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Chemical composition and volatile profiles of edible insect oils and meat-based broths

- Volatile profiles of food materials -

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This is to certify that we have examined the
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ABSTRACT

Chemical composition and volatile profiles of edible insect oils and meat-based broths

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The growing demand for sustainable food ingredients has led to increased interest in the utilization of edible insects as alternative food sources. In this study, oils were extracted from four edible insect species: *Tenebrio molitor* larvae, *Gryllus bimaculatus*, *Locusta migratoria*, and *Zophobas atratus* larvae, using supercritical fluid extraction (SFE) and ultrasound-assisted extraction (UAE). Extraction conditions for SFE were optimized using response surface methodology (RSM), with optimal yield achieved at 400 bar, 55 °C, and a 3-hour extraction time. The extracted oils exhibited favorable nutritional characteristics, including high polyunsaturated to saturated fatty acid ratios and low atherogenic and thrombogenic indices. Volatile compounds were extracted using headspace solid-phase microextraction arrow (HS-SPME-Arrow) and analyzed by gas chromatography - mass

spectrometry (GC/MS). Oils obtained via SFE showed significantly higher total volatile content compared to those from UAE ($p < 0.05$), and several compounds previously associated with favorable volatile notes were identified. These findings suggest that edible insect oils have strong potential as high-quality lipid ingredients with nutritional benefits and distinctive volatile profiles.

The volatile composition of meat-based broths was also characterized to support the development of processed food products. Three SPME-Arrow fibers were compared based on peak area, with divinylbenzene/carboxen/polydimethylsiloxane fiber showing the highest extraction efficiency ($p < 0.05$). Extraction parameters were optimized using RSM, resulting in 20 min equilibration, 30 min extraction, and an extraction temperature of 40 °C. A total of 18 volatile compounds were identified using HS-SPME-Arrow-GC/MS, with aldehydes representing the most abundant class. Eleven key volatiles, including hexanal, octanal, heptanal, nonanal, and decanal, were selected based on variable importance in projection (VIP) scores greater than 1. Comparative analysis with beef flavoring ingredients revealed shared compounds contributing to fatty notes, while distinctive compounds such as pyrazines and methional, known for roasted notes, were detected exclusively in the flavoring samples. These results provide valuable insights into enhancing the volatile characteristics of Korean-style home meal replacement products through targeted compound profiling.

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CHAPTER I

Optimization and comparative analysis of quality characteristics and volatile profiles in edible insect oils extracted using supercritical fluid extraction and ultrasound-assisted extraction methods

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ABSTRACT

Edible insects are gaining global attention as a sustainable alternative source. In this study, insect oils were extracted using supercritical fluid extraction and ultrasound-assisted extraction. The supercritical fluid extraction method was optimized via response surface methodology under conditions of 400 bar pressure, 55 °C, and a 3-h extraction time. Oils from *Tenebrio molitor* and *Locusta migratoria* demonstrated high nutritional quality, with elevated polyunsaturated fatty acid/saturated fatty acid ratios and low arteriosclerosis and thrombosis indices. Volatile compounds were extracted using headspace solid-phase microextraction arrow and analyzed via gas chromatography-mass spectrometry. Supercritical fluid extraction extracts had significantly higher total volatile concentrations than ultrasound-assisted extraction extracts ($p < 0.05$). Moreover, volatile compounds associated with consumer-preferred aromas were identified in *T. molitor* and *L. migratoria* oils. These findings confirm the quality characteristics and volatile profiles of insect oils, underscoring their potential for developing novel edible oils and enhancing the value of edible insect by-products.

Keywords:

Edible insect oil, Supercritical fluid extraction, Ultrasound-assisted extraction, Lipid analysis, Quality characteristics, Fatty acid analysis, Volatile compound analysis

Ⅰ. INTRODUCTION

The global edible oil market faces numerous challenges, including price volatility and sustainability concerns (Li et al., 2024a). Supply disruptions in major exporting countries can result in shortages and safety risks. Therefore, sustainable oil sources are crucial for enhancing the resilience of the global food system. Edible insects present a promising solution, as their industrial production emits lower levels of CO_2 and ammonia and requires fewer resources such as water and land compared to conventional livestock (Lee et al., 2024). However, consumer acceptance of insect-based foods remains low. Studies suggest that acceptance will increase when insects are incorporated as ingredients in food products rather than consumed in their whole form (Li et al., 2024b). Most research on edible insects has focused on their protein, often leading to the removal of lipids through defatting (Lee et al., 2024). However, lipids are the second-largest component of edible insects after protein (Jantzen da Silva Lucas et al., 2020). Extracting insect oils can enhance the value of these by-products and promote their integration into human diets. Furthermore, edible insects may contribute to the United Nations (UN) Sustainable Development Goals (SDGs). Despite their significant lipid content, research on edible insect oils remains limited.

Oil extraction methods have a profound impact on oil quality. Traditional techniques like Soxhlet extraction and hydrodistillation have limitations due to their use of high-purity solvents, lengthy processing times, and low extraction efficiency (Otero et al., 2020). Emerging

technologies, such as supercritical fluid extraction (SFE) and ultrasound-assisted extraction (UAE), offer improved efficiency and reduced environmental impact. SFE is employed to extract oil from various materials and is widely used in the food industry because it is compatible with green processing concepts, providing non-toxic, solvent-free residues and low energy consumption (Ma et al., 2024a). CO_2, a commonly used supercritical solvent, is ideal due to its availability, low cost, non-toxicity, chemical inertness, and ease of removal from extracts (Ou et al., 2024). UAE, characterized by low cost, simple implementation, high efficiency, reduced extraction time, and optimal temperature control, leverages acoustic cavitation to disrupt cell structures and enhance extraction (Rodríguez-Rodríguez et al., 2024). Although some papers focus on various oil extraction methods, there is still limited information available on SFE and UAE using insects recognized as edible in South Korea. Furthermore, the quality characteristics and volatile profiles of these oils have not been comprehensively explored.

Efficient extraction methods are essential for analyzing volatile compounds in food. Solid-phase microextraction (SPME), a common headspace technique, is favored for its speed, convenience, sensitivity, automation, and reproducibility (Zhang et al., 2023). The recently developed SPME-Arrow technique offers considerably larger peak areas (4 to 40 times) than traditional SPME and improves fiber stability by enclosing it in a metal tube (Lee et al., 2019). These advantages make SPME-Arrow highly suitable for analyzing volatile compounds in various

foods. In this study, SPME-Arrow was employed for volatile compound extraction.

An estimated 2 billion people in over 113 countries consume edible insects, with more than 2,100 species identified worldwide (Jantzen da Silva Lucas et al., 2020). The growing interest in edible insects has been largely influenced by the Food and Agriculture Organization (FAO) recognition of insects as a sustainable and nutritious food source for human (van Huis et al., 2013). The global edible insect market was valued at USD 1.35 billion in 2024 and is projected to grow at a compound annual growth rate of 25.1% from 2025 to 2030 (Grand View Research, 2024). Since 2012, the Korean edible insect market, particularly for human consumption, has achieved notable progress supported by government initiatives and successful research efforts. Currently, a total of ten edible insect species, including *Tenebrio molitor* larvae, *Gryllus bimaculatus*, *Locusta migratoria*, and *Zophobas atratus* larvae, are approved as food ingredients in South Korea. However, while numerous studies on *T. molitor* larvae exist (Lee et al., 2024; Li et al., 2024a; Lee et al., 2022; Otero et al., 2020), limited information is available on *G. bimaculatus*, *L. migratoria*, and *Z. atratus* larvae. In particular, *L. migratoria* was newly registered as an edible insect in the Korean Food Standards Codex in 2021, leading to a lack of comprehensive reports on its potential applications and properties. Moreover, despite the growing interest in edible insects, most of the ongoing research focuses primarily on their protein content and nutritional value, with relatively less attention given to their flavor profiles. The accurate identification and

quantification of volatile compounds are essential for the comprehensive characterization of edible insect oils. These compounds remarkably contribute to defining the chemical composition of the oils and have important implications for their functional properties and potential applications across various industries, including food. Establishing reliable analytical methods to quantify volatile compounds is crucial to advancing our understanding and utilization of edible insect oils.

This study aims to assess the quality characteristics and volatile profiles of oils extracted from these four species using SFE and UAE. The extracted oils were evaluated for acid value (AV), peroxide value (PV), iodine value (IV), and fatty acid composition. Additionally, volatile compounds were analyzed using headspace solid-phase microextraction arrow, followed by gas chromatography-mass spectrometry (HS-SPME-Arrow-GC/MS). This research contributes to enhancing the value of edible insect by-products and promoting their application in human diets.

Ⅱ. MATERIALS AND METHODS

2.1. Samples

Four edible insects were obtained from an insect farm (Jeongeup, South Korea): *T. molitor* larvae, *G. bimaculatus*, *L. migratoria*, and *Z. atratus* larvae. The breeding ground for the edible insects was maintained at a temperature of 25 - 30 °C and a relative humidity of 60 - 65%, with fermented sawdust provided on the floor. Edible insects were reared in breeding cages measuring 270 × 450 × 100 mm and fed wheat bran. After the breeding period, the insects underwent a 2-day fasting period to empty their intestines, followed by freezing and sterilization treatments. The edible insects were freeze-dried (FD8508, Ilshin BioBase Co. Ltd., Dongducheon, South Korea), ground (SFM-C3501KP, Shinil, Cheonan, South Korea), and used in subsequent experiments. The edible insect powder was stored in an ultra-low temperature freezer (TSE600D, Thermo Fisher Scientific, USA) at -80 °C until further use.

2.2. Chemicals and reagents

For SFE, CO_2 with a purity of ≥ 99.9% (CS gastec, Hwaseong, South Korea) was used. UAE was performed using ethyl alcohol with a purity of ≥ 99.9% (Samchun Chemicals, Seoul, South Korea). The reagents used for quality characterization and fatty acid analysis were obtained from Daejung (Seoul, South Korea), and undecanoic methyl ester was purchased from Sigma-Aldrich (St. Louis, MO, USA). Internal standards for volatile analysis (2,2-dimethylpropanoic acid, 1-hexyl alcohol-d_{13}, octanal, 2-methylpyrazine, phenyl acetate, toluene-d_8, 3-octanone, and 3,4-dimethyl phenol) and C_7-C_{40} alkane standard mixtures were obtained from Sigma-Aldrich. All chemical standards for the 47 types of volatile compounds detected in the edible insect oils were of analytical grade and obtained from Sigma-Aldrich.

2.3. Preparation of stock and standard solutions

Odorless coconut oil (Evercoco, Hwaseong, South Korea) was used to determine the linearity and limits of detection (LOD) and quantification (LOQ). Stock solutions of volatile standards were prepared by dissolving precisely weighed amounts of the standard compounds in methyl alcohol (HPLC grade, Fisher Scientific, Waltham, Massachusetts, USA). The ISTD mixture was added at the same concentration to standard solutions of different concentrations. For analysis, 0.5 g of odorless coconut oil (blank matrix) was spiked with 10 μL of the standard solution at each concentration using a syringe.

2.4. Experimental design of SFE

The Box-Behnken design (BBD) for response surface methodology (RSM) was used to optimize the three significant factors of the optimum processing conditions. BBD consists of three key steps: conducting an experiment based on the experimental design, calculating the coefficients of the mathematical model, and evaluating the model's suitability (Kim et al., 2022). The oil extraction yield of *T. molitor* was the reaction variable, and extraction pressure (X1: 200 - 400 bar), extraction temperature (X2: 45 - 65 °C), and extraction time (X3: 1 - 3 h) were determined as the independent variables (**Table 1.S1**). The experiments were randomized to reduce the effect of unexplained variability in the observed responses caused by extraneous factors. The complete design was performed in 17 combinations with five replicates of the center points. The quadratic equation used for the fitted model is as follows:

$$Y = \beta_0 + \Sigma_i^3 \beta_i X_i + \Sigma_i^3 \beta_{ii} X_i^2 + \Sigma_{(i \neq j)}^3 \beta_{ij} \beta_i X_j$$

Y is the response function; β_0 is the intercept; β_i is the coefficients of linear; β_{ii} is the coefficients of quadratic; β_{ij} is the coefficients of interaction terms; X_i and X_j are the independent variables.

Statistical analysis was performed using Design Expert statistical software version 7 (Stat-Ease, Minneapolis, MN, USA). A three-dimensional response surface was obtained to graphically show the relationships among the parameters, responses, and exact optimum values.

Table 1.S1. Box-Behnken design modeling for supercritical fluid extraction

Xi	Variables	Levels		
		Low (-1)	Middle (0)	High (+1)
X1	Extraction pressure (bar)	200	300	400
X2	Extraction temperature (℃)	45	55	65
X3	Extraction time (h)	1	2	3

2.5. Supercritical fluid extraction

Freeze-dried edible insect powder was sieved using a 10-mesh sieve (Chen et al., 2008), and 100 g of the sieved powder was placed into a 1 L extractor (SFE001FK, Focos, Hwaseong, Korea). The extraction time was 3 h, and the equipment diagram is shown in **Figure 1.S1**. CO_2 was heated using a preheater and entered the extractor, where it reached a supercritical state to extract the oil from the edible insects. The flow rate of CO_2 was set at 70 mL/min. When the pressure is reduced through the back pressure regulator, CO_2 reverts to a gaseous state in the separator, and the oil is collected at the bottom. The gaseous CO_2 was then cooled, liquefied, and returned to the extractor via a recycling system. After oil extraction, centrifugation was performed at 3,220 × *g* for 10 min. The oils extracted using the SFE method were designated as TS for *T. molitor* oil, GS for *G. bimaculatus* oil, LS for *L. migratoria* oil, and ZS for *Z. atratus* oil.

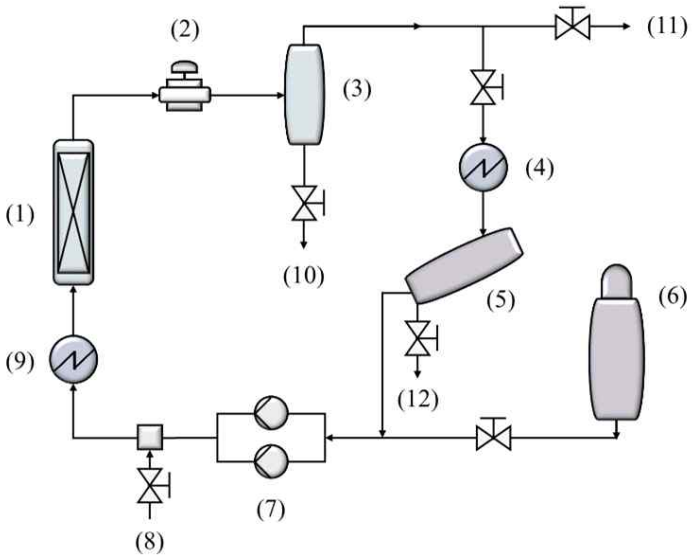

Figure 1.S1. Diagram of the supercritical fluid extraction equipment. (1) extractor; (2) back pressure regulator; (3) separator; (4) chiller; (5) working tank; (6) liquid carbon dioxide bomb; (7) pump; (8) co-solvent; (9) heater; (10) extract; and (11) and (12) vents.

2.6. Ultrasound-assisted extraction

The UAE method was established based on the optimized conditions and results presented in **Table 1.S2** and **Figure 1.S2**. Five-grams freeze-dried edible insect powder, sieved twice through a 14-mesh sieve, was placed in a beaker containing 75 mL of ethyl alcohol and extracted using ultrasonic equipment (Q125 Sonicator, Qsonica, Newtown, CT, USA). The extraction conditions were an amplitude level of 70% and an extraction time of 30 min. To prevent overheating of the ultrasonic equipment during extraction, pulsation mode was employed, alternating between 30 s on and 30 s off. After centrifuging the edible insect oils at 3,220 × g for 10 min, solvent of supernatant was removed by evaporation under vacuum at 35 °C using a rotary evaporator (Laborata 4000; Heidolph Instruments, Schwabach, Germany). The oils extracted using the UAE method were designated TU for *T. molitor* oil, GU for *G. bimaculatus* oil, LU for *L. migratoria* oil, and ZU for *Z. atratus* oil.

Table 1.S2. Box-Behnken design modeling for ultrasound-assisted extraction

Xi	Variables	Levels		
		Low (-1)	Middle (0)	High (+1)
X1	Solvent-to-sample ratio	5	15	25
X2	Extraction time (min)	20	30	40
X3	Amplitude level (%)	55	70	85
X4	Sieve size (mesh)	0	14	28

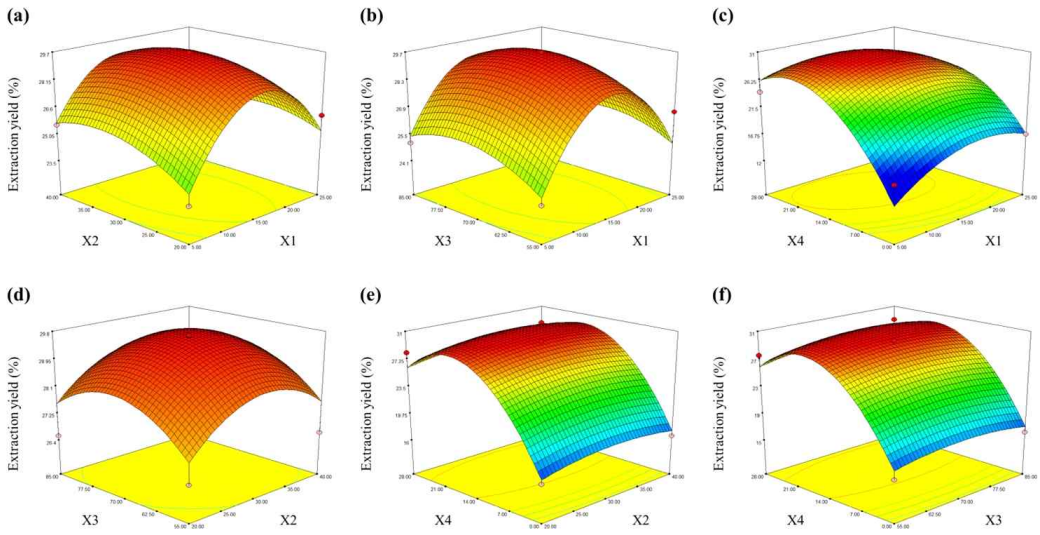

Figure 1.S2. Optimization of the ultrasound-assisted extraction method via response surface methodology. The response variable is the extraction yield of oil, and independent variables encompass solvent-to-sample ratio (X1), extraction time (X2), amplitude level (X3), and sieve size (X4).

2.7. Quality characteristics

Oil extraction yields were calculated using the weights of the freeze-dried edible insect powder as follows:

$$\text{Extraction yield (\%)} = \frac{w_1 - w_0}{S} \times 100$$

where w_1 is the weight of the flask containing the extracted oil (g); w_0 is the weight of the flask (g); and S is the weight of the freeze-dried edible insect powder (g).

The acid value (AV) was determined by modifying the AOAC method (2005) using 1 g of oil sample. The indicator was a 1% phenolphthalein solution, and the titrant was 0.1 N potassium hydroxide ethanolic standard solution. The peroxide value (PV) was measured using the AOAC method (AOAC, 2005). A starch solution was used as an indicator, and the sample was treated with a 0.01 N sodium thiosulfate solution. The iodine value (IV) was determined by the Wijs method. IV was performed using iodine chloride and titrated with a 0.1 N sodium thiosulfate solution.

2.8. Fatty acid analysis

Fatty acids were extracted according to the Korea Food Code (MFDS, 2021). Briefly, an oil sample (25 mg) and 1 mL of undecanoic acid methyl ester (internal standard) were placed in a clear vial, followed by the addition of 2 mL of 0.5 N methanolic sodium hydroxide solution, and the mixture was vortexed. It was then heated in a 100 °C heating block for 10 min, cooled on ice for 3 min, and 2 mL of 14% trifluoroborane-methanol solution was added. The solution was vortexed again, heated at 100 °C for 10 min, and cooled for 3 min. Subsequently, 1 mL of an isooctane solution and 2 mL of a saturated sodium chloride solution were added, and the mixture was vortexed for 1 min. After cooling to room temperature, the separated isooctane layer was collected for further analysis.

The fatty acid analysis was measured via gas chromatography-flame ionization detector (Agilent Technologies, Santa Clara, CA, USA). The injection volume of the oil sample was 2 μL, with a 100:1 split ratio, and the injector temperature was set at 225 °C. The oven temperature was maintained at 100 °C for 5 min and then raised at 10 °C/min to 175 °C where it was maintained for 10 min. The temperature was then increased at 5 °C/min to 210 °C, held for another 10 min, and further increased at 5 °C/min to 230 °C, where it was held for 25 min. The detector temperature was 285 °C. The carrier gas was nitrogen, which was passed through a CP-Sil 88 for FAME column (100 m × 0.25 mm × 0.2 μm) at a flow rate of 1.0 mL/min. The gas flow rates were 40, 40,

and 400 mL/min for the make-up gas (nitrogen), hydrogen, and air, respectively. The fatty acid methyl ester (FAME) standard mixture (Supelco 37 Component FAME Mix #47885-U; Supelco™, Philadelphia, PA, USA) was used for analysis. Undecanoic acid (C11:0) was used as the internal standard, with a concentration of 100 μg/mL, consistent in both the standard mixture and the sample. The retention times and percentages of relative peak areas of the FAME standard mixture were used for the qualitative and quantitative analysis of fatty acids. In addition, the quantified contents were then expressed as percentages of the total fatty acid content. The formula used for fatty acid analysis is as follows (MFDS, 2021):

$$R_i = \frac{Ps_i \times W_{C11:0}}{Ps_{C11:0} \times W_i}$$

R_i, response factor of fatty acid i (FA_i); Ps_i, peak area of FA_i in standard; $W_{C11:0}$, amount of C11:0 in standard (mg); $Ps_{C11:0}$, peak area of C11:0 in standard; and W_i, amount of FA_i in standard (mg).

$$\text{Fatty acid (g/100 g)} = \frac{Pt_i \times Wt_{C11:0} \times 1.0067 \times F_{FAi} \times 100}{Pt_{C11:0} \times R_i \times W_{spl}}$$

Pt_i, peak area of FA_i; $Wt_{C11:0}$, amount of C11:0 (mg); F_{FAi}, conversion factor of FA_i; $Pt_{C11:0}$, peak area of C11:0; and W_{spl}, amount of sample (mg).

