, 5 alcohols, 5 terpenes, 3 phenols, 3 acids, 3 ketones, and 12 others were merely detectable by GC-Q-TOF/MS and GC-Orbitrap-MS. The flavor characteristics of the differential esters were floral, fruity, herbaceous, and fresh green, especially alpha-terpinyl acetate. Similarly, nonanal and hexanal endow the smell of grease and meaty aroma, thus further resulting in greater flavor intensity. It is worth noting that trans-beta-ocimene, trans-isoeugenol, piperitone, feniculin, (E)-ligustilide, cinnamyl alcohol, methyl 4-methoxybenzoate, and xanthoxylin originated from spice (data not shown). 2,3,5-Trimethylpyrazine and tetramethylpyrazine possess roasted and cocoa aromas, which were also the typical aromas of doenjang [21], baijiu [22], and so on. In addition, copaene was the isomeride of (S)-(−)-β-pinene, which blends with pine fresh, as well as α-terpinolene.

The 311 aroma compounds were classified into five sensory descriptors in terms of their flavor characters. As shown in Figure 5(a), spice, grease, floral, and fruity aroma were the primary aroma characteristics. The flavor profiles were roughly the same, with discrepancy in concentration, which may be associated with compound diversity. In this study, spearman correlation analysis was carried out to further reveal the correlation between the sensory descriptors and the 24 flavor compounds (relative content >1%) which were determined and classified by those three methods (Figure 5(b)). As a whole, 21 pairs of positively and extremely significant correlations were identified, while 16 negative pairs ($p < 0.01$, R spearman >0.6). It is in accordance with the interactions between systematic flavor compounds [23]. Results showed that anethole, sabinene, and so on contribute to the spice aroma which is the dominant flavor characteristic of butter hotpot. The smell of grease feature is related to eucalyptol, linalool, and so on.

3.5. Differential Flavor Compounds Determined by GC-Q-TOF/MS and GC-Orbitrap-MS Results. According to the flavor compounds determined by GC-MS, alcohols, phenols, hydrocarbons, and others were the predominant classifications of butter hotpot. Therefore, GC-Q-TOF/MS and GC-Orbitrap-MS methods were performed to identify the more flavor compounds that cannot be determine by GC-MS. As shown in Figure 6, phenylethyl alcohol possesses a floral aroma, which may derive from the material broad bean sauce [1]. The level of trans-isoeugenol is usually used to elucidate the effects of the barriers' maturity and the geographical origin of peppers [24]. Interestingly, the isomeride of copaene and trans-beta-ocimene could be detected by GC-MS, such as beta-pinene and (E, Z)-2,6-dimethylocta-2, 4, 6-triene; it may