Additionally, nutritional quality indices were calculated based on the fatty acid profiles of the edible insect oils.

$$AI = \frac{C_{12:0} + (4 \times C_{14:0}) + C_{16:0}}{MUFA + PUFA}$$

$$TI = \frac{C_{12:0} + C_{16:0} + C_{18:0}}{(0.5 \times MFA) + (0.5 \times n-6PUFA) + (3 \times n-3PUFA) + \frac{n-3PUFA}{n-6PUFA}}$$

AI, arteriosclerosis index; TI, thrombosis index; MUFA, the sum of monounsaturated fatty acids; and PUFA, the sum of polyunsaturated fatty acids of the sample.

2.9. HS-SPME-Arrow-GC/MS analysis

The extraction and analysis of volatile compounds were modified based on a previous study (Nam et al., 2024). Extraction was performed using 120 μm divinylbenzene carboxen polydimethyl-siloxane (DVB/CAR/PDMS) SPME-Arrow fiber (PAL System, Zwingen, Switzerland) with an autosampler (PAL RSI 85, PAL System). Gas chromatography-mass spectrometry analysis was processed using 8890 gas chromatography coupled with 7000E Triplus quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Briefly, 0.5 g of the edible insect oil was added into a 20 mL headspace vial, and 10 μL of the ISTD mixture was spiked. The concentration of ISTD mixture spiked to each sample was as follows: 100 μg/mL each of 2,2-dimethylpropanoic acid, 1-hexyl alcohol-d_{13}, octanal, and 2-methylpyrazine and 5 μg/mL each of phenyl acetate, toluene-d_8, 3-octanone, and 3,4-dimethyl phenol. The sample was equilibrated at 500 rpm for 10 min. Subsequently, extraction was performed at 1,000 rpm for 30 min. Both equilibration and extraction of the sample were performed at 40 °C. The injector temperature was 220 °C, and desorption was performed in splitless mode for 5 min. Volatile compounds were separated using HP-5MS column (60 m × 0.25 mm I.D. × 0.25 μm film thickness, Agilent Technologies). The flow rate of helium used as the carrier gas was 1.0 mL/min. The oven program was set as previously described (Cha et al., 2024). The oven temperature was held at 45 °C for 10 min, then increased to 100 °C at a rate of 2 °C/min, followed by a ramp of 3 °C/min until reaching 230 °C, where it was held for 3 min.

The MS transfer line and ion source were 280 °C and 230 °C, respectively. Electron impact ionization was performed at an ionization energy of 70 eV. Data were acquired in two segments including full scan mode (30 – 550 *m/z*) and selected ion monitoring (SIM) mode.

2.10. Qualification and quantification of volatile compounds

Volatile compounds were identified by matching their mass spectral ratios with those in the National Institute of Standards and Technology (NIST) 2020 library (R-match score ≥ 800). The retention index (RI) was calculated using an alkane standard mixture (C_7-C_{40}).

$$RI_X = 100 \times [n + (N-n)\frac{\log(t'_{r(X)}) - \log(t'_{r(n)})}{\log(t'_{r(N)}) - \log(t'_{r(n)})}]$$

RI is the Retention index of compound X; n is the number of carbon atoms in the smaller n-alkane eluted before compound X; N is the number of carbon atoms in the larger n-alkane eluted after compound X; t′ is the Retention time of the compound.

The volatile compounds were quantified using a regression equation. The standard solution used to construct the regression equation was prepared by mixing the standards of volatile compounds within the same group. The internal standard for each group was added at the same concentration as in the sample. The mixture was then dissolved and brought to volume using methyl alcohol (Wimonmuang & Lee, 2024). Regression equations for each compound were constructed using the ratio of the peak areas (analyte/ISTD). The peak area of each compound was determined by extracting the quantifier ions. The ISTD added for the quantification of each volatile compound group was as follows: 2,2-dimethylpropanoic acid for acids; 1-hexyl alcohol-d_{13} for alcohols and

furans; octanal for aldehydes; 2-methylpyrazine for pyrazines; phenyl acetate for esters, lactones, and indoles; toluene-d_8 for hydrocarbons and sulfur compounds; 3-octanone for ketones; and 3,4-dimethyl phenol for phenols.

2.11. Statistical analysis

All experiments were performed in triplicate (n=3), and the data are expressed as the mean ± standard deviation. The means and standard deviations were calculated using Microsoft Office Excel 2024 (Microsoft Corporation, Redmond, WA, USA). All statistical analyses were performed using the SPSS Statistics version 26 (IBM Inc., Chicago, IL, USA). The differences between the means were evaluated using analysis of variance, followed by Duncan's test to determine significant differences between groups ($p < 0.05$).

Ⅲ. RESULTS AND DISCUSSION

3.1. Optimization of the SFE method

Response surface methodology (RSM), combined with the Box-Behnken design (BBD), was used to optimize the SFE method, with the extraction yield as the response variable and extraction pressure (X1), temperature (X2), and time (X3) as the independent variables. The relationship between the three parameters and the extraction yield is described as follows:

$$Y = 19.99 + 5.53X_1 - 0.43X_2 + 6.10X_3 - 0.85X_1X_2 + 0.77X_1X_3 - 0.79X_2X_3 - 2.78X_1^2 - 1.34X_2^2 - 1.79X_3^2$$

The model response was significant (F = 95.92, $p < 0.0001$), with a high coefficient of determination (R^2 = 0.9920). In the quadratic equation, the linear terms (X1, X2, X3) represent the main effects, whereas the cross-product terms (X1X2, X1X3, and X2X3) represent interaction effects. Extraction pressure (X1) and time (X3) were identified as significant factors ($p < 0.0001$), with both showing positive effects on extraction yield. Conversely, temperature (X2) exhibited a negative effect. Additionally, extraction pressure (X1) and time (X3) demonstrated a positive interaction, whereas temperature (X2) showed a negative interaction with both pressure (X1) and time (X3). Three-dimensional response surface plots depicting these interactions are shown in **Figure 1.1**. The oil extraction yield was maximized at 400 bar pressure and 55 °C (**Figure 1.1a**), at 400 bar pressure and 3-h extraction time (**Figure 1.1b**), and at 55 °C and 3-h extraction time (**Figure 1.1c**). Based on

these results, the optimal extraction conditions were determined to be 400 bar pressure, 55 °C, and 3-h extraction time.

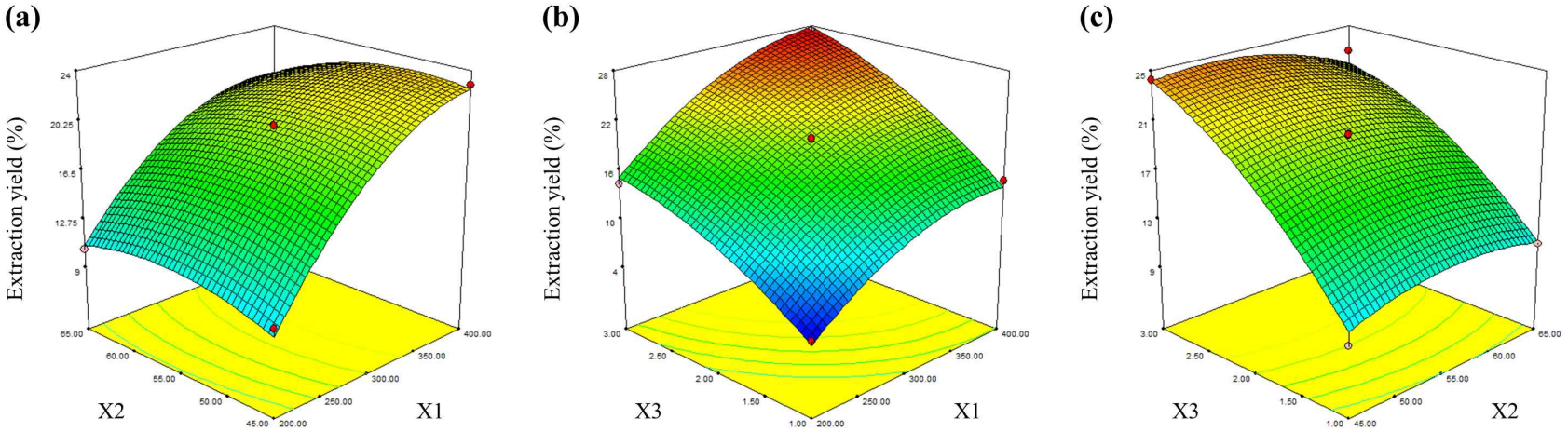

Figure 1.1. Optimization of the supercritical fluid extraction method using response surface methodology. The response variable is the extraction yield of oil, and independent variables encompassed extraction pressure (X1), extraction temperature (X2), and extraction time (X3). Interactions between (a) X1 and X2, (b) X1 and X3, and (c) X2 and X3 are presented.

3.2. Quality characteristics

The extraction yields of *T. molitor* oil using the two extraction methods were 25.43 ± 0.30% (SFE) and 26.99 ± 0.08% (UAE). The yield differences in oil extraction are linked to the oil's solubility in the chosen solvent. Ethyl alcohol is an efficient solvent for extracting oils (Castejón et al., 2018), but its separation after extraction increases both time and energy demands. In contrast, CO_2, which exists as a gas at room temperature, allows for significant removal without leaving residues after system decompression, resulting in a solvent-free extract (Ma et al., 2024a). Therefore, both SFE and UAE are considered suitable methods for recovering edible insect oils, each offering distinct advantages. UAE is recognized for its high extraction efficiency, enabling the recovery of oils effectively and rapidly (Maser et al., 2024; Rodríguez-Rodríguez et al., 2024). Meanwhile, SFE demonstrates sufficient solubilizing capabilities for oil molecules and yields oils that are solvent-free in the final product, eliminating the need for additional solvent removal steps (Ou et al., 2024).

The acid value (AV), peroxide value (PV), and iodine value (IV) for the extracted oils are presented in **Table 1.1**. AV and PV are important indicators of oil quality, with hydroperoxides generated during oxidation and followed by polymerization and degradation (Ma et al., 2024a). The AV is proportional to the amount of free fatty acids formed from hydroperoxide degradation, while PV indicates the initial production of peroxides, serving as a measure of oil oxidation. Both AV and PV were

significantly higher in SFE oils compared to UAE oils ($p < 0.05$). The higher values of SFE oils can be attributed to the high extraction temperature and long extraction times. The high AVs of SFE oils may be due to the increased solubility of free fatty acids in supercritical CO_2 (Ma et al., 2024a). The PVs of all samples were below the Codex standard of 15 meq/kg for vegetable oils (Alimentarius, 2023). The IVs of *T. molitor*, *L. migratoria*, and *Z. atratus* oils were consistent with Codex standards for peanut oil (77 – 107 g/100 g) (Alimentarius, 2023). Additionally, *T. molitor* oil aligned with standards for rice bran and pistachio oil, while *L. migratoria* oil conformed to standards for almond and rapeseed oil (Alimentarius, 2023). In contrast, the IVs of *G. bimaculatus* oil were 70.17 g/100 g (GS) and 69.30 g/100 g (GU), conforming to the Codex standards for rendered pork fat (60 – 72 g/100 g) (Alimentarius, 1999). There were no significant differences in IVs between the oils extracted by SFE and UAE ($p > 0.05$).

Table 1.1. Acid, peroxide, and iodine values of oils derived from four edible insect species (*Tenebrio molitor*, *Gryllus bimaculatus*, *Locusta migratoria*, and *Zophobas atratus*) using two extraction methods

	TS	TU	GS	GU	LS	LU	ZS	ZU
AV (mg/g)	61.06 ± 0.01^{d}	56.32 ± 1.56^{e}	77.96 ± 0.02^{a}	72.33 ± 0.03^{b}	66.75 ± 0.01^{c}	62.09 ± 0.46^{d}	57.04 ± 0.33^{e}	53.46 ± 0.75^{f}
PV (meq/kg)	4.07 ± 0.29^{e}	3.23 ± 3.29^{f}	14.56 ± 9.59^{a}	10.04 ± 3.30^{b}	8.89 ± 7.01^{c}	6.40 ± 0.49^{d}	2.07 ± 0.29^{g}	0.40 ± 4.00^{h}
IV (g/100 g)	90.55 ± 0.10^{b}	90.34 ± 0.21^{b}	70.17 ± 1.00^{d}	69.30 ± 0.08^{d}	101.83 ± 0.46^{a}	101.18 ± 0.97^{a}	80.64 ± 1.00^{c}	80.33 ± 0.24^{c}

$^{a-h}$Duncan's multiple range test shows that values in the row with different letters differ significantly at $p < 0.05$. AV, acid value; PV, peroxide value; IV, iodine value; TS, *Tenebrio molitor* oil prepared using SFE; TU, *Tenebrio molitor* oil prepared using UAE; GS, *Gryllus bimaculatus* oil prepared using SFE; GU, *Gryllus bimaculatus* oil prepared using UAE; LS, *Locusta migratoria* oil prepared using SFE; LU, *Locusta migratoria* oil prepared using UAE; ZS, *Zophobas atratus* oil prepared using SFE; ZU, *Zophobas atratus* oil prepared using UAE.

3.3. Fatty acid analysis

The composition of fatty acids is a key indicator of oil and fat quality. The GC/FID chromatograms of the FAME standard mixture and edible insect oil are presented in **Figure 1.S3**. **Table 1.2** presents the fatty acid profiles of edible insect oils extracted using the two extraction methods (SFE and UAE). Oleic acid (C18:1), linoleic acid (C18:2, n-6), and palmitic acid (C16:0) were the predominant fatty acids in *T. molitor*, *G. bimaculatus*, and *Z. atratus* oils, consistent with previous studies on edible insect oils (Lee et al., 2022; Lee et al., 2023; Otero et al., 2020). The major fatty acid composition and content of *T. molitor* oil was aligned with the Codex standard for rice bran oil (Alimentarius, 2023). A previous study found that oleic acid (C18:1) is the major monounsaturated fatty acid (MUFA) in peanut oil, with contents ranging from 39.34% to 46.91% (Dun et al., 2019). In this study, oleic acid (C18:1) was also the dominant MUFA in *T. molitor* oil, with contents of 42.58% (TS) and 42.11% (TU), showing results similar to those found in peanut oil. *L. migratoria* oil exhibited significantly higher levels of α-linolenic acid (C18:3, n-3) compared to the other insect oils ($p < 0.05$), with α-linolenic acid being the major fatty acid in this oil, a finding consistent with previous research (Lee et al., 2023). Furthermore, linoleic acid (C18:2, n-6) and α-linolenic acid (C18:3, n-3) constituted 60.24% (SFE) and 60.12% (UAE) of the total fatty acids in *L. migratoria* oil, closely resembling the fatty acid profile of walnut oil, in which these acids account for over 65% of total fats (Ma et al., 2024b). In the *L. migratoria* oils, the linoleic acid (C18:2, n-6) and α-linolenic acid (C18:3,

n-3) contents were 60.24% (LS) and 60.12% (LU), respectively, similar to those found in walnut oil. Essential fatty acids include the omega-3 and omega-6 fatty acids, which serve as precursors of other fatty acids. The omega-3 fatty acids include α-linolenic acid (18:3, n-3), docosahexaenoic acid (DHA, 22:6, n-3), and eicosapentaenoic acid (EPA, 20:5, n-3). α -linolenic acid (18:3, n-3) is important because it is a precursor of DHA and EPA. Linoleic acid (C18:2, n-6), one of several omega-6 fatty acids, is a precursor to arachidonic acid (20:4), which is essential for cellular, muscle, immune, and nervous system functions (Geranpour et al. 2020). Therefore, edible insect oils may serve as a good source of α-linolenic acid (C18:3, n-3) and linoleic acid (C18:2, n-6), which are essential fatty acids that cannot be synthesized by the human body. These fatty acids are thought to offer health benefits, including the prevention of cancer and cardiovascular diseases (Jantzen da Silva Lucas et al., 2020; Ma et al., 2024b). Notably, the fatty acid composition of oils extracted via SFE and UAE showed comparable profiles when analyzed within the same species.

The polyunsaturated fatty acid to saturated fatty acid (PUFA/SFA) ratio is used to assess the nutritional value of oils and their impact on cardiovascular health, with a higher PUFA/SFA ratio indicating a more favorable health outcome (Karsli, 2021). According to Wood et al. (2004), the recommended PUFA/SFA ratio in the human diet is at least 0.45. In this study, the PUFA/SFA ratio ranged from 0.55 to 2.33, exceeding the minimum recommendation. The arteriosclerosis index (AI) and thrombosis index (TI) indicate the risk of coronary artery disease, with lower values

associated with better health outcomes (Otero et al., 2020). Among the four insect oils studied, *T. molitor* and *L. migratoria* oils had PUFA/SFA ratios above 1, along with low AI and TI values, indicating high nutritional quality. *L. migratoria* oil had the highest PUFA/SFA ratio and the lowest AI and TI values ($p < 0.05$), suggesting a particularly favorable nutritional profile. Despite these promising findings, research on *L. migratoria* oil remains limited, and this study provides valuable contributions to the growing field of edible insect research. No significant differences were observed in the AI and TI values of the insect oils based on the extraction method.

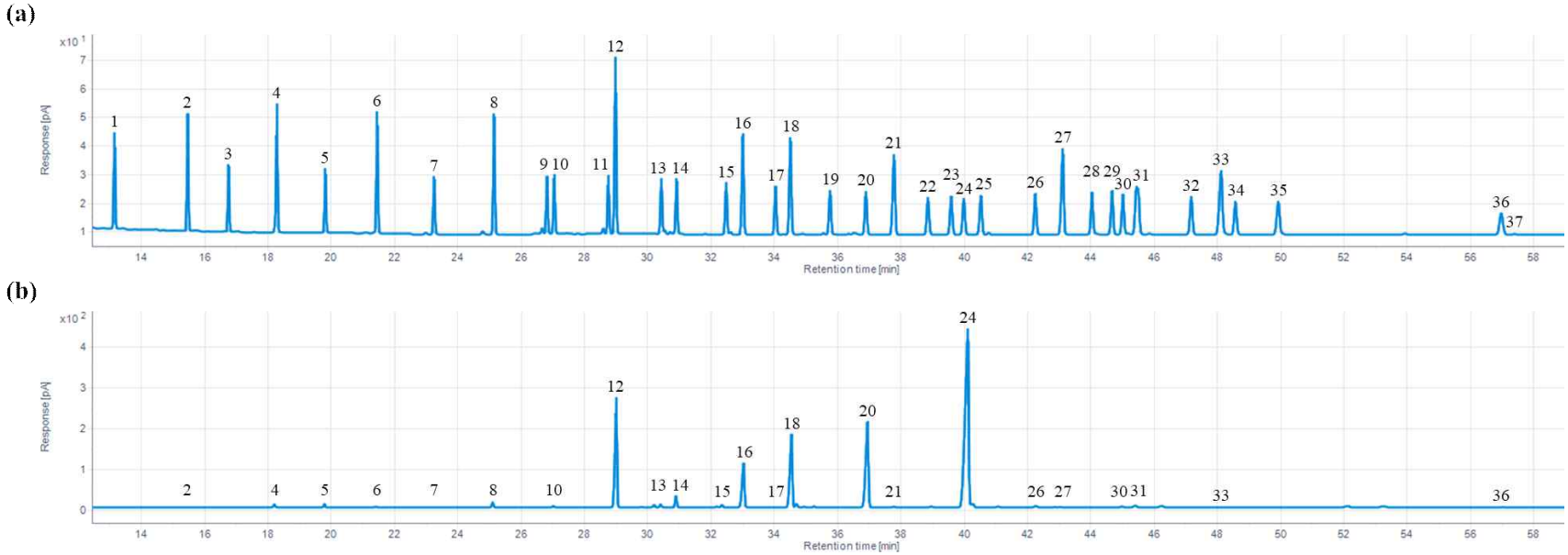

Figure 1.S3. Gas chromatography coupled with a flame ionization detector (GC/FID) chromatogram of fatty acids. (a) 37-FAME standards and (b) *Locusta migratoria* oil prepared using SFE. 1, C4:0; 2, C6:0; 3, C8:0; 4, C10:0; 5, C11:0; 6, C12:0; 7, C13:0; 8, C14:0; 9, C14:1; 10, C15:0; 11, C15:1; 12, C16:0; 13, C16:1; 14, C17:0; 15, C17:1; 16, C18:0; 17,

C18:1n9t; 18, C18:1n9c; 19, C18:2n6t; 20, C18:2n6c; 21, C20:0; 22, C18:3n6; 23, C20:1n9; 24, C18:3n3; 25, C21:0; 26, C20:2; 27, C22:0; 28, C20:3n6; 29, C22:1n9; 30, C20:3n3; 31, C20:4n6; 32, C23:0; 33, C22:2; 34, C24:0; 35, C20:5n3; 36, C24:1; and 37, C22:6n3.

Table 1.2. Fatty acid profile (%) of oils derived from four edible insect species (*Tenebrio molitor*, *Gryllus bimaculatus*, *Locusta migratoria*, and *Zophobas atratus*) using two extraction methods

Fatty acids	TS	TU	GS	GU	LS	LU	ZS	ZU
C6:0	nd	nd	nd	nd	0.02 ± 0.00^{b}	0.02 ± 0.00^{b}	0.66 ± 0.00^{a}	0.65 ± 0.00^{a}
C10:0	0.40 ± 0.02^{e}	0.40 ± 0.01^{e}	0.50 ± 0.01^{c}	0.54 ± 0.02^{b}	0.45 ± 0.00^{d}	0.45 ± 0.01^{d}	0.58 ± 0.00^{a}	0.57 ± 0.00^{a}
C12:0	0.39 ± 0.01^{a}	0.34 ± 0.00^{b}	0.28 ± 0.01^{c}	0.25 ± 0.00^{d}	0.15 ± 0.00^{e}	0.14 ± 0.00^{f}	0.12 ± 0.01^{g}	0.11 ± 0.00^{g}
C13:0	0.08 ± 0.00^{a}	0.08 ± 0.00^{a}	nd	nd	0.02 ± 0.00^{d}	0.01 ± 0.00^{e}	0.05 ± 0.00^{b}	0.04 ± 0.00^{c}
C14:0	3.95 ± 0.02^{b}	4.03 ± 0.09^{a}	0.93 ± 0.00^{e}	0.87 ± 0.00^{f}	0.55 ± 0.01^{g}	0.52 ± 0.00^{g}	1.78 ± 0.00^{c}	1.69 ± 0.00^{d}
C14:1	0.02 ± 0.00^{b}	0.02 ± 0.00^{b}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	nd	nd	nd	nd
C15:0	0.16 ± 0.00^{c}	0.16 ± 0.00^{c}	0.11 ± 0.00^{d}	0.11 ± 0.00^{d}	0.24 ± 0.00^{b}	0.22 ± 0.03^{b}	0.54 ± 0.00^{a}	0.53 ± 0.00^{a}
C16:0	18.06 ± 0.01^{f}	18.21 ± 0.02^{e}	28.80 ± 0.09^{c}	28.49 ± 0.01^{d}	13.51 ± 0.04^{h}	13.95 ± 0.02^{g}	31.50 ± 0.05^{b}	31.85 ± 0.01^{a}
C16:1	1.94 ± 0.00^{a}	1.61 ± 0.00^{b}	1.39 ± 0.00^{c}	1.32 ± 0.00^{d}	0.44 ± 0.01^{g}	0.38 ± 0.00^{h}	0.72 ± 0.00^{e}	0.59 ± 0.00^{f}
C17:0	0.18 ± 0.00^{g}	0.19 ± 0.00^{f}	0.23 ± 0.00^{d}	0.22 ± 0.00^{e}	1.45 ± 0.00^{b}	1.53 ± 0.01^{a}	0.77 ± 0.00^{c}	0.77 ± 0.00^{c}
C17:1	0.16 ± 0.00^{c}	0.15 ± 0.00^{c}	nd	nd	0.39 ± 0.00^{a}	0.34 ± 0.00^{b}	nd	nd
C18:0	2.64 ± 0.00^{h}	3.08 ± 0.01^{g}	7.70 ± 0.04^{d}	8.15 ± 0.02^{c}	9.51 ± 0.05^{b}	9.89 ± 0.04^{a}	5.95 ± 0.02^{f}	6.30 ± 0.00^{e}
C18:1 (trans)	nd	nd	0.16 ± 0.00^{b}	0.21 ± 0.00^{a}	0.02 ± 0.00^{c}	0.03 ± 0.00^{c}	nd	nd
C18:1	42.58 ± 0.03^{a}	42.11 ± 0.09^{b}	26.13 ± 0.06^{d}	25.46 ± 0.01^{e}	11.69 ± 0.01^{f}	11.13 ± 0.08^{g}	33.09 ± 0.03^{c}	33.03 ± 0.01^{c}
C18:2 (trans)	nd	nd	0.16 ± 0.00^{a}	0.15 ± 0.00^{a}	nd	nd	nd	nd
C18:2	27.61 ± 0.02^{d}	27.99 ± 0.03^{c}	31.75 ± 0.07^{b}	32.38 ± 0.02^{a}	15.14 ± 0.01^{h}	15.50 ± 0.02^{g}	23.21 ± 0.04^{f}	22.83 ± 0.01^{e}
C20:0	0.12 ± 0.00^{f}	0.12 ± 0.00^{f}	0.39 ± 0.00^{a}	0.39 ± 0.00^{a}	0.20 ± 0.00^{c}	0.25 ± 0.01^{b}	0.14 ± 0.00^{e}	0.15 ± 0.00^{d}
C20:1	0.16 ± 0.00^{a}	0.16 ± 0.00^{a}	nd	nd	nd	nd	0.08 ± 0.00^{b}	0.08 ± 0.00^{b}
C18:3	1.35 ± 0.00^{c}	1.15 ± 0.00^{e}	1.22 ± 0.01^{d}	1.19 ± 0.00^{de}	45.10 ± 0.02^{a}	44.62 ± 0.06^{b}	0.75 ± 0.00^{f}	0.73 ± 0.00^{f}
C20:2	0.07 ± 0.00^{c}	0.06 ± 0.02^{c}	0.15 ± 0.00^{b}	0.16 ± 0.00^{b}	0.33 ± 0.00^{a}	0.32 ± 0.00^{a}	0.04 ± 0.00^{d}	0.04 ± 0.00^{d}

C22:0	0.01 ± 0.00^{g}	0.01 ± 0.00^{g}	0.04 ± 0.00^{d}	0.05 ± 0.00^{c}	0.09 ± 0.00^{b}	0.11 ± 0.00^{a}	0.02 ± 0.00^{f}	0.03 ± 0.00^{e}
C22:1	nd	nd	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	nd	nd	nd	nd
C20:3	nd	nd	0.02 ± 0.00^{c}	0.02 ± 0.00^{c}	0.21 ± 0.00^{a}	0.20 ± 0.00^{b}	nd	nd
C20:4	nd	nd	0.01 ± 0.00^{c}	0.01 ± 0.00^{c}	0.19 ± 0.00^{a}	0.15 ± 0.00^{b}	nd	nd
C23:0	0.12 ± 0.00^{a}	0.13 ± 0.00^{a}	nd	nd	nd	nd	nd	nd
C22:2	nd	nd	nd	nd	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	nd	nd
C24:1	nd	nd	nd	nd	0.27 ± 0.00^{a}	0.24 ± 0.01^{b}	nd	nd
PUFA /SFA	1.11 ± 0.00^{c}	1.09 ± 0.00^{d}	0.85 ± 0.00^{f}	0.86 ± 0.00^{e}	2.33 ± 0.00^{a}	2.25 ± 0.00^{b}	0.57 ± 0.00^{g}	0.55 ± 0.00^{h}
AI	0.46 ± 0.00^{e}	0.47 ± 0.00^{d}	0.54 ± 0.00^{b}	0.53 ± 0.00^{c}	0.22 ± 0.00^{f}	0.22 ± 0.00^{f}	0.67 ± 0.00^{a}	0.67 ± 0.00^{a}
TI	0.61 ± 0.00^{e}	0.64 ± 0.00^{d}	1.11 ± 0.01^{c}	1.12 ± 0.00^{c}	0.15 ± 0.00^{g}	0.16 ± 0.00^{f}	1.27 ± 0.00^{b}	1.30 ± 0.00^{a}

$^{a-h}$Duncan's multiple range test shows that values in the row with different letters differ significantly at $p < 0.05$. nd, not detected; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; AI, arteriosclerosis index; TI, thrombotic index; TS, *Tenebrio molitor* oil prepared using SFE; TU, *Tenebrio molitor* oil prepared using UAE; GS, *Gryllus bimaculatus* oil prepared using SFE; GU, *Gryllus bimaculatus* oil prepared using UAE; LS, *Locusta migratoria* oil prepared using SFE; LU, *Locusta migratoria* oil prepared using UAE; ZS, *Zophobas atratus* oil prepared using SFE; ZU, *Zophobas atratus* oil prepared using UAE.

3.4. Establishing a quantitative method

The quantification of volatile compounds was achieved through the evaluation of linearity, using regression equations, and determining the LOD and LOQ. The LOD refers to the minimum concentration of a substance that can be reliably detected, while the LOQ indicates the minimum concentration that can be accurately quantified by the analytical method. Linearity was assessed by preparing six different concentrations through dilution of stock solutions of the volatile compounds. The lowest concentration in the calibration curve was above the LOQ and encompassed the full range of concentrations found in the samples. The LOD and LOQ were determined using the standard deviation of the y-intercepts (σ) and the slope (S) from the regression analysis. The calculations were performed using the equations LOD = 3σ /S and LOQ = 10σ/S (Kim et al., 2024; Zhang et al., 2024; Kim et al., 2022). **Table 1.3** presents the calibration parameters for 47 volatile compounds, with all regression equations showing correlation coefficients (R^2) greater than 0.9899. Notably, the LOD for hydrocarbons, ketones, 2-pentylfuran, and 2,5-dimethylpyrazine was below 10 ng/g, and the LOQ was below 20 ng/g, indicating a high level of detection sensitivity. These results align with the guidelines set by the Codex Alimentarius (2015).

Table 1.3. Regression equations and limits of detection (LOD) and quantification (LOQ) of volatile compounds in edible insect oils

Compounds	Quantifier ions (*m/z*)	Qualifier ions (*m/z*)	Linearity			LOD (ng/g)	LOQ (ng/g)
			Regression equation	R^2	Range (ng/g)		
Acids							
2-methylbutanoic acid	74	57,41	y = 4.432x - 3.013	0.9899	20-20000	3.24	10.79
2-methylpropanoic acid	88	43,73	y = 0.402x - 0.0087	0.9999	20-7000	2.85	9.52
3-methylbutanoic acid	87	60,43	y = 1.5584x - 1.2918	0.9989	500-30000	41.45	138.17
acetic acid	60	43,29	y = 20.772x - 11.97	0.9994	300-200000	65.95	219.83
butanoic acid	73	60,55	y = 1.6492x - 0.5506	0.9940	100-10000	19.91	66.36
decanoic acid	129	73,60	y = 0.052x + 0.0054	0.9940	1000-15000	127.88	426.26
heptanoic acid	73	60,43	y = 0.1907x + 0.1587	0.9981	2000-30000	593.79	1979.32
nonanoic acid	73	60,41	y = 0.1405x - 0.1526	0.9997	2000-80000	401.34	1337.81
octanoic acid	115	60,101	y = 0.5996x - 2.5717	0.9970	100-150000	23.74	79.13
pentanoic acid	73	60,55	y = 1.411x - 0.3304	0.9930	50-5000	10.61	35.37
propanoic acid	74	45,28	y = 4.8046x - 1.3446	0.9999	300-120000	80.22	267.40
Alcohols							
1-decanol	83	55,112	y = 0.024x - 0.0019	0.9992	20-5000	5.23	17.43
2,3-butanediol	45	57,75	y = 0.003x - 0.0201	0.9970	1000-200000	204.64	682.12
2-phenylethanol	122	91,92	y = 0.6113x - 0.3178	0.9998	10-100000	1.01	3.36

Aldehydes							
(E)-2-nonenal	96	70,83	y = 0.0267x - 0.007	0.9978	50-15000	13.53	45.10
(E)-2-octenal	83	55,70	y = 0.1959x - 0.0263	0.9983	50-5000	13.78	45.92
2-methylbutanal	57	41,86	y = 0.1486x - 0.0126	0.9984	200-8000	55.33	184.43
3-methylbutanal	58	44,71	y = 0.2171x - 0.0732	0.9992	50-20000	10.44	34.81
benzaldehyde	106	77,51	y = 4.7424x - 0.012	0.9993	20-1000	5.55	18.50
decanal	57	43,41	y = 0.0687x - 0.0114	0.9979	100-3000	11.82	39.40
heptanal	70	41,55	y = 1.3454x - 0.0761	0.9998	100-3000	23.02	76.72
hexanal	72	44,56	y = 0.5698x - 0.265	0.9994	50-40000	14.86	49.55
nonanal	98	57,70	y = 0.209x - 0.0173	0.9990	50-6000	8.35	27.84
pentanal	58	44,57	y = 0.9277x + 0.0727	0.9992	100-4000	22.07	73.58
phenylacetaldehyde	120	91,65	y = 0.8205x - 0.0166	0.9912	20-1000	6.00	20.01
Esters							
ethyl octanoate	127	88,101	y = 5.4518x - 1.656	0.9975	50-10000	7.27	24.25
methyl decanoate	143	74,87	y = 2.3201x + 0.1603	0.9978	20-1000	5.43	18.11
methyl hexanoate	99	74,87	y = 112.77x - 0.055	0.9988	5-100	1.28	4.28
methyl octanoate	127	74,158	y = 0.4835x - 0.0043	0.9996	50-5000	9.84	32.80
methyl phenylacetate	150	90,65	y = 8.0161x - 0.0846	0.9995	10-1000	3.05	10.18
methyl salicylate	152	120,92	y = 0.8482x - 0.4024	0.9921	100-10000	16.98	56.61
Furan							
2-pentylfuran	81	95,138	y = 0.236x + 0.0672	0.9992	10-20000	1.19	3.96
Hydrocarbons							

d-limonene	136	68,93	y = 1.0887x - 0.0676	0.9967	5-2000	1.34	4.45
o-xylene	106	91,77	y = 8.6549x + 0.0322	0.9996	20-4000	4.68	15.61
p-xylene	106	91,77	y = 11.321x + 0.1008	0.9999	10-13000	2.92	9.72
Indole							
indole	117	90,63	y = 0.9206x + 0.0549	0.9992	100-4000	19.01	63.35
Ketones							
2-decanone	71	58,43	y = 22.794x - 0.2171	0.9961	20-400	5.73	19.09
2-heptanone	58	71,114	y = 76.563x - 0.2886	0.9990	5-400	1.32	4.39
2-nonanone	71	43,58	y = 80.029x + 0.0533	0.9992	5-200	1.17	3.90
acetophenone	120	105,77	y = 151.82x - 0.2115	0.9985	5-600	1.19	3.97
Lactones							
γ-butyrolactone	86	42,56	y = 17.045x - 1.4453	0.9984	20-4000	4.03	13.43
γ-nonanolactone	99	85,29	y = 2.8907x - 0.6397	0.9930	50-4000	13.64	45.46
Phenols							
2-methoxyphenol	124	81,109	y = 4.0895x - 0.1926	0.9970	10-5000	1.92	6.40
phenol	94	66,65	y = 11.261x - 1.2951	0.9942	100-10000	25.28	84.27
Pyrazine							
2,5-dimethylpyrazine	108	42,81	y = 0.4479x - 0.0254	0.9989	10-4000	1.26	4.22
Sulfur compounds							
dimethyl sulfone	94	79,15	y = 1.0793x - 1.1585	0.9983	50-50000	14.17	47.22
methional	104	76,61	y = 0.3496x - 0.0525	0.9982	50-5000	12.02	40.07

R^2, Correlation coefficient; LOD=3σ/S and LOQ=10σ/S.

3.5. Volatile compound analysis

The volatile compounds analyzed by HS-SPME-Arrow-GC/MS are listed in **Table 1.4** and **Figure 1.S4**. A total of 47 volatile compounds were detected in the edible insect oils, classified into 12 classes: acids (11), alcohols (3), aldehydes (11), esters (6), furans (1), hydrocarbons (3), indoles (1), ketones (4), lactones (2), phenols (2), pyrazines (1), and sulfur compounds (2). Oils extracted from *T. molitor* (TS), *G. bimaculatus* (GS), *L. migratoria* (LS), and *Z. atratus* (ZS) using the SFE method contained 22, 37, 34, and 34 volatile compounds, respectively. Oils extracted using the UAE method from *T. molitor* (TU), *G. bimaculatus* (GU), *L. migratoria* (LU), and *Z. atratus* (ZU) contained 12, 26, 17, and 23 volatile compounds, respectively. The total concentrations of volatile compounds were higher in the SFE-extracted oils compared to those extracted by UAE.

The oils extracted using the SFE method contained a greater variety of volatile compounds and exhibited higher overall concentrations than those extracted by UAE. Compounds such as 2-methylbutanoic acid, acetic acid, benzaldehyde, methyl salicylate, phenol, and dimethyl sulfone were found in oils extracted by both methods. 2-Methylbutanoic acid imparts buttery, cheesy, and sour notes, while acetic acid contributes acidic, fruity, and vinegary notes. The almond-like aroma in insect oils may be attributed to benzaldehyde, a compound also present in peanut, rapeseed, and soybean oils (Lee et al., 2023). Methyl salicylate adds almond, caramel, and fresh notes, while phenol imparts sharp, smoky,

and spicy notes. Dimethyl sulfone, associated with burnt and sulfuric notes, is likely derived from the insects' diet, as it is an oxidation product of dimethyl sulfoxide, a compound naturally present in grains, vegetables, and fruits (Perez-Santaescolastica et al., 2023). The concentration of these volatile compounds varied depending on the insect species and the extraction method used.

As in previous studies (Lee et al., 2023; Perez-Santaescolastica et al., 2023), acetic acid was detected as a major compound in most edible insects, with the highest concentration observed in GS. Additionally, *G. bimaculatus* oils contained a high concentration of propanoic acid, which imparts fat, pungent, and raspberry notes. In *Z. atratus* oils, significantly higher concentrations of octanoic acid (acid and cheese) and nonanoic acid (fatty, green, and sour) were found ($p < 0.05$). Both extraction methods contained the same components, such as 2-methylpropanoic acid, decanoic acid, and propanoic acid in *G. bimaculatus* and *Z. atratus* oils.

Alcohols are well recognized for their intense sensory characteristics, including fruity, floral, and fatty notes (Lee et al., 2019). In this study, 2,3-butanediol (creamy, floral, and fruity), detected exclusively in UAE oils, was the most abundant alcohol, with the highest concentration in GU (177.208 μg/g), followed by LU (135.772 μg/g). ZU and TU had similar concentrations, at 29.430 μg/g and 26.622 μg/g, respectively. 1-Decanol, which imparts fat and oil notes, was detected only in *Z. atratus* oils, whereas 2-phenylethanol (cornflake and honey) was

significantly higher in *G. bimaculatus* oils ($p < 0.05$).

A total of 11 aldehydes were detected, with 3-methylbutanal, hexanal, pentanal, and phenylacetaldehyde found only in SFE oils and (E)-2-nonenal, 2-methylbutanal, and decanal detected only in GS. Aldehydes extensively influence both fragrances and flavors, typically contributing fatty notes (Dun et al., 2019). Hexanal and nonanal are both products of lipid oxidation, with hexanal derived from linoleic acid and nonanal from oleic acid (Liu et al., 2019). This may explain the high concentrations of hexanal and nonanal, as linoleic acid and oleic acid are the primary fatty acids in edible insect oils (**Table 1.2**). Furthermore, benzaldehyde (almond, berry, and bitter), another oxidative degradation byproduct of oleic acid and linoleic acid, exhibited its highest concentration in TS, which is consistent with findings in peanut oil (Lee et al., 2023). Additionally, phenylacetaldehyde, which had its highest concentration in TS, was detected only in SFE oils. Known as a precursor of floral notes, phenylacetaldehyde is also associated with the Maillard reaction in nuts. It is produced from phenylalanine via polyphenol oxidase and has been reported in freshly roasted almonds (Valdés García et al., 2021). Benzaldehyde and phenylacetaldehyde have been reported as major volatile compounds in roasted sesame oil, following pyrazines (Yin et al., 2021).

Esters, which impart pleasant fruity notes, are primarily produced through the esterification of alcohols and organic acids (Nam et al., 2024). *Z. atratus* oils showed the highest concentration of ethyl

octanoate, likely due to the high level of octanoic acid in this species, as esters are generally formed by the esterification of carboxylic acids (Lee et al., 2021). These results are consistent with previous studies on the volatile analysis of *Z. atratus* (Perez-Santaescolastica et al., 2023). Methyl octanoate (fruit, orange, and sweet) was higher in SFE oils, whereas methyl salicylate (almond, caramel, and fresh) was significantly higher in UAE oils ($p < 0.05$).

The only furan identified in this study was 2-pentylfuran, which imparts buttery, floral, and fruity notes and was found exclusively in SFE oils. 2-Pentylfuran has also been reported in peanut oil (Dun et al., 2019) and is derived from the oxidation of linolenic acid and other n-6 fatty acids. It is known to significantly contribute to aroma due to its low odor threshold (Ma et al., 2024b; Perez-Santaescolastica et al., 2023).

In the hydrocarbon group, three compounds—d-limonene, o-xylene, and p-xylene—were detected. The concentration of d-limonene, which imparts citrus and mint notes, was significantly higher in the SFE oils ($p < 0.05$), with LS showing the highest concentration. D-limonene, a major component of citrus essential oil, is used as an additive in various foods and exhibits anti-cancer properties (Lee et al., 2021). Additionally, o-xylene (geranium) and p-xylene (cold meat fat and sweet) were found only in SFE oils and have been identified in various foods, including edible insects, insect oils, and walnut oil (Lee et al., 2021; Lee et al., 2022; Lee et al., 2023; Ma et al., 2024b).

Four ketones, identified as minor compounds, were detected. The most

abundant was 2-heptanone, found in all samples except *T. molitor* oils. Additionally, 2-decanone and 2-nonanone were detected in *G. bimaculatus* and *L. migratoria* oils, while acetophenone (almond, animal, and floral) was found exclusively in *L. migratoria* oils.