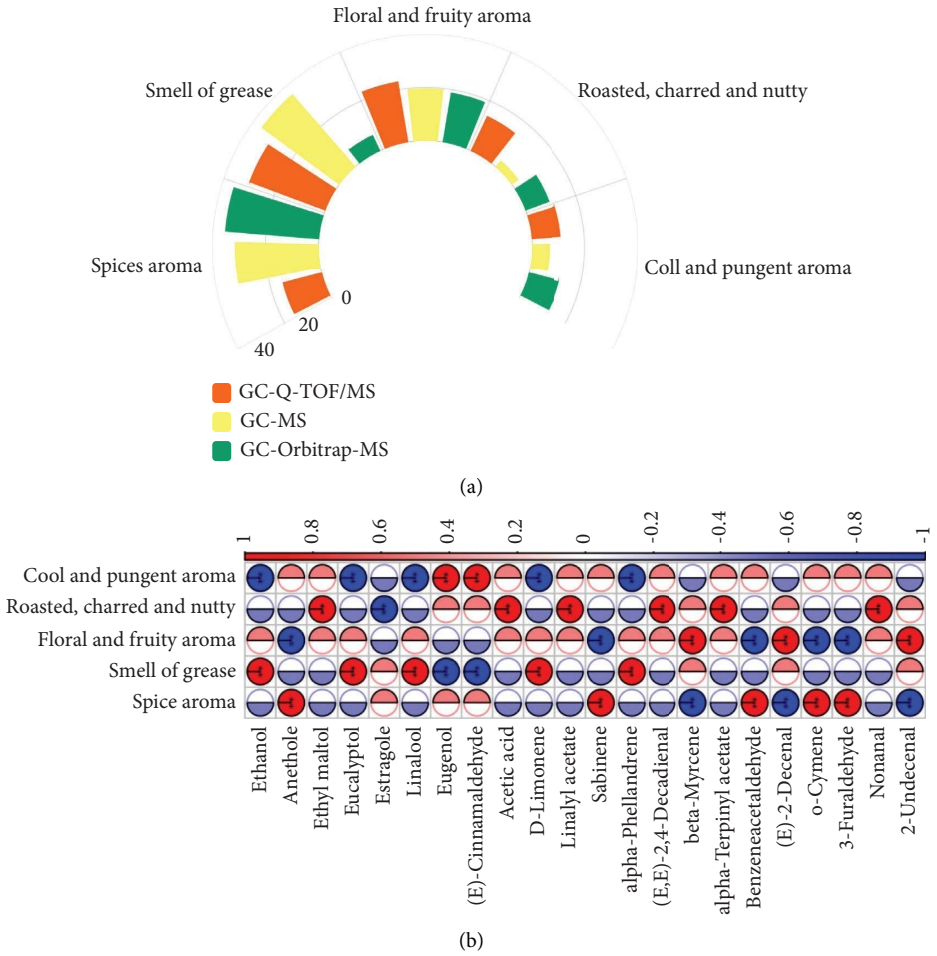

Figure 5: (a) Profiles of aroma compounds determined by those three methods. (b) Correlation analysis of the five sensory descriptors and dominant aroma compounds. GC-MS, GC-Q-TOF/MS, and GC-Orbitrap-MS were used to analyze the potential aroma compounds of butter hotpot, and those were clustered into five descriptors in terms of their aroma characters.

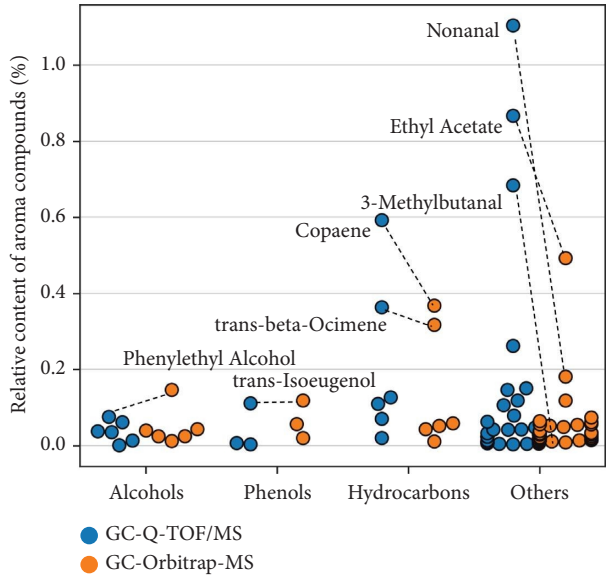

Figure 6: Differential aroma compounds determined by GC-Q-TOF/MS and GC-Orbitrap-MS methods compared to GC-MS. Those aroma compounds are detectable by GC-Q-TOF/MS and GC-Orbitrap-MS, with discrepancies in the relative content.

relate to the high resolution of both detection methods. Similarly, the same flavor characters (smell of grease) of nonanal can be endowed by (E, E)-2, 4-decadienal, (E)-2-octenal, and so on, all of them can originate from beef tallow [25]. Ethyl acetate was the esterification product of acetic acid and ethanol; it shows the same fruity aroma as 3-methylbutanal.