Two lactones were detected: γ-butyrolactone and γ-nonanolactone. γ-butyrolactone was present in all samples except LU, whereas γ-nonanolactone was detected only in GS. Notably, γ-butyrolactone (caramel, fruity, and roasted nut) was significantly higher in TS ($p < 0.05$). The γ-butyrolactone core structure is found in numerous natural products with biological activities, including antibiotic, antitumor, and antifungal effects. It is also valued in the pharmaceutical and food industries for its fragrance and flavor properties (Kumru et al., 2018).

The compound 2,5-dimethylpyrazine, which imparts burnt, cocoa, and coffee notes, was detected exclusively in SFE oils. Notably, its concentration was significantly higher in LS ($p < 0.05$). 2,5-dimethylpyrazine has been reported in various oils, including edible insect oil, peanut oil, and roasted sesame oil (Dun et al., 2019; Lee et al., 2022; Lee et al., 2023; Yin et al., 2021), and has been strongly correlated with roasted and nutty notes (Yin et al., 2021). Heterocyclic compounds such as pyrazines and furans are volatile compounds formed via the Maillard reaction in various heat-treated foods (Ma et al., 2024b). Nitrogen-containing pyrazines, in particular, contribute to nutty and roasted notes, which are key factors influencing consumer preferences (Lee et al., 2023).

Two sulfur compounds were identified: methional and dimethyl sulfone. Methional was detected exclusively in SFE oils, with a significantly high concentration in LS ($p < 0.05$). Methional, which imparts potato, roasted, and soy notes, has been detected in almonds and is also a Maillard reaction product (Valdés García et al., 2021). Dimethyl sulfone, characterized by burnt and sulfur notes, was present in high concentrations in *G. bimaculatus* oils.

Indole, known for its floral note at low concentrations and strong fecal note at higher levels (Kim et al., 2021), was detected in all edible insect oils except *T. molitor*. Phenolic compounds, such as 2-methoxyphenol and phenol, which impart smoky notes, were extracted using both methods.

Table 1.4. Volatile compound concentration levels (μg/g) in oils derived from four edible insect species (*Tenebrio molitor*, *Gryllus bimaculatus*, *Locusta migratoria*, and *Zophobas atratus*) using two extraction methods

Compounds	TS	TU	GS	GU	LS	LU	ZS	ZU	Aroma description
Acids									
2-methyl butanoic acid	4.793 ± 0.143^{e}	3.918 ± 0.212^{f}	19.115 ± 0.082^{a}	8.347 ± 0.339^{c}	8.679 ± 0.429^{c}	12.181 ± 0.720^{b}	4.454 ± 0.142^{ef}	6.585 ± 0.138^{d}	butter, cheese, sour
2-methyl propanoic acid	nd	nd	6.617 ± 0.226^{a}	0.373 ± 0.010^{d}	nd	nd	4.099 ± 0.219^{c}	5.333 ± 0.398^{b}	burnt, butter, cooked
3-methyl butanoic acid	nd	nd	24.887 ± 2.394^{a}	8.035 ± 0.277^{d}	9.884 ± 0.464^{c}	13.756 ± 0.273^{b}	3.219 ± 0.194^{f}	6.084 ± 0.406^{e}	acid, cheese, fermented
acetic acid	97.451 ± 0.476^{c}	50.901 ± 0.678^{d}	189.108 ± 7.235^{a}	32.772 ± 0.446^{e}	115.950 ± 9.010^{b}	97.118 ± 2.172^{c}	54.574 ± 0.146^{d}	48.537 ± 0.593^{d}	acid, fruit, vinegar
butanoic acid	8.139 ± 0.525^{a}	3.239 ± 0.031^{c}	nd	nd	nd	nd	3.636 ± 0.278^{c}	7.183 ± 0.343^{b}	butter, cheese, must
decanoic acid	nd	nd	2.777 ± 0.203^{c}	1.696 ± 0.013^{d}	nd	nd	6.286 ± 0.473^{b}	14.629 ± 0.036^{a}	dust, fat, grass,
heptanoic acid	nd	nd	22.245 ± 1.553^{a}	1.718 ± 0.036^{b}	nd	nd	nd	nd	apricot, floral, sweat
nonanoic acid	7.727 ± 0.188^{d}	nd	14.681 ± 1.327^{c}	7.068 ± 0.401^{d}	15.086 ± 0.993^{c}	nd	17.258 ± 0.713^{b}	59.236 ± 0.668^{a}	fat, green, sour
octanoic acid	3.033 ± 0.045^{de}	3.224 ± 0.087^{de}	8.783 ± 0.633^{c}	3.694 ± 0.044^{cde}	5.812 ± 0.465^{cd}	nd	125.374 ± 7.521^{b}	138.027 ± 3.107^{a}	acid, cheese
pentanoic acid	nd	nd	nd	nd	3.276 ± 0.205^{a}	nd	nd	nd	cheese, pungent, sweat

propanoic acid	nd	nd	119.190 ± 3.893[a]	45.360 ± 2.390[b]	nd	nd	6.234 ± 0.111[d]	12.788 ± 0.140[c]	fat, pungent, raspberry
Alcohols									
1-decanol	nd	nd	nd	nd	nd	nd	1.537 ± 0.315[b]	4.977 ± 0.396[a]	fat, oil
2,3-butanediol	nd	26.622 ± 1.755[c]	nd	177.208 ± 9.968[a]	nd	135.772 ± 5.091[b]	nd	29.430 ± 1.633[c]	cream, floral, fruit
2-phenylethanol	nd	nd	61.857 ± 1.083[a]	33.376 ± 2.593[b]	1.794 ± 0.123[c]	0.490 ± 0.025[c]	0.339 ± 0.002[c]	1.661 ± 0.136[c]	corn flakes, honey
Aldehydes									
(E)-2-nonenal	nd	nd	8.449 ± 0.594[a]	nd	nd	nd	nd	nd	beany, cucumber
(E)-2-octenal	nd	nd	3.153 ± 0.099[a]	0.316 ± 0.016[c]	0.616 ± 0.038[b]	nd	nd	nd	fat, fish oil, green
2-methylbutanal	nd	nd	2.809 ± 0.132[a]	nd	nd	nd	nd	nd	chocolate, cocoa, hazelnut
3-methylbutanal	6.973 ± 0.113[a]	nd	5.779 ± 0.133[c]	nd	6.233 ± 0.450[b]	nd	1.456 ± 0.112[d]	nd	almond, chocolate, cocoa
benzaldehyde	0.812 ± 0.006[a]	0.049 ± 0.004[g]	0.677 ± 0.008[b]	0.307 ± 0.022[e]	0.472 ± 0.005[d]	0.568 ± 0.020[c]	0.114 ± 0.003[f]	0.098 ± 0.008[f]	almond, berry, bitter
decanal	nd	nd	0.659 ± 0.036[a]	nd	nd	nd	nd	nd	fat, floral, fried
heptanal	0.190 ± 0.001[c]	nd	1.101 ± 0.032[a]	0.131 ± 0.004[e]	0.317 ± 0.010[b]	nd	0.155 ± 0.002[d]	nd	citrus, dry fish
hexanal	1.851 ± 0.008[d]	nd	20.303 ± 0.715[a]	nd	5.116 ± 0.404[b]	nd	2.779 ± 0.339[c]	nd	apple, cut grass

nonanal	0.383 ± 0.010^{c}	nd	1.981 ± 0.042^{a}	0.251 ± 0.003^{d}	0.898 ± 0.087^{b}	nd	0.228 ± 0.013^{d}	nd	citrus, cucumber, fat
pentanal	nd	nd	1.949 ± 0.046^{b}	nd	2.323 ± 0.038^{a}	nd	nd	nd	almond, chemical, green
phenylacetaldehyde	0.682 ± 0.005^{a}	nd	0.148 ± 0.004^{b}	nd	0.118 ± 0.001^{c}	nd	0.110 ± 0.002^{d}	nd	berry, floral, flower
Esters									
ethyl octanoate	1.674 ± 0.002^{d}	1.670 ± 0.001^{d}	nd	nd	1.956 ± 0.015^{c}	1.699 ± 0.009^{d}	9.256 ± 0.060^{b}	9.910 ± 0.155^{a}	apricot, banana, brandy
methyl decanoate	nd	nd	nd	nd	nd	nd	0.157 ± 0.008^{a}	0.060 ± 0.003^{b}	fresh, soap, wax
methyl hexanoate	nd	nd	0.089 ± 0.003^{a}	nd	0.059 ± 0.001^{b}	nd	0.058 ± 0.000^{b}	nd	ester, fresh, fruit
methyl octanoate	nd	nd	0.145 ± 0.002^{c}	0.067 ± 0.001^{c}	0.139 ± 0.029^{c}	0.057 ± 0.001^{c}	2.958 ± 0.187^{a}	1.637 ± 0.110^{b}	fruit, orange, sweet
methyl phenylacetate	nd	nd	nd	0.176 ± 0.001^{a}	nd	nd	nd	nd	honey, jasmine, sweet
methyl salicylate	0.471 ± 0.005^{f}	1.387 ± 0.083^{d}	1.067 ± 0.015^{e}	9.000 ± 0.392^{b}	0.698 ± 0.027^{f}	2.835 ± 0.118^{c}	0.648 ± 0.008^{f}	9.780 ± 0.226^{a}	almond, caramel, fresh
Furan									
2-pentylfuran	0.212 ± 0.010^{d}	nd	17.544 ± 0.455^{a}	nd	4.405 ± 0.164^{b}	nd	0.737 ± 0.009^{c}	nd	butter, floral, fruit
Hydrocarbons									
d-limonene	0.459 ± 0.005^{c}	0.105 ± 0.002^{f}	1.246 ± 0.014^{b}	0.191 ± 0.014^{e}	1.440 ± 0.034^{a}	0.317 ± 0.012^{d}	0.115 ± 0.003^{f}	nd	citrus, mint
o-xylene	0.787 ± 0.010^{c}	nd	3.361 ± 0.112^{b}	nd	3.836 ± 0.040^{a}	nd	0.109 ± 0.005^{d}	nd	geranium

p-xylene	1.835 ± 0.011^{c}	nd	7.726 ± 0.199^{b}	nd	9.463 ± 0.089^{a}	nd	0.156 ± 0.005^{d}	nd	cold meat fat, sweet
Indole									
indole	nd	nd	2.932 ± 0.194^{b}	0.661 ± 0.050^{e}	0.842 ± 0.019^{d}	0.498 ± 0.030^{e}	2.023 ± 0.077^{c}	4.601 ± 0.189^{a}	animal, burnt, fecal
Ketones									
2-decanone	nd	nd	0.369 ± 0.014^{a}	0.247 ± 0.001^{c}	0.275 ± 0.004^{b}	0.243 ± 0.002^{c}	nd	nd	fat, fruit
2-heptanone	nd	nd	0.387 ± 0.006^{a}	0.293 ± 0.000^{cd}	0.332 ± 0.001^{b}	0.291 ± 0.000^{de}	0.296 ± 0.000^{c}	0.289 ± 0.000^{e}	cinnamon, fruit, green
2-nonanone	nd	nd	0.067 ± 0.003^{a}	nd	0.028 ± 0.000^{b}	nd	nd	nd	fragrant, fruit, green
acetophenone	nd	nd	nd	nd	0.219 ± 0.001^{b}	0.224 ± 0.001^{a}	nd	nd	almond, animal, flower
Lactones									
γ-butyrolactone	2.929 ± 0.092^{a}	2.089 ± 0.047^{c}	2.054 ± 0.019^{c}	1.689 ± 0.047^{e}	1.907 ± 0.044^{d}	nd	2.656 ± 0.116^{b}	2.030 ± 0.039^{c}	caramel, fruit, roasted nut
γ-nonanolactone	nd	nd	0.684 ± 0.006^{a}	nd	nd	nd	nd	nd	apricot, cocoa, coconut
Phenols									
2-methoxyphenol	nd	nd	2.568 ± 0.124^{c}	4.905 ± 0.095^{b}	2.762 ± 0.045^{c}	5.638 ± 0.456^{a}	0.268 ± 0.006^{de}	0.544 ± 0.018^{d}	bacon, cream, smoke
phenol	2.675 ± 0.023^{e}	1.473 ± 0.035^{f}	9.000 ± 0.224^{a}	8.803 ± 0.221^{a}	3.766 ± 0.225^{d}	2.509 ± 0.041^{e}	5.662 ± 0.135^{c}	7.959 ± 0.292^{b}	sharp, smoke, spice
Pyrazine									

2,5-dimethyl pyrazine	0.109 ± 0.001[c]	nd	nd	nd	1.202 ± 0.002[a]	nd	0.586 ± 0.005[b]	nd	burnt, cocoa, coffee
Sulfur compounds									
dimethyl sulfone	1.948 ± 0.049[e]	3.049 ± 0.041[d]	39.824 ± 1.262[a]	13.353 ± 0.429[b]	4.548 ± 0.065[c]	1.449 ± 0.011[e]	2.010 ± 0.002[e]	1.804 ± 0.016[e]	burnt, sulfur
methional	1.771 ± 0.125[b]	nd	nd	nd	2.939 ± 0.228[a]	nd	1.335 ± 0.020[c]	nd	potato, roast, soy

[a-g]Duncan's multiple range test shows that values in the row with different letters differ significantly at $p < 0.05$; Aroma descriptions were provided using the online databases from https://www.vcf-online.nl/VcfHome.cfm. nd, not detected; TS, *Tenebrio molitor* oil prepared using SFE; TU, *Tenebrio molitor* oil prepared using UAE; GS, *Gryllus bimaculatus* oil prepared using SFE; GU, *Gryllus bimaculatus* oil prepared using UAE; LS, *Locusta migratoria* oil prepared using SFE; LU, *Locusta migratoria* oil prepared using UAE; ZS, *Zophobas atratus* oil prepared using SFE; ZU, *Zophobas atratus* oil prepared using UAE.

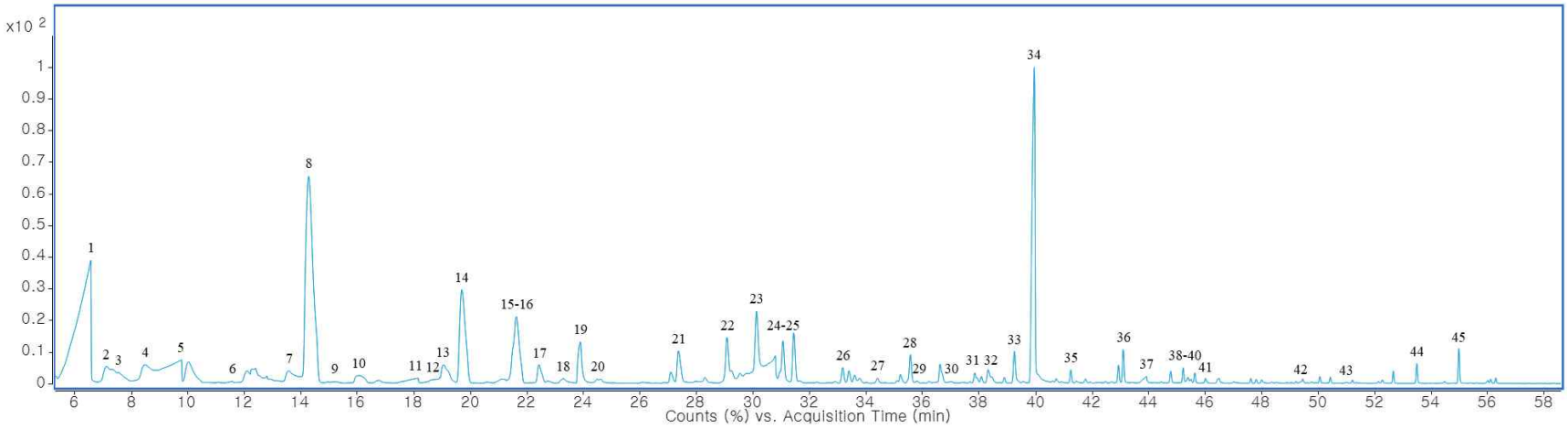

Figure 1.S4. Gas chromatography-mass spectrometry (GC/MS) chromatograms of volatile compounds in *Gryllus bimaculatus* oil prepared using supercritical fluid extraction (SFE). 1, acetic acid; 2, 3-methylbutanal; 3, 2-methylbutanal; 4, pentanal; 5, propanoic acid; 6, 2-methylpropanoic acid; 7, toluene-d_8 (IS); 8, hexanal; 9, 2,2-dimethylpropanoic acid; 10, 2-methylpyrazine (IS); 11, 3-methylbutanoic acid; 12, 2-methylbutanoic acid; 13, 1-hexyl alcohol-d_{13} (IS); 14, p-xylene; 15, 2-heptanone; 16, o-xylene; 17, heptanal; 18, γ-butyrolactone; 19, dimethyl

sulfone; 20, methyl hexanoate; 21, benzaldehyde; 22, phenol; 23, 2-penthylfuran; 24, 3-octanone (IS); 25, octanal (IS); 26, d-limonene; 27, phenylacetaldehyde; 28, (E)-2-octenal; 29, phenyl acetate (IS); 30, heptanoic acid; 31, 2-methoxyphenol; 32, 2-nonanone; 33, nonanal; 34, 2-phenylethanol; 35, methyl octanoate; 36, (E)-2-nonenal; 37, octanoic acid; 38, 3,4-dimethyl phenol (IS); 39, 2-decanone; 40, methyl salicylate; 41, decanal; 42, nonanoic acid; 43, indole; 44, decanoic acid; and 45, γ-nonalactone.

3.6. Hierarchical clustering analysis

A heatmap was utilized for a comprehensive evaluation of the correlation analysis (**Figure 1.S5**). The color gradient represented the concentration levels of these compounds, ranging from maximum (dark red) to minimum (dark blue). Volatile compounds associated with roasted notes, such as 2,5-dimethylpyrazine and methional, displayed a red pattern for LS. Additionally, d-limonene (citrus and mint), known for its anti-cancer properties, exhibited a red pattern in LS. Although the volatile compound content in *T. molitor* oils was the lowest among the four insect species, benzaldehyde (almond, berry, and bitter), phenylacetaldehyde (berry and floral), and γ-butyrolactone (caramel, fruit, and roasted nut) had the highest concentrations in TS. Regardless of the extraction method, compounds that exhibited similar red patterns across insect species included 2-phenylethanol in *G. bimaculatus* and octanoic acid and ethyl octanoate in *Z. atratus*.

To the best of our knowledge, limited information is available on the volatile compounds in edible insect oils. This study revealed diverse volatile compound profiles in oils extracted from *T. molitor*, *G. bimaculatus*, *L. migratoria*, and *Z. atratus*, many of which have been reported as key compounds in various food products. Our findings demonstrate that TS and LS contain volatile compounds that contribute sensory notes associated with consumer preferences.

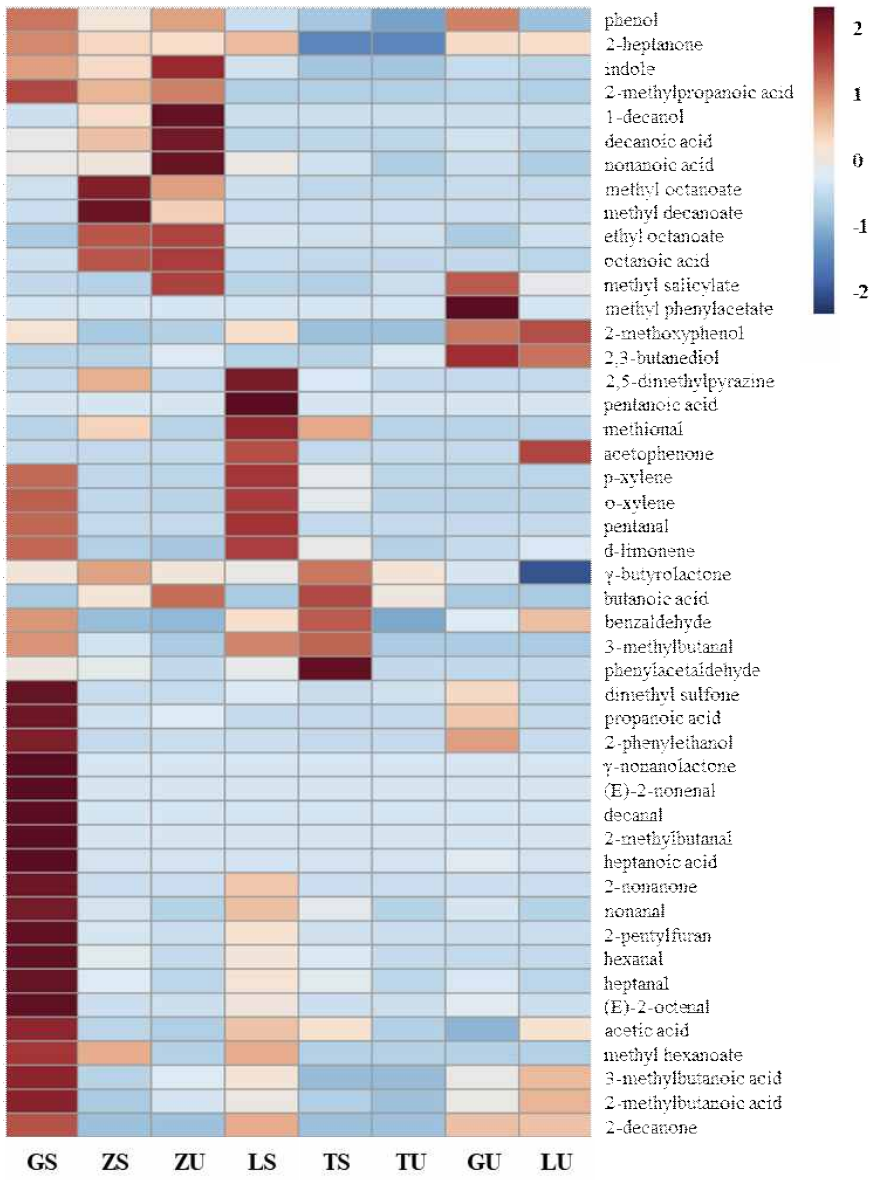

Figure 1.S5. Heatmap of changes in volatile compounds detected in the four edible insect oils obtained using two extraction methods. TS, *Tenebrio molitor* oil prepared using SFE; TU, *Tenebrio molitor* oil prepared using UAE; GS, *Gryllus bimaculatus* oil prepared using SFE;

GU, *Gryllus bimaculatus* oil prepared using UAE; LS, *Locusta migratoria* oil prepared using SFE; LU, *Locusta migratoria* oil prepared using UAE; ZS, *Zophobas atratus* oil prepared using SFE; ZU, *Zophobas atratus* oil prepared using UAE.