There were 117 aroma compounds merely identified by GC-Q-TOF/MS in comparison to the GC-MS and GC-Orbitrap-MS results, accounting for 11.35% of total VCs. Caryophyllene, gamma-terpineol, (R-(R*, R*))-2,3-butanediol, and (+)-α-terpineol with the high level. Among them, caryophyllene can originate from most spices (data not shown), with a note of spices, woody, and tangerines. The isomer of gamma-terpineol is (+)-α-terpineol, they showed high contributions due to their low threshold, the same is true of (R-(R*, R*))-2,3-butanediol and (S-(R*, R*))-2,3-butanediol. In addition, the aroma cluster of GC-Q-TOF/MS was highlighted in floral and fruity aroma, which was partly related to 19 compounds (gamma-terpineol, 2,3-butanediol, eugenol acetate, etc.). Thereinto, eugenol acetate, citronellyl formate, geranyl formate, and fenchyl acetate were synthesized by esterification reaction during the production process. Similarly, the high intensity of roasted, charred, and nutty was partially associated with 7 compounds (furfural, pentadecanal, 3-nonen-2-one, etc.). Furthermore, some sulfocompounds have been merely detected by GC-Q-TOF/MS, which included methanethiol, dipropyl disulfide, (E)-1-(prop-1-en-1-yl)-2-propyldisulfane, propyl mercaptan, methyl disulfide, (Z)-1-(prop-1-en-1-yl)-2-propyldisulfane, dimethyl sulfide, and (Z)-1-methyl-2-(prop-1-en-1-yl) disulfane; most of them probably derived from onion and ginger [26]. Compared to GC-MS and GC-Q-TOF/MS results, 39 differential aroma compounds were identified by GC-Orbitrap-MS, accounting for 4.06% of total VCs, indicating that most compounds can be covered by the other two methods. Spice aroma was dominant in flavor profiles of GC-Orbitrap-MS, associating to L-fenchonem, (Z)-anethole, (R)-cuparene, and spathulenol in part. L-Ethyl lactate and 1-octen-3-ol have a certain contribution to the cool and pungent aroma descriptor those were the major odor-active compounds of baijiu [27] and soybean products [28], respectively.

4. Conclusion

In summary, the universal flavor characteristics of butter hotpots were revealed by multiple mass spectroscope techniques. A wide range of volatile contents (369.39 ± 38.96 mg/kg~2739.32 ± 439.97 mg/kg) was detected in samples because of the different brands. Among the 25 co-detected compounds, 11 differential compounds (VIP > 1) were calculated by PLS-DA, those derived from ingredients. Subsequently, GC-Q-TOF-MS and GC-Orbitrap-MS methods were used to identify the more potential aroma compounds compared to GC-MS. Results showed that phenylethyl alcohol, trans-isoeugenol, copaene, nonanal, and so on, were the more aroma compounds identified. There were 117 compounds only detected by GC-Q-TOF/MS, accounting for 11.35% of all volatiles. The 117 compounds which accounted for 11.35% of all volatiles were only detected by GC-Q-TOF-MS, especially 37 sulfocompounds. In particular, the relative content of caryophyllene and gamma-terpineol was relatively high. Similarly, GC-Orbitrap-MS further confirmed that L-fenchonem, L-ethyl lactate, 1-octen-3-ol, and so on were the differential compounds for the overall aroma of butter hotpot. Overall, those compounds have a certain effect on the flavor profiles of spices, grease, and floral and fruity aromas. This study will provide a theoretical basis for improving the quality of butter hotpots and flavor control in the hotpot industry.

Data Availability

The authors are unable or have chosen not to specify which data and materials have been used.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

The supplementary material file included one figure and four tables. Figure was the chromatogram of butter hotpot detected by GC-Q-TOF/MS and GC-Orbitrap-MS; table of formula and rough proportions of HG-12 hotpot sample; table of variable importance in projection (VIP) values of the differential aroma compounds conducted by PLS-DA; table of sensory evaluation criteria of butter hotpot; table of volatile compounds and contents of sample HG-12 detected by GC-MS, GC-Q-TOF/MS, and GC-Orbitrap-MS. (*Supplementary Materials*)

References

[1] M. L. Yang, J. Huang, R. Q. Zhou et al., "Characterization of the flavor in traditional Pixian Doubanjiang by polyphasic quantitative detection technology," *Food Research International*, vol. 138, Article ID 109753, 2020.

[2] J. Sun, M. J. Ma, B. G. Sun et al., "Identification of characteristic aroma components of butter from Chinese butter hotpot seasoning," *Food Chemistry*, vol. 338, Article ID 127838, 2021.

[3] M. G. Yu, S. Y. Wan, H. L. Song et al., "Sensory-based identification of aroma-active compounds in Hotpot seasoning before and after boiling," *Molecules*, vol. 26, no. 19, p. 5727, 2021.

[4] A. Raza, H. Song, J. Raza, P. Li, K. Li, and J. Yao, "Formation of beef-like odorants from glutathione-enriched yeast extract

via Maillard reaction," *Food & Function*, vol. 11, no. 10, pp. 8583–8601, 2020.

[5] G. H. Qin, S. T. Tao, Y. F. Cao et al., "Evaluation of the volatile profile of 33 Pyrus ussuriensis cultivars by HS-SPME with GC-MS," *Food Chemistry*, vol. 134, no. 4, pp. 2367–2382, 2012.

[6] M. G. Yu, T. Li, S. Y. Wan et al., "Sensory-directed establishment of sensory wheel and characterization of key aroma-active compounds for spicy tallow hot pot seasoning," *Food Chemistry*, vol. 405, Article ID 134904, 2023.

[7] M. G. Yu, T. Li, S. Y. Wan et al., "Study of aroma generation pattern during boiling of hot pot seasoning," *Journal of Food Composition and Analysis*, vol. 114, Article ID 104844, 2022.

[8] N. A. Bokulich, J. H. Thorngate, P. M. Richardson, and D. A. Mills, "Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 1, pp. E139–E148, 2014.

[9] Y. N. Xia, Y. Zhao, J. L. Wang, J. Z. Li, and Q. Shuang, "Evaluation of flavor characteristics of Sichuan hotpot condiments by GC-MS, electronic nose and electronic tongue," *Food Technology*, vol. 46, no. 03, pp. 267–275, 2021.

[10] D. Y. Liu, C. Yang, L. Bai et al., "Analysis of volatile compounds in Jinhua ham using three extraction methods combined with gas chromatography-time-of-flight mass spectrometry," *Foods*, vol. 11, no. 23, p. 3897, 2022.

[11] Y. R. Liu, N. Li, X. Y. Li et al., "A high-resolution Orbitrap Mass spectral library for trace volatile compounds in fruit wines," *Scientific Data*, vol. 9, no. 1, p. 496, 2022.

[12] X. Gao, C. Cui, H. Zhao, M. Zhao, L. Yang, and J. Ren, "Changes in volatile aroma compounds of traditional Chinese-type soy sauce during moromi fermentation and heat treatment," *Food Science and Biotechnology*, vol. 19, no. 4, pp. 889–898, 2010.

[13] Y. Xu, Y. P. Chen, S. L. Deng et al., "Application of sensory evaluation, GC-TOF-MS, and E-nose to discriminate the flavor differences among five distinct parts of the Chinese blanched chicken," *Food Research International*, vol. 137, Article ID 109669, 2020.

[14] M. M. Mazida, M. M. Salleh, and H. Osman, "Analysis of volatile aroma compounds of fresh chilli (*Capsicum annuum*) during stages of maturity using solid phase microextraction (SPME)," *Journal of Food Composition and Analysis*, vol. 18, no. 5, pp. 427–437, 2005.

[15] Y. Ma, J. Y. Tian, Y. B. Chen, M. Chen, Y. Liu, and A. Wei, "Volatile oil profile of *Prickly Ash* (Zanthoxylum) pericarps from different locations in China," *Foods*, vol. 10, no. 10, p. 2386, 2021.