Ⅳ. CONCLUSION

In this study, the SFE method was optimized using response surface methodology, and four edible insect oils were extracted using two different methods (SFE and UAE). The extracted oils were analyzed for quality characteristics, fatty acids via GC/FID analysis, and volatile compounds using HS-SPME-Arrow-GC/MS analysis. The AVs and PVs of all samples were significantly higher after SFE than UAE ($p < 0.05$), likely due to the higher extraction temperature and longer extraction time. The oils were deemed suitable for consumption, with PVs below 15 meq/kg and PUFA/SFA ratios greater than 0.45. *T. molitor* oils being comparable to rice bran oil and peanut oil in terms of IV and fatty acid composition. The primary fatty acid in *L. migratoria* oil was α-linolenic acid, an essential fatty acid, with *L. migratoria* oil exhibiting the highest PUFA/SFA ratio and the lowest AI and TI among the species analyzed ($p < 0.05$). A total of 47 volatile compounds were identified, with higher concentrations in SFE oils compared to UAE oils. SFE oils notably contained Maillard reaction products such as 2-pentylfuran, 2,5-dimethylpyrazine, methional, and phenylacetaldehyde. Furthermore, TS was characterized by higher levels of compounds associated with nutty and fruity notes (benzaldehyde, phenylacetaldehyde, and γ-butyrolactone), whereas LS had elevated levels of compounds linked to citrus and roasted notes (d-limonene, 2,5-dimethylpyrazine, and methional). These findings highlight the potential of edible insect oils, particularly those derived from *T. molitor* and *L. migratoria*, as viable alternatives to conventional edible oils due to their advantageous nutritional profiles and

valuable contribution to food upcycling initiatives.

The oil extraction process of edible insects typically involves a defatting step, which facilitates oil recovery and enables the extraction of valuable protein resources. Notably, the analysis of volatile compounds in edible insect oils remains limited, with the present study being the first to quantify the concentrations of these compounds using two distinct extraction methods, SFE and UAE. However, one of the primary challenges in promoting edible insect oils is overcoming the low consumer acceptance caused by the unappealing visual characteristics of insects. Additionally, the distinct off-flavors, such as indole, associated with insect-derived oils present another limitation when compared to the sensory profiles of traditional edible oils. Addressing these sensory drawbacks requires the development of advanced processing technologies aimed at mitigating off-flavors. Future research should prioritize the optimization of processing methodologies aimed at reducing undesirable volatile compounds, thereby enhancing the sensory attributes of edible insect oils and facilitating greater consumer acceptance. By overcoming these challenges, edible insect oils can be positioned as a sustainable and nutritionally valuable alternative within the global food landscape.

REFERENCES

Alimentarius, C. (1999). Codex standard for named animal fats. *Codex Stan*, *211*, 1 - 6.

Alimentarius, C. (2015). Codex alimentarius commission: Procedural manual. Rome: Joint FAO/WHO Food Standards Programme.

Alimentarius, C. (2023). Codex standard for named vegetable oils. *Codex Stan*, *210*, 1 - 19.

AOAC (2005). *Official methods of analysis* (eighteenth edn.). Washington, DC: Association of Official Analytical Chemists.

Castejón, N., Luna, P., & Señoráns, F. J. (2018). Alternative oil extraction methods from *Echium plantagineum* L. seeds using advanced techniques and green solvents. *Food Chemistry*, *244*, 75 - 82. https://doi.org/10.1016/j.foodchem.2017.10.014.

Cha, J. Y., Han, J. J., Heo, J. A., Yu, H. H., Kim, Y. J., Jang, H. W., Kim, M. R., & Choi, Y. S. (2024). Variation of volatile compounds and sensory profile for *Protaetia brevitarsis* larvae fermented with lactic acid bacteria and yeast. *Food Chemistry*, *452*, 139480. https://doi.org/10.1016/j.foodchem.2024.139480.

Chen, C. R., Wang, C. H., Wang, L. Y., Hong, Z. H., Chen, S. H., Ho, W. J., & Chang, C. M. J. (2008). Supercritical carbon dioxide extraction and deacidification of rice bran oil. *Journal of Supercritical Fluids*, *45*, 322 - 331. https://doi.org/10.1016/j.supflu.2008.01.006.

Dun, Q., Yao, L., Deng, Z., Li, H., Li, J., Fan, Y., & Zhang, B. (2019). Effects of hot and cold-pressed processes on volatile compounds of peanut oil and corresponding analysis of characteristic flavor components. *LWT*, *112*, 107648. https://doi.org/10.1016/j.lwt.2018.11.084.

Geranpour, M., Assadpour, E., & Jafari, S. M. (2020). Recent advances in the spray drying encapsulation of essential fatty acids and functional oils. *Trends in Food Science and Technology*, *102*, 71 - 90. https://doi.org/10.1016/j.tifs.2020.05.028.

Grand View Research. (2024). *Edible insects market size, share & trends analysis report by product (whole insects, insect powder), by application (animal feed, protein bars), by region, and segment forecasts, 2023 - 2030*. Retrieved from https://www.grandviewresearch.com/industry-analysis/edible-insects-market. Accessed January 15, 2025.

Jantzen da Silva Lucas, A., Menegon de Oliveira, L., da Rocha, M., & Prentice, C. (2020). Edible insects: An alternative of nutritional, functional and bioactive compounds. *Food Chemistry*, *311*, 126022. https://doi.org/10.1016/j.foodchem.2019.126022.

Karsli, B. (2021). Comparative analysis of the fatty acid composition of commercially available fish oil supplements in Turkey: Public health risks and benefits. *Journal of Food Composition and Analysis*, *103*, 104105. https://doi.org/10.1016/j.jfca.2021.104105.

Kim, J. Y., Lee, H. E., Kim, Y. J., Yang, J. W., Lee, S. J., & Jung, Y. H.

(2021). Development of a post-processing method to reduce the unique off-flavor of *Allomyrina dichotoma*: Yeast fermentation. *LWT*, *150*, 111940. https://doi.org/10.1016/j.lwt.2021.111940.

Kim, T. E., Lee, M. H., Kim, B. K., Lee, J. H., Chun, Y. G., & Jang, H. W. (2024). Optimization of the QuEChERS-UPLC-APCI-MS/MS method for the analysis of vitamins D and K nanoencapsulated in yogurt. *Journal of Chromatography A*, *1734*, 465275. https://doi.org/10.1016/j.chroma.2024.465275.

Kim, T. E., Yoo, G., Lee, M. H., Kim, B. K., & Jang, H. W. (2022). Novel QuEChERS-ultra-performance liquid chromatography - atmospheric-pressure chemical ionization tandem mass spectrometry method for the simultaneous determination of vitamin D and vitamin K in vitamin-fortified nanoemulsions. *Food Chemistry*, *389*, 133009. https://doi.org/10.1016/j.foodchem.2022.133009.

Kumru, C., Classen, T., & Pietruszka, J. (2018). Enantioselective, Catalytic One-Pot Synthesis of ɣ-Butyrolactone-Based Fragrances. *ChemCatChem*, *10*, 4917 – 4926. https://doi.org/10.1002/cctc.201801040.

Lee, G. E., Kim, Y. J., Jang, H. B., Cha, J. Y., Choi, Y. S., & Jang, H. W. (2023). Comparison of oil quality characteristics of *Locusta migratoria*, *Allomyrina dichotoma*, and *Tenebrio molitor* obtained using different extraction methods. *Journal of Insects as Food and Feed*, *9*, 1631 – 1642. https://doi.org/10.1163/23524588-20230039.

Lee, J. H., Cha, J. Y., Kim, T. K., Choi, Y. S., & Jang, H. W. (2021).

Effects of a defatting process on the thermal stabilities and volatile compound profiles of proteins isolated from *Protaetia brevitarsis* larvae. *LWT*, *151*, 112095. https://doi.org/10.1016/j.lwt.2021.112095.

Lee, J. H., Kim, Y. J., Kim, T. K., Song, K. M., & Choi, Y. S. (2024). Effect of ethanol treatment on the structural, techno-functional, and antioxidant properties of edible insect protein obtained from *Tenebrio molitor* larvae. *Food Chemistry*, *437*, 137852. https://doi.org/10.1016/j.foodchem.2023.137852.

Lee, J. Y., Kim, W. S., Lee, Y. Y., Choi, Y. S., Choi, H. W., & Jang, H. W. (2019). Solid phase microextraction arrow for the volatile organic compounds in soy sauce. *Journal of Separation Science*, *42*, 2942 - 2948. https://doi.org/10.1002/jssc.201900388.

Lee, M. H., Kim, T. K., Cha, J. Y., Yong, H. I., Kang, M. C., Jang, H. W., & Choi, Y. S. (2022). Physicochemical characteristics and aroma patterns of oils prepared from edible insects. *LWT*, *167*, 113888. https://doi.org/10.1016/j.lwt.2022.113888.

Li, A., Dewettinck, K., Verheust, Y., Van de Walle, D., Raes, K., Diehl, B., & Tzompa-Sosa, D. A. (2024a). Edible insects as a novel source of lecithin: Extraction and lipid characterization of black soldier fly larvae and yellow mealworm. *Food Chemistry*, *452*, 139391. https://doi.org/10.1016/j.foodchem.2024.139391.

Li, Q., Zhang, C., Liu, W., Li, B., Chen, S., Wang, H., Li, Y., & Li, J. (2024b). Characterization and exploration of dynamic variation of volatile

compounds in vine tea during processing by GC-IMS and HS-SPME/GC-MS combined with machine learning algorithm. *Food Chemistry*, *460*, 140580. https://doi.org/10.1016/j.foodchem.2024.140580.

Liu, H., Wang, Z., Zhang, D., Shen, Q., Pan, T., Hui, T., & Ma, J. (2019). Characterization of key aroma compounds in Beijing roasted duck by gas chromatography-olfactometry-mass spectrometry, odor-activity values, and aroma-recombination experiments. *Journal of Agricultural and Food Chemistry*, *67*, 5847-5856. https://doi.org/10.1021/acs.jafc.9b01564.

Ma, X., Huang, C., Zheng, C., Wang, W., Ying, H., & Liu, C. (2024a). Effect of oil extraction methods on walnut oil quality characteristics and the functional properties of walnut protein isolate. *Food Chemistry*, *438*, 138052. https://doi.org/10.1016/j.foodchem.2023.138052.

Ma, X., Zheng, C., Zhou, Q., Huang, C., Wang, W., Huang, Y., & Liu, C. (2024b). Comparison evaluation pretreatments on the quality characteristics, oxidative stability, and volatile flavor of walnut oil. *Food Chemistry*, *448*, 139124. https://doi.org/10.1016/j.foodchem.2024.139124.

Maser, W. H., Maiyah, N., Karnjanapratum, S., Nukthamna, P., Thompson, A. K., Huda, N., Ali, A. M. M., & Bavisetty, S. C. B. (2024). Antidiabetic Property Optimization from Green Leafy Vegetables Using Ultrasound-Assisted Extraction to Improve Cracker Production. *Preventive Nutrition and Food Science*, *29*(1), 47. https://doi.org/10.3746/pnf.2024.29.1.47.

MFDS (2021). General Test Methods (in Korea). Retrieved from

https://various.foodsafetykorea.go.kr/fsd/#/ext/Document/FC. Accessed September 1, 2024.

Nam, J. K., Lee, J. Y., & Jang, H. W. (2024). Quality characteristics and volatile compounds of plant-based patties supplemented with biji powder. *Food Chemistry: X*, *23*, 101576. https://doi.org/10.1016/j.fochx.2024.101576.

Otero, P., Gutierrez-Docio, A., Del Hierro, J. N., Reglero, G., & Martin, D. (2020). Extracts from the edible insects *Acheta domesticus* and *Tenebrio molitor* with improved fatty acid profile due to ultrasound assisted or pressurized liquid extraction. *Food Chemistry*, *314*, 126200. https://doi.org/10.1016/j.foodchem.2020.126200.

Ou, H., Zuo, J., Gregersen, H., & Liu, X. Y. (2024). Combination of supercritical CO_2 and ultrasound for flavonoids extraction from *Cosmos sulphureus*: Optimization, kinetics, characterization and antioxidant capacity. *Food Chemistry*, *435*, 137598. https://doi.org/10.1016/j.foodchem.2023.137598.

Perez-Santaescolastica, C., De Winne, A., Devaere, J., & Fraeye, I. (2023). Comparing the aromatic profile of seven unheated edible insect species. *Food Research International*, *164*, 112389. https://doi.org/10.1016/j.foodres.2022.112389.

Rodríguez-Rodríguez, E., Herrero-Lodares, C., Sánchez-Prieto, M., Olmedilla-Alonso, B., Sánchez-Moreno, C., & De Ancos, B. (2024). Sustainable extraction methods of carotenoids from mango (*Mangifera indica* L. 'Kent') pulp: Ultrasound assisted extraction and green solvents.

Food Chemistry, *450*, 139253. https://doi.org/10.1016/j.foodchem.2024.139253.

Valdés García, A., Sánchez Romero, R., Juan Polo, A., Prats Moya, S., Maestre Pérez, S. E., & Beltrán Sanahuja, A. (2021). Volatile profile of nuts, key odorants and analytical methods for quantification. *Foods*, *10*, 1611. https://doi.org/10.3390/foods10071611.

van Huis, A., van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G., & Vantomme, P. (2013). Edible insects: Future prospects for food and feed security. Rome: Food and Agriculture Organization of the United Nations. Retrieved from https://www.cabidigitallibrary.org/doi/full/10.5555/20133217074.

Wimonmuang, K., & Lee, Y. S. (2024). Absolute contents of aroma-affecting volatiles in cooked rice determined by one-step rice cooking and volatile extraction coupled with standard-addition calibration using HS-SPME/GC - MS. *Food Chemistry*, *440*, 138271. https://doi.org/10.1016/j.foodchem.2023.138271.

Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., Sheard, P. R., & Enser, M. (2004). Effects of fatty acids on meat quality: A review. *Meat Science*, *66*, 21 - 32. https://doi.org/10.1016/S0309-1740(03)00022-6.

Yin, W. T., Ma, X. T., Li, S. J., Wang, X. D., Liu, H. M., & Shi, R. (2021). Comparison of key aroma-active compounds between roasted and cold-pressed sesame oils. *Food Research International*, *150*, 110794.

https://doi.org/10.1016/j.foodres.2021.110794.

Zhang, J., Chen, Y., Ni, M., Hou, C., Qiao, X., & Wang, T. (2024). A novel halloysite nanotubes-based hybrid monolith for in-tube solid-phase microextraction of polar cationic pesticides. *Food Chemistry*, *458*, 140205. https://doi.org/10.1016/j.foodchem.2024.140205.

Zhang, Y., Stöppelmann, F., Zhu, L., Liang, J., Rigling, M., Wang, X., Jin, Q., & Zhang, Y. (2023). A comparative study on flavor trapping techniques from the viewpoint of odorants of hot-pressed rapeseed oil. *Food Chemistry*, *426*, 136617. https://doi.org/10.1016/j.foodchem.2023.136617.

CHAPTER Ⅱ

Characterization of the key volatile compounds in meat-based broths using HS-SPME-Arrow-GC/MS

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ABSTRACT

Meat-based broths are increasingly popular with Korean consumers; however, their volatile profiles remain underexplored. In this study, headspace solid-phase microextraction-Arrow (HS-SPME-Arrow) combined with gas chromatography/mass spectrometry (GC/MS) was employed to identify and quantify the volatile compounds in several commercial meat-based broths. The analytical method was optimized using Box - Behnken design-based response surface methodology and validated through calibration curves, limits of detection and quantification, and recovery analyses to ensure sensitivity and accuracy. Eighteen volatile compounds were identified, with aldehydes being the most abundant. Several of these compounds, including aldehydes contributing fatty characteristics, were also identified in beef extract and powder. In contrast, pyrazines and methional were found exclusively in the beef flavoring ingredients. This study offers valuable insight into the development of Korean-style home meal replacement products by identifying key volatile compounds in meat-based broths and comparing their volatile profiles with those of beef flavoring ingredients.

Keywords:

Meat-based broth, Volatile compounds, Headspace solid-phase microextraction-Arrow, Method optimization, Key volatile compound

Ⅰ. INTRODUCTION

The demand for pre-prepared meals intended for home consumption, such as ready-to-eat meals and home meal replacement (HMR) products, has increased steadily in recent decades, partly due to the growing number of single-person households. In the Korean market, recent developments in the HMR sector have emphasized soup- and stew-based products, particularly those that include meat or meat-derived components (Ahn et al., 2024). Bone broth has emerged as a fundamental base of these products, as it offers both rich flavor and important nutrients, including several amino acids and minerals that support physiological functions in humans (Zhang et al., 2017). For example, certain types of bone broth and soup have been reported to exhibit antioxidant properties, promote bone growth, and prevent osteoporosis (Meng et al., 2022).

Given the importance of flavor in shaping consumer preference and purchasing behavior, understanding the volatile profiles of HMR products is crucial. However, although over 1,000 volatile compounds have been identified in different meats and meat-derived products (Ahamed et al., 2023), investigations into the volatile profiles of meat-based broths remain scarce. Most studies on meat-based broths have focused on characterizing non-volatile compounds, such as free amino acids and 5′-nucleotides (Yue et al., 2024; Wang et al., 2020). Meanwhile, little research has been conducted on the quantification of volatile compounds in meat-based broths and the identification of key contributors to their

volatile profiles. Moreover, previous studies on bone broths have primarily focused on laboratory-prepared samples (Meng et al., 2022; Zhang et al., 2017), with limited attention given to the analysis of volatile compounds in commercial products.

The accurate identification and quantification of volatile compounds in bone broths is essential for understanding and enhancing the volatile profiles of HMR products. Headspace solid-phase microextraction-Arrow (HS-SPME-Arrow) combined with gas chromatography/mass spectrometry (GC/MS) is a reliable and sensitive technique for characterizing volatile compounds in foods (Lee et al., 2021; Lee et al., 2019). Headspace extraction is a solvent-free method with high sensitivity and selectivity (Nam et al., 2024), while solid-phase microextraction (SPME) offers increased speed, automation, and reproducibility (Song et al., 2019). SPME-Arrow is a more recent advancement, integrating the benefits of SPME and stir bar sorptive extraction. Owing to its larger sorbent volume and improved fiber stability, SPME-Arrow significantly enhances the limits of detection (LODs) and GC/MS peak intensities, making it an effective tool for analyzing volatile compounds with high sensitivity and reproducibility (Nam et al., 2024; Lee et al., 2021; Lee et al., 2019).

In this study, HS-SPME-Arrow-GC/MS was used for the extraction, identification, and quantification of volatile compounds in Korean-style meat-based broths and beef flavoring ingredients, with the aim of systematically characterizing the volatile compounds in meat-based

broths, identifying key compounds, and understanding the volatile profiles of commercially available HMR products. The development and validation of analytical methods is crucial for advancing scientific understanding. Therefore, we also performed comprehensive method validation using calibration curves, LODs, limits of quantification (LOQs), and recovery assessment. This study provides the first characterization of key volatile compounds in Korean-style meat-based broth, showcasing its distinct composition. The results offer insight into how the volatile compounds in meat-based broths and beef flavoring ingredients contribute to the overall volatile profiles of meat-based products.

Ⅱ. MATERIALS AND METHODS

2.1. Sample preparation

Five commercial meat-based broths were obtained from a local supermarket in Seoul, Korea. The stated ingredients of each broth are listed in **Table 2.S1**. Prior to analysis, the broth samples were heated while sealed in their original packaging by submerging them in boiling water for 5 min. Beef extract and beef powder were provided by Seoul Flavor & Fragrance Co. (Seoul, Korea) and analyzed without modification.

Table 2.S1. Ingredient composition of commercial meat-based broth samples

Sample	Ingredients
MB1	Purified water, beef bone extract, garlic extract, beef broth base, black pepper
MB2	Purified water, beef bone extract
MB3	Purified water, fatty brisket slices, brisket extract, beef broth powder, radish extract powder, flavor enhancer, amino base, mixed vegetable base, complex seasoning mixture
MB4	Purified water, beef bone concentrate, amino base, beef extract powder, green onion extract, flavor enhancer, liquid seasoning sauce, refined salt, mixed vegetable base, garlic extract concentrate
MB5	Purified water, beef bone extract, flavor enhancer, refined salt

2.2. Chemicals and reagents

All chemical standards for the detected volatile compounds and all internal standards (phenyl acetate, 1-hexyl alcohol-d_{13}, toluene-d_8, 3-octanone, and 2,4,6-trimethylpyridine) were of analytical grade and obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade water and methanol were purchased from Fisher Scientific (PA, USA).

2.3. Optimization of volatile compound extraction

To determine the optimal sample processing conditions, extraction experiments were conducted with different sample pretreatment (cooking) methods, volumes of saturated NaCl solution, and SPME-Arrow fiber types. Specifically, pretreatment was performed by techniques such as submersion, microwave heating, and boiling; samples were prepared with 0, 0.5, and 1 mL of saturated NaCl; and three types SPME-Arrow fiber, namely, DVB/PDMS, CAR/PDMS, and DVB/CAR/PDMS (where DVB = divinylbenzene, CAR = carboxen, and PDMS = polydimethylsiloxane), were compared. All SPME-Arrow fibers had a phase length of 1.1 mm × 20 mm and phase thickness of 120 μm. The experimental conditions were optimized based on the GC/MS peak areas.

Next, to determine the optimal SPME-Arrow extraction conditions, the equilibration time (10, 20, and 30 min), extraction time (20, 30, and 40 min), and extraction temperature (30, 40, and 50 °C) were optimized using a Box - Behnken design (BBD) combined with response surface methodology (RSM).

2.4. Volatile compound analysis

Volatile compounds were extracted using a DVB/CAR/PDMS SPME-Arrow fiber (PAL System, Zwingen, Switzerland; phase length: 1.1 mm × 20 mm, phase thickness: 120 μm) in combination with an autosampler (PAL RSI 85, PAL System). For the meat-based broths, 0.5 mL of broth was added to a 20 mL headspace vial, along with 1 mL of a saturated NaCl solution and 10 μL of an internal standard mixture. Similarly, for the beef flavoring ingredients, 0.5 g of beef extract or beef powder was added to the headspace vial, along with 10 μL of the same internal standard mixture. The final amounts of internal standards in each sample were 0.1 μg each of 1-hexyl alcohol-d_{13}, toluene-d_8, and 2,4,6-trimethylpyridine, and 0.02 μg each of phenyl acetate and 3-octanone. The samples were equilibrated at 500 rpm for 20 min, followed by extraction at 1000 rpm for 30 min. Equilibration and extraction were both conducted at 40 °C. The injector temperature was set to 220 °C, and desorption was carried out in splitless mode for 5 min.

GC/MS analysis was conducted using an 8890 gas chromatograph coupled with a 7000E triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The volatile compounds were separated using an HP-5MS column (60 m × 0.25 mm I.D. × 0.25 μm film thickness, Agilent Technologies). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature was held at 45 °C for 5 min; increased from 45 to 100 °C at a rate of 2

°C/min; increased from 100 to 153 °C at 3 °C/min; and finally ramped to 230 °C at 20 °C/min and held for 3 min. The MS transfer line and ion source temperatures were 280 and 230 °C, respectively. Electron impact ionization was conducted with an ionization energy of 70 eV. Data acquisition was performed in two segments: full-scan mode (30 - 550 *m/z*) and selected-ion monitoring (SIM) mode.

2.5. Identification and quantification of volatile compounds

The volatile compounds were identified by comparing their mass spectral ratios with those in the National Institute of Standards and Technology (NIST) 2020 library, using a reverse match factor (R. Match) of ≥ 800. The retention index (RI) was calculated based on an alkane standard mixture (C_6 - C_{40}).

$$RI_X = 100 \times [n + (N-n)\frac{\log(t'_{r(X)}) - \log(t'_{r(n)})}{\log(t'_{r(N)}) - \log(t'_{r(n)})}],$$

where RI_X is the retention index of compound X; n is the number of carbon atoms in the smaller n-alkane eluted before compound X; N is the number of carbon atoms in the larger n-alkane eluted after compound X; and t′ is the retention time of the compound.

The volatile compounds were quantified using regression equations. These equations were established based on the ratio of peak areas (analyte/internal standard). The peak area of each compound was determined by extracting the quantifier ions. The internal standards used for the quantification of each volatile compound group were as follows: phenyl acetate for acids and esters; 1-hexyl alcohol-d_{13} for alcohols and furans; toluene-d_8 for aldehydes, hydrocarbons and sulfur compounds; 3-octanone for ketones; and 2,4,6-trimethylpyridine for pyrazines.

2.6. Method validation

The HS-SPME-Arrow-GC/MS method was validated using a modified version of the technique reported by Ye et al. (2022). First, an odorless matrix was prepared by mixing 100 mL of meat-based broth with 100 mL of methanol, followed by rotary evaporation for 2 h to remove volatile compounds. Next, stock solutions of volatile standards were prepared by accurately weighing the standard compounds and dissolving them in methanol. Internal standards were added at consistent concentrations across all standard solutions. Finally, 10 μL of each standard solution was spiked into 0.5 mL of odorless meat-based broth.

The validation criteria included the linearity, LOD, LOQ, and recovery. Calibration curves were constructed for each volatile compound by plotting the mass of the standard compound on the x-axis and the standard compound/internal standard peak area ratio on the y-axis. Based on these calibration curves, the concentration of each volatile compound in the sample was determined. The LOD and LOQ were calculated as $3\sigma/S$ and $10\sigma/S$, respectively, where σ is the standard deviation of the y-intercept and S is the slope obtained from regression analysis (Nam et al., 2025; Kim et al., 2024). The recovery was assessed at three different mass levels for each compound, as detailed in Table S2. The recovery (%) was calculated as follows:

Recovery (%) = Measured concentration/Spiked concentration × 100.

2.7. Statistical analysis

All experiments were conducted in triplicate (n=3), and the data are presented as the mean ± standard deviation. All statistical analyses were conducted using SPSS Statistics (version 29, IBM Inc., Chicago, IL, USA), with statistical significance set at $p < 0.05$ for all tests. RSM analysis was performed using Design-Expert statistical software (version 7, Stat-Ease, Minneapolis, MN, USA). Multivariate statistical analyses were conducted using MetaboAnalyst (version 6, https://www.metaboanalyst.ca/).

Ⅲ. RESULTS AND DISCUSSION

3.1. Optimization of extraction parameters for volatile compound analysis

The volatile compounds in meat-based broths are classified into several groups, with aldehydes being the most dominant (Guan et al., 2025; Zhang et al., 2017). To tailor the extraction process to the matrix characteristics of meat-based broths, the extraction conditions were optimized based on the GC/MS peak areas (AU × 10^5) of aldehydes and total volatile compounds (**Figure. 2.1**).

To determine the most effective pretreatment method for meat-based broths, techniques such as submersion, microwave heating, and boiling were used. Volatile compounds were subsequently analyzed through HS-SPME-Arrow-GC/MS, and the total peak areas (AU × 10^5) were compared (**Figure. 2.1a**). Among the identified volatile compounds, aldehydes were the predominant group. Their total peak area was highest in samples prepared via submersion cooking; therefore, this technique was established as the optimal pretreatment method. Next, the effect of the volume of saturated NaCl solution was evaluated. Samples were prepared with 0, 0.5, and 1 mL NaCl. The samples with 1 mL NaCl demonstrated the highest total peak area for both aldehydes and overall volatile compounds, thereby establishing this as the optimal volume of saturated NaCl solution (**Figure. 2.1b**).

Three types of SPME-Arrow fibers (DVB/CAR/PDMS, DVB/PDMS,

and CAR/PDMS) were evaluated. All fibers had a phase length of 1.1 mm × 20 mm and phase thickness of 120 μm. As shown in **Figure. 2.1c**, the DVB/CAR/PDMS SPME-Arrow fiber provided significantly higher ($p < 0.05$) GC/MS peak areas (aldehydes: 3432.45 AU × 10^5, total: 5959.45 AU × 10^5) than the DVB/PDMS fiber (aldehydes: 1582.24 AU × 10^5, total: 2196.87 AU × 10^5) and CAR/PDMS fiber (aldehydes: 1643.81 AU × 10^5, total: 2731.83 AU × 10^5). The selection of an appropriate coating material is critical for optimizing SPME-Arrow extraction. DVB/CAR/PDMS fibers, consisting of three distinct sorbent phases, have a high affinity for both polar and non-polar compounds, resulting in superior extraction performance (Ahamed et al., 2023). DVB/CAR/PDMS fibers have been used for the volatile analysis of a range of foods, as documented in prior research (Li et al., 2024b; Liu et al., 2024a; Liu et al., 2024b; Zhao et al., 2024; Meng et al., 2022; Zhang et al., 2017). Based on these findings, the DVB/CAR/PDMS SPME-Arrow fiber was selected for use in this study.

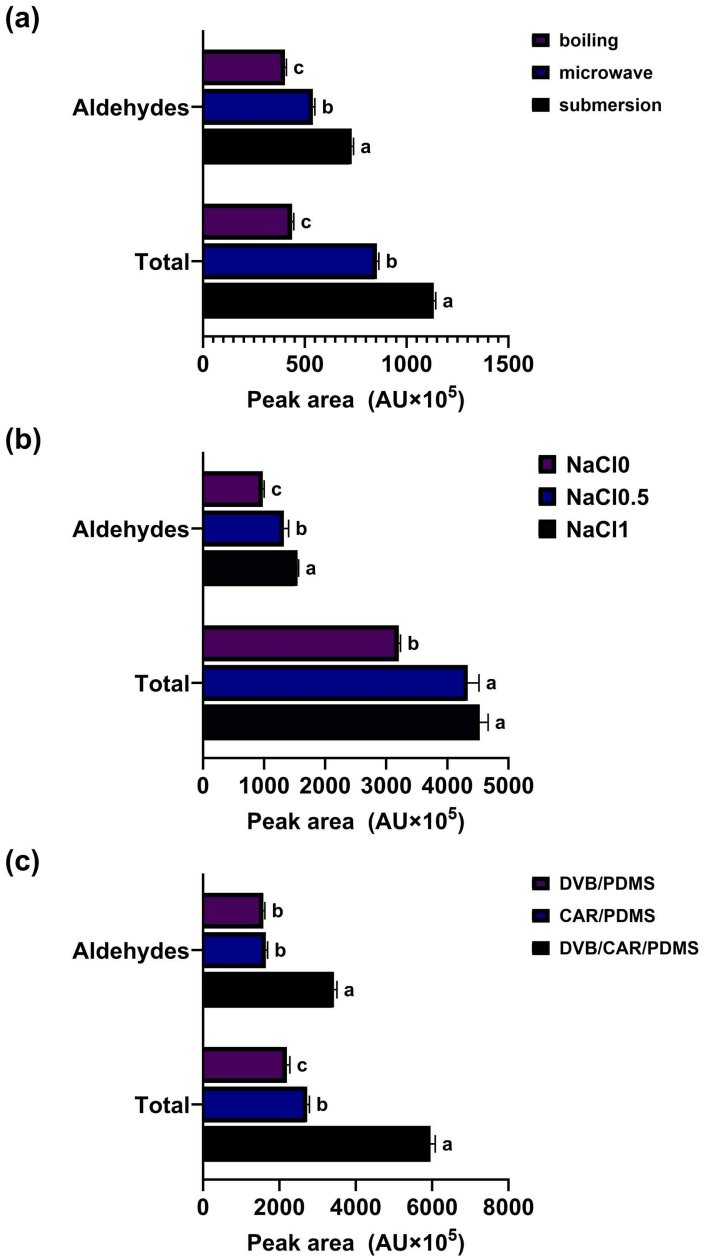

Figure 2.1. Peak areas (AU × 10^5) of HS-SPME-Arrow-GC/MS spectra when using different extraction conditions. (a) Cooking method, (b) saturated NaCl solution volume, and (c) SPME-Arrow fiber type.

3.2. Optimization of SPME-Arrow extraction conditions

The extraction conditions were optimized using RSM in combination with BBD. The equilibration time (X1), extraction time (X2), and extraction temperature (X3) were selected as the independent variables, and the total area (AU × 10^5) of aldehydes was set as the response variable (Y). The relationship among X1, X2, X3, and Y is described as follows:

$$Y = 434.71 + 10.28X_1 + 25.75X_2 + 18.99X_3 - 15.42X_1X_2 + 14.82X_1X_3 - 6.03X_2X_3 - 31.11X_1^2 - 61.26X_2^2 - 58.23X_3^2$$

The model response was significant (F = 55.47, p < 0.0001), with a high coefficient of determination (R^2 = 0.9862). Three-dimensional response surface plots are shown in **Figure. 2.2**. Y was maximized at X1 = 20 and X2 = 30 (**Figure. 2.2a**); at X1 = 20 and X3 = 40 (**Figure. 2.2b**); and at X2 = 30 and X3 = 40 (**Figure. 2.2c**). Based on these results, the optimal extraction conditions were an equilibration time of 20 min, extraction time of 30 min, and extraction temperature of 40 °C.

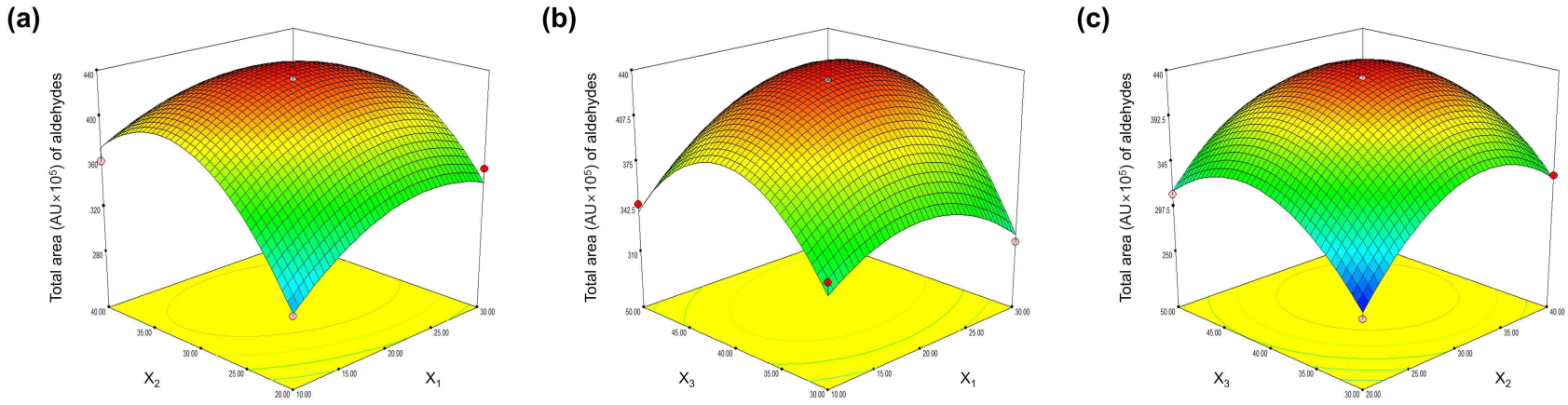

Figure 2.2. Response surface plots for total peak area (AU × 10^5) of aldehydes in HS-SPME-Arrow-GC/MS spectra. The independent variables are equilibration time (X1), extraction time (X2), and extraction temperature (X3). (a) X1 and X2, (b) X1 and X3, and (c) X2 and X3.

3.3. Method validation of volatile analysis

The calibration and validation results of 27 volatile compounds are presented in **Table 2.1**. The R^2 values for all volatiles were greater than 0.995, indicating excellent linearity. The LODs and LOQs ranged from 0.03 to 23.59 ng and from 0.11 to 78.62 ng, respectively (LODs: 0.11 – 0.19 ng for alcohols, 0.03 – 3.31 ng for aldehydes, 3.59 ng for ethyl acetate (an ester), 0.27 – 5.61 ng for furans, 0.08 – 14.44 ng for hydrocarbons, 0.41 – 21.38 ng for ketones, 2.86 – 14.33 ng for pyrazines, and 23.59 ng for methional (a sulfur compound); LOQs: 0.36 – 0.65 ng for alcohols, 0.11 – 11.05 ng for aldehydes, 11.97 ng for ethyl acetate, 0.92 – 18.71 ng for furans, 0.28 – 48.15 ng for hydrocarbons, 1.36 – 71.27 ng for ketones, 9.52 – 47.76 ng for pyrazines, and 78.62 ng for methional). These results demonstrate that the HS-SPME-Arrow-GC/MS method is suitable and effective for the analysis of volatile compounds. Moreover, the recoveries ranged from 90.69% to 109.54% (**Table S2**), confirming the reliability of the method, and the RSDs ranged from 0.13% to 2.29%, demonstrating that the method was highly reproducible. Therefore, the optimized HS-SPME-Arrow-GC/MS method was suitable for analyzing volatile compounds in meat-based broths and beef flavoring ingredients.

Table 2.1. Validation parameters (calibration curves parameters, limits of detection and quantification) of the optimized HS-SPME-Arrow-GC/MS method

Compound	Calibration curve	R^2	Linear range (ng)	LOD (ng)	LOQ (ng)
Alcohols					
1-heptanol	$y = 0.0163x - 0.0089$	0.9997	0.5 - 40	0.11	0.36
1-octanol	$y = 0.0063x - 0.0043$	0.9997	1 - 30	0.19	0.65
Aldehydes					
(*E*)-2-decenal	$y = 0.0007x - 0.0004$	0.9997	1 - 20	0.28	0.93
(*E*)-2-nonenal	$y = 0.0008x + 0.0001$	0.9997	0.3 - 5	0.07	0.23
2-methylbutanal	$y = 0.00003x + 0.0001$	0.9997	2 - 75	0.47	1.58
3-methylbutanal	$y = 0.0002x - 0.0003$	0.9994	10 - 300	2.88	9.60
benzaldehyde	$y = 0.0105x - 0.0234$	0.9982	2 - 40	0.60	1.99
decanal	$y = 0.0005x - 0.0002$	0.9993	0.5 - 20	0.10	0.34
heptanal	$y = 0.0029x - 0.0011$	0.9999	0.5 - 50	0.03	0.11
hexanal	$y = 0.0002x - 0.0074$	0.9991	15 - 2000	3.31	11.05
nonanal	$y = 0.0042x - 0.0022$	0.9997	0.5 - 40	0.13	0.42
octanal	$y = 0.0004x - 0.0031$	0.9993	5 - 300	1.25	4.16
phenylacetaldehyde	$y = 0.0001x - 0.0009$	0.9999	10 - 300	1.23	4.11
Esters					

ethyl acetate	$y = 0.005x - 0.0009$	0.9999	15 - 250	3.59	11.97
Furans					
2-acetylfuran	$y = 0.0079x - 0.1903$	0.9990	20 - 500	5.61	18.71
2-furfural	$y = 0.0013x - 0.0141$	0.9996	20 - 500	4.16	13.88
2-pentylfuran	$y = 0.0567x - 0.0123$	0.9998	1 - 15	0.27	0.92
Hydrocarbons					
d-limonene	$y = 0.0027x - 0.1294$	0.9997	50 - 1000	14.44	48.15
o-xylene	$y = 0.0188x - 0.0084$	0.9989	0.5 - 20	0.08	0.28
p-xylene	$y = 0.0173x - 0.0041$	0.9994	0.5 - 40	0.15	0.50
Ketones					
2-butanone	$y = 0.0005x - 0.0107$	0.9999	100 - 2000	21.38	71.27
acetophenone	$y = 0.0095x - 0.0058$	0.9998	1.5 - 40	0.41	1.36
Pyrazines					
2,3-dimethylpyrazine	$y = 0.0077x - 0.1462$	0.9991	25 - 200	6.18	20.59
2,5-dimethylpyrazine	$y = 0.0064x - 0.4127$	0.9958	50 - 1000	14.33	47.76
methylpyrazine	$y = 0.0042x - 0.0082$	0.9998	10 - 500	2.86	9.52
trimethylpyrazine	$y = 0.0090x + 0.0085$	0.9997	20 - 1000	5.73	19.08
Sulfur compounds					
methional	$y = 0.00003x - 0.0017$	0.9994	100 - 500	23.59	78.62

R^2, coefficient of determination; LOD, limit of detection; LOQ, limit of quantification.