[16] A. Y. Ko, M. Musfiqur Rahman, A. M. Abd El-Aty et al., "Identification of volatile organic compounds generated from healthy and infected powdered chili using solvent-free solid injection coupled with GC/MS: application to adulteration," *Food Chemistry*, vol. 156, pp. 326–332, 2014.

[17] M. J. Jordan, V. Lax, M. C. Rota, S. Loran, and J. A. Sotomayor, "Effect of bioclimatic area on the essential oil composition and antibacterial activity of *Rosmarinus officinalis* L," *Food Control*, vol. 30, no. 2, pp. 463–468, 2013.

[18] J. Wang, L. Chen, Y. Liu, T. M. Olajide, Y. R. Jiang, and W. M. Cao, "Identification of key aroma-active compounds in beef tallow varieties using flash GC electronic nose and GC×GC-TOF/MS," *European Food Research and Technology*, vol. 248, pp. 1733–1747, 2022.

[19] A. H. Aktas, "Spectrometric multi component determination of maltol, ethyl maltol, vanillin and ethyl vanillin in foods by multi linear regression calibration, classical least square and inverse least square methods," *Asian Journal of Chemistry*, vol. 22, no. 5, pp. 3719–3728, 2010.

[20] D. D. Garruti, W. D. Mesquita, H. C. R. Magalhaes, I. M. D. Araujo, and R. D. C. A. Pereira, "Odor-contributing volatile compounds of a new Brazilian tabasco pepper cultivar analyzed by HS-SPME-GC-MS and HS-SPME-GC-O/FID," *Food Science and Technology*, vol. 41, no. 3, pp. 696–701, 2021.

[21] D. M. Han, B. H. Chun, H. M. Kim, and C. O. Jeon, "Characterization and correlation of microbial communities and metabolite and volatile compounds in doenjang fermentation," *Food Research International*, vol. 148, Article ID 110645, 2021.

[22] Y. M. Yu, Y. Nie, S. Chen, and Y. Xu, "Characterization of the dynamic retronasal aroma perception and oral aroma release of Baijiu by progressive profiling and an intra-oral SPME combined with GC × GC-TOFMS method," *Food Chemistry*, vol. 405, Article ID 134854, 2023.

[23] A. Nose, T. Hamasaki, M. Hojo, R. Kato, K. Uehara, and T. Ueda, "Hydrogen bonding in alcoholic beverages (distilled spirits) and water-ethanol mixtures," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 18, pp. 7074–7081, 2005.

[24] A. Rivera-Pérez, R. López-Ruiz, R. Romero-González, and A. Garrido Frenich, "A new strategy based on gas chromatography-high resolution mass spectrometry (GC-HRMS-Q-Orbitrap) for the determination of alkenylbenzenes in pepper and its varieties," *Food Chemistry*, vol. 321, Article ID 126727, 2020.

[25] S. Q. Song, X. M. Zhang, K. Hayat et al., "Formation of the beef flavour precursors and their correlation with chemical parameters during the controlled thermal oxidation of tallow," *Food Chemistry*, vol. 124, no. 1, pp. 203–209, 2011.

[26] S. M. Choi, D. J. Lee, J. Y. Kim, and S. T. Lim, "Volatile composition and sensory characteristics of onion powders prepared by convective drying," *Food Chemistry*, vol. 231, pp. 386–392, 2017.

[27] J. Y. Ren, G. Liu, Y. F. Chen et al., "Enhanced production of ethyl lactate in *Saccharomyces cerevisiae* by genetic modification," *Journal of Agricultural and Food Chemistry*, vol. 68, no. 47, pp. 13863–13870, 2020.

[28] K. Matsui, H. Takemoto, T. Koeduka, and T. Ohnishi, "1-Octen-3-ol is formed from its glycoside during processing of soybean (Glycine max L. Merr) seeds," *Journal of Agricultural and Food Chemistry*, vol. 66, no. 28, pp. 7409–7416, 2018.