Table 2.S2. Recovery (%) of volatile compounds using the optimized HS-SPME-Arrow-GC/MS method

Compound	Spiking 1			Spiking 2			Spiking 3		
	Mass (ng)	Recovery (%)	RSD (%)	Mass (ng)	Recovery (%)	RSD (%)	Mass (ng)	Recovery (%)	RSD (%)
Alcohols									
1-heptanol	1.5	98.90 ± 0.31	0.31	5	99.90 ± 0.64	0.64	35	103.62 ± 0.52	0.5
1-octanol	2.5	103.84 ± 0.69	0.67	10	100.97 ± 0.96	0.95	25	97.94 ± 0.67	0.69
Aldehydes									
(*E*)-2-decenal	2.5	109.29 ± 0.33	0.3	5	102.87 ± 0.69	0.67	7.5	94.70 ± 0.32	0.34
(*E*)-2-nonenal	1	92.29 ± 0.50	0.54	2	95.52 ± 0.70	0.73	4	99.26 ± 0.69	0.7
2-methylbutanal	15	101.42 ± 0.57	0.56	30	97.50 ± 0.32	0.33	45	95.42 ± 0.54	0.56
3-methylbutanal	15	104.39 ± 0.42	0.4	50	100.44 ± 0.70	0.7	100	93.70 ± 0.31	0.33
benzaldehyde	5	108.96 ± 0.68	0.63	10	101.99 ± 0.34	0.33	20	93.82 ± 0.26	0.28
decanal	2.5	93.86 ± 0.35	0.37	5	101.69 ± 0.67	0.65	10	109.54 ± 0.58	0.53
heptanal	5	101.01 ± 0.16	0.16	10	97.97 ± 0.26	1.29	45	92.36 ± 0.34	0.36
hexanal	100	108.96 ± 0.65	0.6	500	103.42 ± 0.99	0.95	1500	98.56 ± 0.32	1.34
nonanal	2.5	106.77 ± 0.62	0.58	5	102.67 ± 0.61	0.59	35	99.64 ± 0.64	0.64
octanal	25	107.56 ± 0.72	0.67	50	104.92 ± 0.61	0.58	250	101.63 ± 0.66	0.65
phenylacetaldehyde	50	109.50 ± 0.54	0.49	100	102.44 ± 0.61	0.59	250	101.11 ± 0.52	0.51
Esters									

ethyl acetate	100	98.05 ± 0.62	0.64	150	102.54 ± 0.25	0.24	200	105.95 ± 0.15	0.14
Furans									
2-acetylfuran	25	109.54 ± 0.15	0.14	200	98.14 ± 0.26	0.26	400	93.16 ± 0.97	1.04
2-furfural	30	96.45 ± 0.38	0.39	150	102.05 ± 0.13	0.13	300	105.54 ± 0.08	1.03
2-pentylfuran	2.5	102.55 ± 0.76	0.74	5	96.72 ± 0.29	0.3	10	90.69 ± 0.74	0.81
Hydrocarbons									
d-limonene	250	101.64 ± 0.42	0.42	500	98.61 ± 0.18	0.19	750	94.54 ± 0.80	0.85
o-xylene	2.5	106.22 ± 0.32	0.3	5	100.20 ± 2.29	2.29	10	96.17 ± 0.16	0.16
p-xylene	2.5	103.73 ± 0.47	0.45	10	99.59 ± 0.02	1.02	20	99.15 ± 0.37	0.37
Ketones									
2-butanone	500	96.31 ± 0.26	0.27	1000	100.01 ± 0.90	0.9	1500	106.25 ± 0.66	0.62
acetophenone	2.5	94.48 ± 0.48	0.51	10	96.24 ± 0.51	0.53	25	99.06 ± 0.26	0.26
Pyrazines									
2,3-dimethylpyrazine	50	106.48 ± 0.80	0.75	75	100.82 ± 0.58	0.58	100	96.59 ± 0.41	0.42
2,5-dimethylpyrazine	250	105.02 ± 0.63	0.6	500	99.85 ± 0.49	0.49	750	93.01 ± 0.44	0.47
methylpyrazine	75	105.43 ± 0.44	0.41	150	99.36 ± 0.40	1.41	250	95.04 ± 0.75	0.79
trimethylpyrazine	40	101.33 ± 0.31	0.31	200	99.16 ± 0.78	0.79	400	99.00 ± 0.80	0.81
Sulfur compounds									
methional	150	93.98 ± 0.91	0.97	250	101.85 ± 0.92	0.91	300	107.00 ± 0.97	0.91

3.4. Characterization of key volatile compounds in meat-based broths

Table 2.2 presents the volatile compounds analyzed using HS-SPME-Arrow-GC/MS. A total of 18 volatile compounds were identified in five meat-based broths (MB1 - MB5), including two alcohols, eleven aldehydes, one ester, one furan, two hydrocarbons, and one ketone. A total of 13, 10, 18, 16, and 8 volatile compounds were identified in MB1, MB2, MB3, MB4, and MB5, respectively.

Among the identified compounds, aldehydes were the most abundant. Benzaldehyde, heptanal, nonanal, and octanal were detected in all five meat-based broths, which is consistent with previous studies on bone soup by Meng et al. (2022) and Zhang et al. (2017). Heptanal, nonanal, and octanal were identified as key volatiles, both in this study and the previous reports, with variable importance in projection (VIP) scores exceeding 1 (**Figure 2.S1**). Notably, these aldehydes have been reported to contribute significantly to the characteristic cooked beef note (Li et al., 2024b). Aldehydes are commonly formed through lipid oxidation and degradation, as well as the Strecker degradation of amino acids (Zou et al., 2018). Owing to their typically low odor threshold values, aldehydes substantially influence the volatile profile of meat-based broths (Wang et al., 2016). Straight-chain aldehydes, including decanal, heptanal, hexanal, nonanal, and octanal, originate from the degradation of unsaturated fatty acids, such as oleic and linoleic acids. Meanwhile, branched aldehydes, such as 2-methylbutanal, 3-methylbutanal, benzaldehyde, and

phenylacetaldehyde, contribute grilled meat notes (Ahamed et al., 2023).

VIP scores derived from partial least squares discriminant analysis (PLS-DA) were used to evaluate the contribution of each compound to the volatile profiles of the meat-based broth samples. Eleven compounds, including hexanal, 1-heptanol, 2-methylbutanal, 3-methylbutanal, 1-octanol, 2-butanone, phenylacetaldehyde, octanal, heptanal, nonanal, and decanal, had VIP scores exceeding 1, suggesting that these compounds contribute to the distinct volatile profiles of the broth samples (**Figure 2.S1**). Consequently, these compounds are suggested as candidate markers for sample differentiation.

Principal component analysis (PCA) was performed based on the concentrations of the 18 volatile compounds in the meat-based broths. The first and second principal components (PC1 and PC2, respectively) accounted for 66.17% and 20.32% of the total variance, respectively. Notably, the compounds with VIP > 1 contributed to the separation of samples in the PCA biplot (**Figure 2.3**). Among them, hexanal, 1-heptanol, 1-octanol, octanal, heptanal, nonanal, and decanal were associated with the localization of MB1 samples on the positive side of PC1. MB1 had the highest detected concentration of hexanal at 3523.74 ng/mL, compared to 914.45 and 233.15 ng/mL for MB2 and MB3, respectively, and none for MB4 and MB5 (**Table 2.2**). Previous reports have identified hexanal as a predominant volatile compound in stewed beef (Zou et al., 2018), highlighting its significance in heat-treated meat products. It is primarily generated through the oxidation of

polyunsaturated fatty acids, such as linoleic acid, under thermal conditions (Nam et al., 2025). MB1 also had higher concentrations of octanal (519.36 ng/mL), heptanal (91.88 ng/mL), nonanal (69.09 ng/mL), and decanal (24.76 ng/mL), which are associated with fatty notes, than other samples ($p < 0.05$). Therefore, MB1 might exhibit stronger fatty characteristics. Meanwhile, 1-heptanol (green and wood notes) and 1-octanol (citrus, fat, fruit, and green notes) have been identified in previous studies on pan-roasted beef and beef soup (Ahamed et al., 2023; Wang et al., 2022).

For MB3 and MB4, which were positioned along the negative direction of PC1 in the PCA biplot, were mainly influenced by 2-methylbutanal, 3-methylbutanal, 2-butanone, and phenylacetaldehyde. These volatile compounds were exclusively detected in MB3 and MB4 (**Table 2.2**). According to Ahamed et al. (2023), 2-methylbutanal (almond, chocolate, cocoa, fermented, and hazelnut notes) and 3-methylbutanal (acrid, almond, chocolate, cocoa, and corn-flakes notes) are commonly found in pan-roasted meats such as beef, pork, and chicken. Phenylacetaldehyde has been reported to impart a rosy note to broth (Wang et al., 2016). Furthermore, 2-butanone (butterscotch, cheese, and fragrant notes) has previously been documented in chicken broth (Yuan et al., 2024; Feng et al., 2018). This compound, known for its fruity note, is also permitted as a direct food additive (Kim et al., 2021).

In conclusion, aldehydes were the predominant type of volatile compound in all five samples. However, differences in their

concentrations contributed to distinct volatile profiles among the samples. The key volatile compounds identified through VIP analysis corresponded well with the clustering patterns observed in PCA, highlighting their importance in distinguishing volatile profiles.

The distinct volatile profiles of the five meat-based broth samples may be related to their ingredients (**Table 2.S1**). The volatile profile of MB1 included significantly higher levels of medium- and long-chain aliphatic aldehydes (hexanal, octanal, heptanal, and nonanal) and corresponding alcohols (1-heptanol and 1-octanol) than those of the other samples. These aldehydes are known products of lipid oxidation, particularly from unsaturated fatty acids, which are abundant in beef (Li et al., 2024a). This suggests that these compounds likely originated from the beef bone extract and beef broth base in MB1. The volatile profiles of MB3 and MB4 contained Strecker aldehydes such as phenylacetaldehyde (117.62 and 116.00 ng/mL, respectively), 2-methylbutanal (93.22 and 67.36 ng/mL, respectively), and 3-methylbutanal (29.23 and 22.59 ng/mL, respectively). These compounds are formed via the Strecker degradation of phenylalanine, isoleucine, and leucine, respectively (Yang et al., 2021). The presence of amino base and vegetable extracts in these samples likely facilitated these reactions during cooking, contributing to their distinct volatile profiles. MB2 and MB5 exhibited a notably simpler volatile profile, which was likely attributed to the lack of ingredients such as amino bases and vegetable extracts. In particular, MB5 had a significantly higher concentration of ethyl acetate (460.70 ng/mL) than the other samples ($p < 0.05$). This ester has been reported to impart

fruity notes to wine and pork broth (Kim et al., 2021; Wang et al., 2016). These results collectively demonstrate that the ingredient composition, especially the presence of amino acid-rich bases and fat-containing components, significantly influences the volatile profile of meat-based broth products. Variations in aldehyde content are closely related to lipid oxidation and Strecker degradation, which vary depending on the ingredient composition.

Table 2.2. Volatile compound concentrations (ng/mL) in five meat-based broths (MB1 - MB5)

Compound	Concentration (ng/mL)					Aroma description†
	MB1	MB2	MB3	MB4	MB5	
Alcohols						
1-heptanol	69.78 ± 0.02[a]	8.92 ± 0.08[b]	2.62 ± 0.03[c]	2.89 ± 0.05[c]	nd	green, wood
1-octanol	53.73 ± 3.90[a]	5.99 ± 0.18[b]	4.99 ± 0.10[b]	6.37 ± 0.05[b]	nd	citrus, fat, fruit, green
Aldehydes						
(*E*)-2-decenal	13.25 ± 0.39[a]	nd	3.91 ± 0.17[c]	11.01 ± 0.17[b]	nd	fat, fish, hay, tallow
(*E*)-2-nonenal	8.68 ± 0.23[a]	nd	1.61 ± 0.13[c]	2.98 ± 0.39[b]	nd	beany, cucumber, cut grass, earth, fat
2-methylbutanal	nd	nd	93.22 ± 2.35[a]	67.36 ± 5.84[b]	nd	almond, chocolate, cocoa, fermented, hazelnut
3-methylbutanal	nd	nd	29.23 ± 1.12[a]	22.59 ± 2.29[b]	nd	acrid, almond, chocolate, cocoa, corn flakes
benzaldehyde	14.81 ± 4.02[c]	15.32 ± 0.41[b]	33.84 ± 0.06[a]	10.30 ± 0.03[e]	13.37 ± 0.14[d]	almond, berry, bitter, bitter almond, burnt sugar
decanal	24.76 ± 1.25[a]	nd	4.47 ± 0.11[b]	4.54 ± 0.10[b]	nd	fat, floral, fried, orange peel, penetrating

heptanal	91.88 ± 1.02[a]	17.42 ± 0.16[b]	7.55 ± 0.14[c]	4.00 ± 0.14[d]	7.56 ± 0.32[c]	citrus, dry fish, fat, green, nut
hexanal	3523.74 ± 113.56[a]	914.45 ± 44.33[b]	233.15 ± 1.91[c]	nd	nd	apple, cut grass, fresh, fruit, grass
nonanal	69.09 ± 1.21[a]	3.49 ± 0.22[c]	13.47 ± 0.26[b]	13.06 ± 0.11[b]	2.87 ± 0.06[c]	citrus, cucumber, fat, floral, green
octanal	519.36 ± 5.02[a]	99.25 ± 5.51[b]	57.85 ± 0.19[cd]	75.27 ± 1.31[bc]	41.31 ± 1.17[d]	citrus, fat, fruit, green, honey
phenylacetaldehyde	nd	nd	117.62 ± 5.07[a]	116.00 ± 4.60[a]	nd	berry, floral, flower, geranium, honey
Esters						
ethyl acetate	nd	nd	309.70 ± 3.87[b]	252.32 ± 3.15[c]	460.70 ± 24.08[a]	balsamic, fruit, grape, pineapple
Furans						
2-pentylfuran	23.30 ± 6.02[a]	11.30 ± 0.62[b]	6.76 ± 0.11[c]	nd	2.04 ± 0.06[d]	butter, floral, fruit, green, green bean
Hydrocarbons						
o-xylene	1.34 ± 0.02[c]	1.61 ± 0.03[c]	18.85 ± 0.18[b]	22.62 ± 0.97[a]	1.59 ± 0.02[c]	geranium
p-xylene	1.97 ± 0.02[c]	3.26 ± 0.04[c]	36.31 ± 0.98[b]	42.90 ± 1.80[a]	2.00 ± 0.04[c]	cold meat fat, sweet
Ketones						
2-butanone	nd	nd	2291.19 ± 49.12[a]	2033.68 ± 18.82[b]	nd	butterscotch, cheese, fragrant

Abbreviations: nd, not detected. [a-e]Values in the same row with different superscript letters are significantly different (Duncan's multiple range test, $p < 0.05$). [†] Aroma descriptions were sourced from the online database at https://www.vcf-online.nl/VcfHome.cfm.

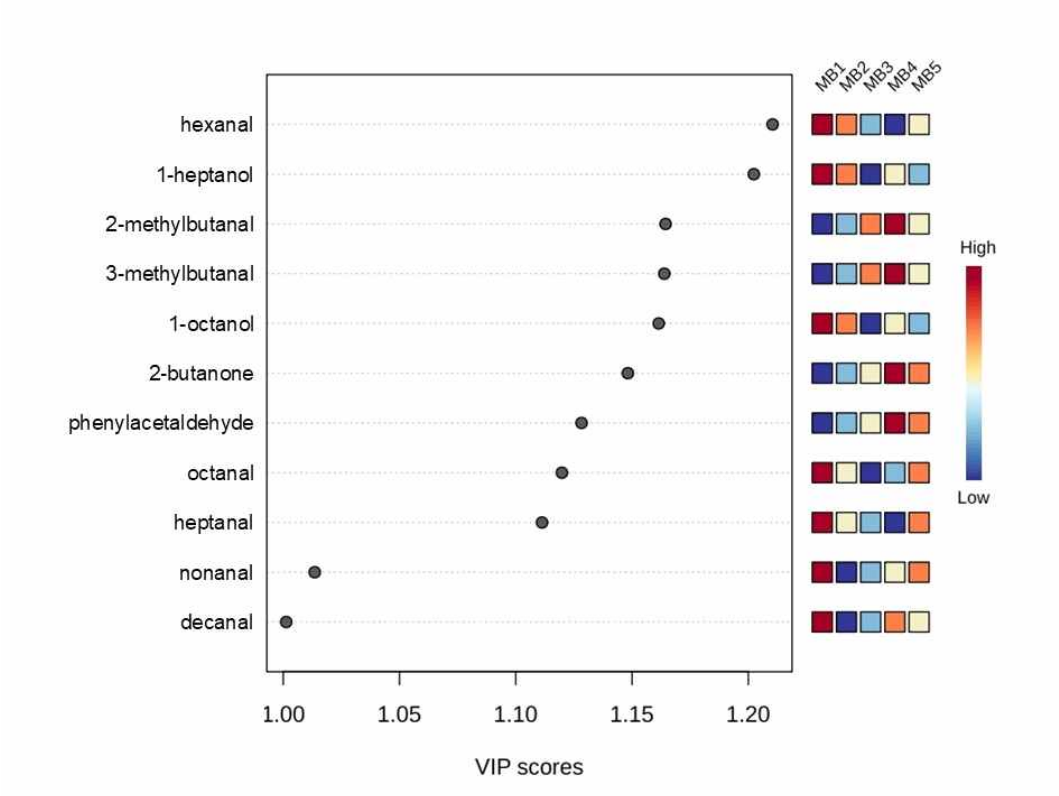

Figure 2.S1. Key volatile compounds in meat-based broths.

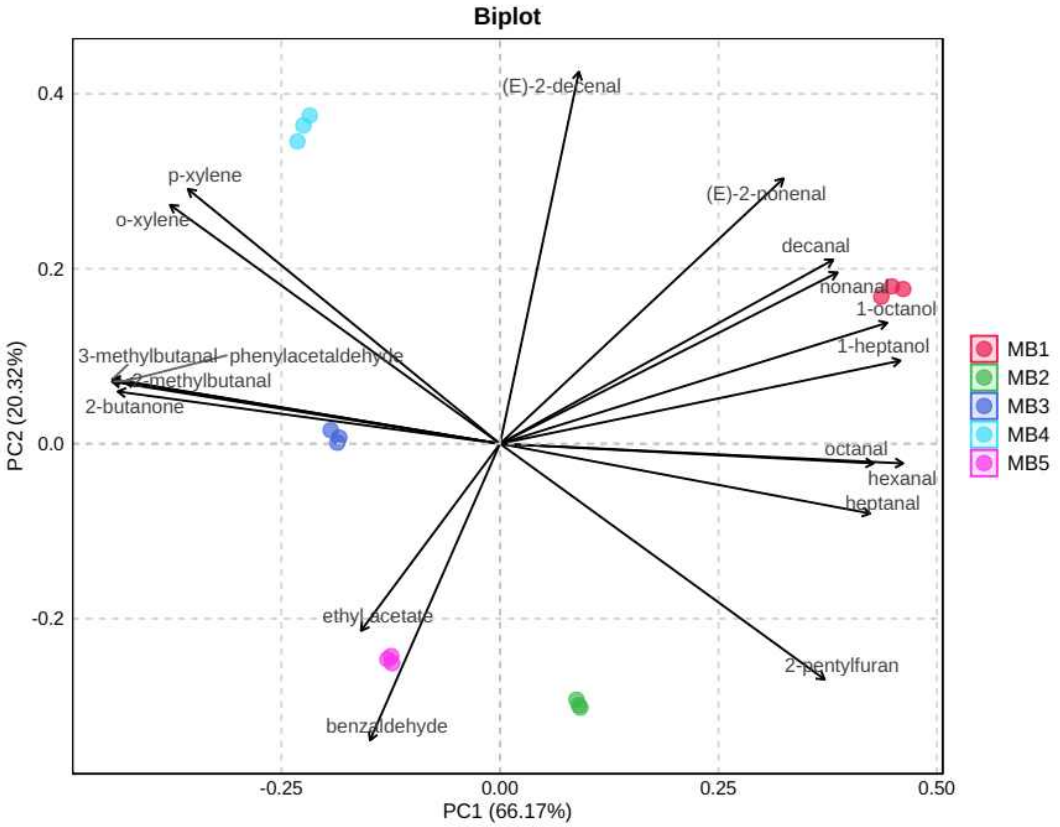

Figure 2.3. PCA Biplot showing relationships between samples and volatile compounds.

3.5. Volatile compounds of beef flavoring ingredients

The identified volatile compounds were compared with those found in two common beef flavoring ingredients, namely, beef extract and beef powder. Although these seasoning ingredients were not present in the tested broth samples, analyzing their volatile profiles offers valuable insight into how similarities and differences in the volatile compounds affect the overall profiles of meat-based products. Furthermore, it is important for exploring how the volatile profiles of meat-based broths could be improved.

Table 2.3 presents the volatile compounds in beef extract and powder, as analyzed using the same HS-SPME-Arrow-GC/MS technique. Seventeen volatile compounds were identified in total, with 12 in beef extract and 16 in beef powder (**Table 2.3**). Several of the aldehydes and hydrocarbons listed in **Table 2.3**, including 3-methylbutanal, benzaldehyde, heptanal, hexanal, nonanal, phenylacetaldehyde, o-xylene, and p-xylene, were also identified in the meat-based broths (**Table 2.2**). Among these, the aldehydes 3-methylbutanal, heptanal, hexanal, nonanal, and phenylacetaldehyde were detected as key volatile compounds of the meat-based broths (VIP > 1).

d-Limonene was detected in high concentrations in beef powder (1026.93 ng/g). It is a key compound of citrus peel essential oil and imparts citrus, lemon, and mint notes. It has previously been detected in beef and beef jerky (Li et al., 2024a; Bai et al., 2022; Luo et al., 2020) and is widely used as a flavoring agent in food products and as a

fragrance component in cosmetics. Furthermore, it is reported to have anticancer, antimicrobial, and cardioprotective properties (Nam et al., 2025; Akhavan-Mahdavi et al., 2022).

2-Acetylfuran and 2-furfural were also detected in high concentrations in beef powder (763.42 and 607.17 ng/g, respectively), as well as in beef extract at lower concentrations (55.69 and 66.86 ng/g, respectively), but not in the meat-based broths. These compounds are both furan derivatives, typically produced through Maillard and sugar degradation reactions. 2-Acetylfuran imparts buttery and meaty notes, as previously reported in studies on cooked meats (Zhang et al., 2022; Sohail et al., 2022). Meanwhile, 2-furfural has notes of almonds, baked potatoes, bread, burnt sugar, and candy. Its presence in beef powder and beef extract aligns with a prior study on the volatile profile of beef (Liu et al., 2024b).

Four pyrazines (2,3-dimethylpyrazine, 2,5-dimethylpyrazine, methylpyrazine, and trimethylpyrazine) and one sulfur compound (methional) were found in the beef flavoring ingredients. Notably, no compounds of these types were detected in any of the commercial meat-based broths. Pyrazines are volatile nitrogen-containing heterocyclic compounds that impart roasted, baked, and nutty notes to foods (Nie et al., 2025; Yu et al., 2021). These compounds are primarily created through the Maillard reaction and greatly improve the volatile profile of meat-based products (Yu et al., 2021). Methional, a sulfur-based volatile compound, has been found in cooked beef, stewed beef, and pork broth,

where it adds pleasant warm-meat, brothy, and cooked-potato notes (Liu et al., 2024a; Wang et al., 2016; Watanabe et al., 2015). Methional greatly enhances the fatty and floral notes of pork broth because it has a low odor threshold (Wang et al., 2016). Unlike the meat-based broths, where aldehydes mainly contributed to the fatty note and sulfur compounds were absent, the presence of a sulfur-containing compound in beef extract suggests a contribution to the meaty note, potentially from thiamine degradation or the Maillard reaction (Zhao et al., 2024).

The Maillard reaction is widely known for its role in forming characteristic meat notes (Chu et al., 2025; Deng et al., 2025). In thermally processed meat, compounds like aldehydes, furans, and pyrazines contribute significantly to the volatile profile (Nie et al., 2024; Sun et al., 2022). Furan derivatives such as 2-acetylfuran and 2-furfural are formed through Maillard and sugar degradation reactions during the thermal processing of meat, and impart sweet, caramel, and smoky notes that improve the sensory quality of meat products (Zhang et al., 2022; Yin et al., 2022). Pyrazines like 2,5-dimethylpyrazine are produced by the interaction of sugars, amino acids, and lipids during the Maillard reaction and impart roasted, nutty, and toasted notes (Sun et al., 2022), thereby enhancing the volatile profile further (Nie et al., 2024). The Maillard reaction also produces various sulfur compounds that enhance the roasted notes and umami taste of beef (Nie et al., 2024; Sun et al., 2022). Therefore, the development of Maillard reaction-based flavoring agents is essential for improving the volatile profile and sensory qualities of meat-based broths.

Table 2.3. Volatile profiles (ng/g) of beef extract and powder

Compound	Concentration (ng/g)		*p*value	Aroma description†
	Beef extract	Beef powder		
Aldehydes				
3-methylbutanal	105.92 ± 3.81[b]	192.14 ± 1.34[a]	**	acrid, almond, chocolate, cocoa, corn flakes
benzaldehyde	7.45 ± 0.04[b]	41.38 ± 2.23[a]	**	almond, berry, bitter, bitter almond, burnt sugar
heptanal	nd	9.98 ± 0.55[a]	**	citrus, dry fish, fat, green, nut
hexanal	nd	159.69 ± 1.24[a]	**	apple, cut grass, fresh, fruit, grass
nonanal	1.13 ± 0.01[b]	8.23 ± 0.30[a]	**	citrus, cucumber, fat, floral, green
phenylacetaldehyde	192.83 ± 15.56[b]	541.33 ± 18.70[a]	*	berry, floral, flower, geranium, honey
Furans				
2-acetylfuran	55.69 ± 0.31[b]	763.42 ± 26.91[a]	**	balsamic, cocoa, coffee, fermented
2-furfural	66.86 ± 3.77[b]	607.17 ± 3.47[a]	**	almond, baked potatoes, bread, burnt sugar, candy
Hydrocarbons				
d-limonene	nd	1026.93 ± 10.53[a]	**	citrus, lemon, mint

o-xylene	1.13 ± 0.01[b]	4.16 ± 0.05[a]	**	geranium
p-xylene	1.24 ± 0.03[b]	9.83 ± 0.18[a]	**	cold meat fat, sweet
Ketones				
acetophenone	3.39 ± 0.13[b]	51.11 ± 0.81[a]	*	almond, animal, floral, flower
Pyrazines				
2,3-dimethylpyrazine	nd	132.20 ± 1.65[a]	**	caramel, cocoa, coffee, dry, hazelnut
2,5-dimethylpyrazine	nd	1118.23 ± 12.16[a]	**	burnt, cocoa, coffee
methylpyrazine	488.95 ± 39.87[a]	177.34 ± 3.10[b]	**	burnt, cocoa, fish, green
trimethylpyrazine	83.90 ± 4.66[b]	767.64 ± 18.15[a]	**	bread, burnt, cocoa, coffee, earth
Sulfur compounds				
methional	327.44 ± 8.57[a]	nd	**	baked potatoes, caramel, cooked potato, earth, meat

Abbreviations: nd, not detected. [a,b]Values in the same row with different superscript letters are significantly different (Student's t-test, *p < 0.01, **p < 0.001). [†] Aroma descriptions were sourced from the online database at https://www.vcf-online.nl/VcfHome.cfm.

Ⅳ. CONCLUSION

This study aimed to identify and quantify key volatile compounds in meat-based broths using HS-SPME-Arrow-GC/MS. The extraction efficiency of three different SPME-Arrow fibers was compared to assess their suitability for extracting volatile compounds from meat-based broths. The DVB/CAR/PDMS fiber had the highest extraction capacity, yielding much higher GC/MS peak intensities than the other fibers ($p < 0.05$). The equilibration time, extraction time, and extraction temperature were optimized using BBD-based RSM, with the optimal conditions being 20 min of equilibration, 30 min of extraction, and an extraction temperature of 40 °C.

Eighteen volatile compounds were identified in five commercial meat-based broths, with aldehydes associated with fatty notes being the primary contributors to the volatile profile. Notably, the first meat-broth sample, MB1, was characterized by higher concentrations of fat-associated aldehydes (hexanal, octanal, heptanal, nonanal, and decanal) and alcohols (1-heptanol and 1-octanol), thereby contributing to a distinct fatty note. In contrast, the volatile profiles of MB3 and MB4 were influenced by the presence of 2-methylbutanal, 3-methylbutanal, phenylacetaldehyde, and 2-butanone, which are typically associated with roasted or broth-like characteristics.

A comparison with beef flavoring ingredients emphasized the potential for enhancing the volatile profile of meat-based broths. Several compounds, including 3-methylbutanal, hexanal, heptanal, nonanal, and

phenylacetaldehyde, were present in both the meat-based broths and beef flavoring ingredients, suggesting that they contribute fatty notes. By contrast, compounds like pyrazines and methional, which add roasted and cooked characteristics, were found only in the beef flavoring ingredients. These compounds are known to form through the Maillard reaction, indicating that the use of Maillard reaction-based seasonings could enhance the volatile profiles of meat broths. Overall, these findings offer important insights for improving the volatile profiles of Korean-style HMR products, especially meat-based broths.

This research primarily focused on the identification and quantification of volatiles using the HS-SPME-Arrow-GC/MS technique; however, the sensory implications of these compounds on consumer preferences were not thoroughly explored. In future research, ingredients or seasonings should be developed using the key volatile compounds identified in this study to improve the sensory profiles of meat-based broths. The use of Maillard reaction-based flavoring agents is expected to greatly enhance the volatile profiles of these products. Focusing on these areas is expected to aid the development of more appealing meat-based broths that align with consumer preferences.

REFERENCES

Ahamed, Z., Seo, J. K., Eom, J. U., & Yang, H. S. (2023). Optimization of volatile compound extraction on cooked meat using HS-SPME-GC-MS, and evaluation of diagnosis to meat species using volatile compound by multivariate data analysis. *LWT*, *188*, 115374. https://doi.org/10.1016/j.lwt.2023.115374.

Ahn, H. S., Yu, S. S., Kim, C. Y., Kim, Y. W., Yoon, Y., Lee, H., & Park, S. H. (2024). Heat penetration and quality attributes of superheated steam sterilization (SHS) home meal replacement (HMR) meat products stew. *LWT*, *191*, 115621. https://doi.org/10.1016/j.lwt.2023.115621.

Akhavan-Mahdavi, S., Sadeghi, R., Esfanjani, A. F., Hedayati, S., Shaddel, R., Dima, C., Malekjani, N., Boostani, S., & Jafari, S. M. (2022). Nanodelivery systems for *d*-limonene; techniques and applications. *Food Chemistry*, *384*, 132479. https://doi.org/10.1016/j.foodchem.2022.132479.

Bai, S., You, L., Ji, C., Zhang, T., Wang, Y., Geng, D., Gao, S., Bi, Y., & Luo, R. (2022). Formation of volatile flavor compounds, maillard reaction products and potentially hazard substance in China stir-frying beef sao zi. *Food Research International*, *159*, 111545. https://doi.org/10.1016/j.foodres.2022.111545.

Chu, X., Du, P., Wang, W., He, H., Hu, P., Liu, Y., Li, M., Jiang, W., & Abd El-Aty, A. M. (2025). L-cysteine modulates the Maillard reaction: Impacts on PhIP and pyrazine formation. *Food Chemistry*, *472*, 142849. https://doi.org/10.1016/j.foodchem.2025.142849.

Deng, S., Cui, H., Hussain, S., Hayat, K., Liu, W., Zhang, X., & Ho, C. T. (2025). Promoted formation of pyrazines by targeted precursor addition to improve aroma of thermally processed methionine-glucose Amadori compound. *Food Chemistry*, *465*, 142033. https://doi.org/10.1016/j.foodchem.2024.142033.

Feng, Y., Cai, Y., Fu, X., Zheng, L., Xiao, Z., & Zhao, M. (2018). Comparison of aroma-active compounds in broiler broth and native chicken broth by aroma extract dilution analysis (AEDA), odor activity value (OAV) and omission experiment. *Food Chemistry*, *265*, 274-280. https://doi.org/10.1016/j.foodchem.2018.05.043.

Guan, H., Zhang, W., Tian, Y., Leng, S., Zhao, S., Liu, D., & Diao, X. (2025). Analysis of the flavor profile of chicken white soup with varying fat addition using GC‐MS, GC-IMS and *E*-nose combined with *E*-tongue. *Food Chemistry: X*, *26*, 102335. https://doi.org/10.1016/j.fochx.2025.102335.

Kim, J., Lee, H. E., Kim, Y., Yang, J., Lee, S. J., & Jung, Y. H. (2021). Development of a post-processing method to reduce the unique off-flavor of *Allomyrina dichotoma*: Yeast fermentation. *LWT*, *150*, 111940. https://doi.org/10.1016/j.lwt.2021.111940.

Kim, T. E., Lee, M. H., Kim, B. K., Lee, J. H., Chun, Y. G., & Jang, H. W. (2024). Optimization of the QuEChERS-UPLC-APCI-MS/MS method for the analysis of vitamins D and K nanoencapsulated in yogurt. *Journal of Chromatography A*, *1734*, 465275. https://doi.org/10.1016/j.chroma.2024.465275.

Lee, J. H., Cha, J. Y., Kim, T. K., Choi, Y. S., & Jang, H. W. (2021). Effects of a defatting process on the thermal stabilities and volatile compound profiles of proteins isolated from *Protaetia brevitarsis* larvae. *LWT*, *151*, 112095. https://doi.org/10.1016/j.lwt.2021.112095.

Lee, J. Y., Kim, W. S., Lee, Y. Y., Choi, Y. S., Choi, H. W., & Jang, H. W. (2019). Solid phase microextraction Arrow for the volatile organic compounds in soy sauce. *Journal of Separation Science*, *42*, 2942 - 2948. https://doi.org/10.1002/jssc.201900388.

Li, H., Chen, J., Zhang, Y., Jiang, Y., Sun, D., Piao, C., Li, T., Wang, J., Li, H., Mu, B., & Li, G. (2024a). Evaluation of the flavor profiles of Yanbian-style sauced beef from differently treated raw beef samples. *Food Chemistry: X*, *22*, 101505. https://doi.org/10.1016/j.fochx.2024.101505.

Li, Z., Frank, D., Ha, M., Hastie, M., & Warner, R. D. (2024b). Hemoglobin and free iron influence the aroma of cooked beef by influencing the formation and release of volatiles. *Food Chemistry*, *437*, 137794. https://doi.org/10.1016/j.foodchem.2023.137794.

Liu, J., Deng, S., Wang, J., Huang, F., Han, D., Xu, Y., Yang, P., Zhang, C., & Blecker, C. (2024a). Comparison and elucidation of the changes in the key odorants of precooked stewed beef during cooking-refrigeration-reheating. *Food Chemistry: X*, *23*, 101654. https://doi.org/10.1016/j.fochx.2024.101654.

Liu, Q., Gu, X., Wen, R., Sun, C., & Yu, Q. (2024b). Changes in meat quality and volatile flavor compounds profile in beef loin during

dry-aging. *LWT*, *205*, 116500. https://doi.org/10.1016/j.lwt.2024.116500.

Luo, Y., Zhao, L., Xu, J., Su, L., Jin, Z., Su, R., & Jin, Y. (2020). Effect of fermentation and postcooking procedure on quality parameters and volatile compounds of beef jerky. *Food Science & Nutrition*, *8*, 2316–2326. https://doi.org/10.1002/fsn3.1515.

Meng, Q., Zhou, J., Gao, D., Xu, E., Guo, M., & Liu, D. (2022). Desorption of nutrients and flavor compounds formation during the cooking of bone soup. *Food Control*, *132*, 108408. https://doi.org/10.1016/j.foodcont.2021.108408.

Nam, J. K., Kwak, B. M., & Jang, H. W. (2025). Optimization and comparative analysis of quality characteristics and volatile profiles in edible insect oils extracted using supercritical fluid extraction and ultrasound-assisted extraction methods. *Food Chemistry*, *474*, 143237. https://doi.org/10.1016/j.foodchem.2025.143237.

Nam, J. K., Lee, J. Y., & Jang, H. W. (2024). Quality characteristics and volatile compounds of plant-based patties supplemented with biji powder. *Food Chemistry: X*, *23*, 101576. https://doi.org/10.1016/j.fochx.2024.101576.

Nie, R., Wang, Z., Wei, X., Zhang, D., Liu, H., & Zhang, C. (2025). Understanding protein-lipid interactions in pyrazine binding under heat treatment: A case study of collagen and triglycerides. *Food Chemistry*, *482*, 144213. https://doi.org/10.1016/j.foodchem.2025.144213.

Nie, R., Zhang, C., Liu, H., Wei, X., Gao, R., Shi, H., Zhang, D., & Wang, Z. (2024). Characterization of key aroma compounds in roasted

chicken using SPME, SAFE, GC-O, GC - MS, AEDA, OAV, recombination-omission tests, and sensory evaluation. *Food Chemistry: X*, *21*, 101167. https://doi.org/10.1016/j.fochx.2024.101167.

Sohail, A., Al-Dalali, S., Wang, J., Xie, J., Shakoor, A., Asimi, S., Shah, H., & Patil, P. (2022). Aroma compounds identified in cooked meat: A review. *Food Research International*, *157*, 111385. https://doi.org/10.1016/j.foodres.2022.111385.

Song, N. E., Lee, J. Y., Lee, Y. Y., Park, J. D., & Jang, H. W. (2019). Comparison of headspace - SPME and SPME-Arrow - GC - MS methods for the determination of volatile compounds in Korean salt - fermented fish sauce. *Applied Biological Chemistry*, *62*, 16. https://doi.org/10.1186/s13765-019-0424-6.

Sun, A., Wu, W., Soladoye, O. P., Aluko, R. E., Bak, K. H., Fu, Y., & Zhang, Y. (2022). Maillard reaction of food-derived peptides as a potential route to generate meat flavor compounds: A review. *Food Research International*, *151*, 110823. https://doi.org/10.1016/j.foodres.2021.110823.

Wang, L., Li, C., Al-Dalali, S., Liu, Y., Zhou, H., Chen, C., Xu, B., & Wang, Y. (2022). Characterization of key aroma compounds in traditional beef soup. *Journal of Food Composition and Analysis*, *114*, 104839. https://doi.org/10.1016/j.jfca.2022.104839.

Wang, L., Qiao, K., Duan, W., Zhang, Y., Xiao, J., & Huang, Y. (2020). Comparison of taste components in stewed beef broth under different

conditions by means of chemical analyzed. *Food Science & Nutrition*, *8*, 955 - 964. https://doi.org/10.1002/fsn3.1376.

Wang, Y., Song, H., Zhang, Y., Tang, J., & Yu, D. (2016). Determination of aroma compounds in pork broth produced by different processing methods. *Flavour and Fragrance Journal*, *31*, 319 - 328. https://doi.org/10.1002/ffj.3320.

Watanabe, A., Kamada, G., Imanari, M., Shiba, N., Yonai, M., & Muramoto, T. (2015). Effect of aging on volatile compounds in cooked beef. *Meat Science*, *107*, 12 - 19. https://doi.org/10.1016/j.meatsci.2015.04.004.

Yang, J., Wu, S., Mai, R., Lin, L., Zhao, W., & Bai, W. (2021). Formation of amino acid-derived volatile compounds in dry-cured mackerel (*Scomberomorus niphonius*): Metabolic pathways involving microorganisms, precursors, and intermediates. *Food Chemistry*, *364*, 130163. https://doi.org/10.1016/j.foodchem.2021.130163.

Ye, Y., Wang, L., Zhan, P., Tian, H., & Liu, J. (2022). Characterization of the aroma compounds of Millet Huangjiu at different fermentation stages. *Food Chemistry*, *366*, 130691. https://doi.org/10.1016/j.foodchem.2021.130691.

Yin, X., Gao, M., Wang, H., Chen, Q., & Kong, B. (2022). Probing the interaction between selected furan derivatives and porcine myofibrillar proteins by spectroscopic and molecular docking approaches. *Food Chemistry*, *397*, 133776. https://doi.org/10.1016/j.foodchem.2022.133776.

Yu, H., Zhang, R., Yang, F., Xie, Y., Guo, Y., Yao, W., & Zhou, W. (2021). Control strategies of pyrazines generation from Maillard reaction. *Trends in Food Science & Technology*, *112*, 795 - 807. https://doi.org/10.1016/j.tifs.2021.04.028.

Yuan, C., Xu, C., Chen, L., Yang, J., Qiao, M., & Wu, Z. (2024). Effect of different cooking methods on the aroma and taste of chicken broth. *Molecules*, *29*, 1532. https://doi.org/10.3390/molecules29071532.

Yue, Z., Lai, J., Li, Q., Yu, Q., He, Y., Liu, J., & Zhu, Y. (2024). Enhancement of nutritional, organoleptic, and umami qualities of chicken soup induced by enzymatic pre-treatment of chicken. *Food Chemistry: X*, *24*, 101914. https://doi.org/10.1016/j.fochx.2024.101914.

Zhang, J., Yang, Z., Yang, Y., Han, L., Yu, Q., Cao, H., & Zhang, W. (2017). Development of a flavor fingerprint by GC-MS with chemometric method for volatile compounds of yak and yellow cattle bone soup. *Food Analytical Methods*, *10*, 943 - 954. https://doi.org/10.1007/s12161-016-0657-5.

Zhang, Z., Blank, I., Wang, B., & Cao, Y. (2022). Changes in odorants and flavor profile of heat processed beef flavor during storage. *Journal of Food Science*, *87*, 5208 - 5224. https://doi.org/10.1111/1750-3841.16363.

Zhao, Y., He, W., Zhan, P., Geng, J., Wang, P., & Tian, H. (2024). A comprehensive analysis of aroma quality and perception mechanism in ginger-infused stewed beef using instrumental analysis, sensory evaluation and molecular docking. *Food Chemistry*, *460*, 140435.

https://doi.org/10.1016/j.foodchem.2024.140435.

Zou, Y., Kang, D., Liu, R., Qi, J., Zhou, G., & Zhang, W. (2018). Effects of ultrasonic assisted cooking on the chemical profiles of taste and flavor of spiced beef. *Ultrasonics Sonochemistry*, *46*, 36 - 45. https://doi.org/10.1016/j.ultsonch.2018.04.005.

ABSTRACT IN KOREAN (국문 요약)

지속 가능한 식품 소재에 대한 수요가 증가함에 따라 식용곤충을 대체 식품 자원으로 활용하려는 관심이 높아지고 있다. 본 연구에서는 *Tenebrio molitor* 유충, *Gryllus bimaculatus*, *Locusta migratoria*, *Zophobas atratus* 유충의 식용곤충 4종으로부터 초임계 유체 추출법(supercritical fluid extraction, SFE)과 초음파 보조 추출법(ultrasound-assisted extraction, UAE)을 이용하여 오일을 추출하였다. SFE 조건은 반응표면분석법(response surface methodology, RSM)을 활용하여 최적화하였으며, 최적 조건은 400 bar, 55도, 3시간으로 도출되었다. 추출된 식용곤충 오일은 불포화지방산/포화지방산 비율이 높고, 동맥경화 및 혈전 지수가 낮아 영양학적으로 우수한 특성을 나타냈다. 휘발성 화합물은 헤드스페이스 고체상 미세추출 애로우(headspace solid-phase microextraction arrow, HS-SPME-Arrow)를 이용하여 추출한 후, 가스크로마토그래피-질량분석기(gas chromatography-mass spectrometry, GC/MS)를 통해 분석하였다. SFE를 통해 얻은 오일은 UAE에 비해 총 휘발성 화합물 함량이 유의하게 높았으며($p < 0.05$), 이전 연구에서 긍정적인 휘발성 특성과 관련된 것으로 보고된 여러 화합물이 확인되었다. 이러한 결과는 식용곤충 오일이 영양적 이점과 특이적인 휘발성 특성을 지닌 고품질 지질 소재로서의 활용 가능성을 제시한다.

또한, 가공식품 개발을 위한 기초자료로 활용하고자 고기 육수의 휘발성 조성에 대한 분석도 수행하였다. 세 가지 SPME-Arrow 섬유의 휘발성 화합물 추출 효율을 비교한 결과, divinylbenzene/carboxen/polydimethylsiloxane 섬유가 가장 높은 추출 효율

을 나타내었다($p < 0.05$). 휘발성 화합물 추출 조건은 RSM을 통해 최적화하였으며, 평형시간 20분, 추출시간 30분, 추출온도 40도가 최적 조건으로 도출되었다. HS-SPME-Arrow-GC/MS 분석을 통해 총 18종의 휘발성 화합물이 확인되었으며, 이 중 알데하이드류가 가장 우세한 화합물 군으로 나타났다. 특히, hexanal, octanal, heptanal, nonanal, decanal 등을 포함한 11종이 VIP 점수 1 이상으로 주요 화합물로 확인되었다. 또한, 소고기 향료와의 비교 분석을 통해 지방 향에 기여하는 공통 화합물을 확인하였으며, 피라진류 및 methional과 같이 구운 향 특성과 관련된 휘발성 화합물은 소고기 향료에서만 검출되었다. 본 연구 결과는 타겟 화합물 기반의 프로파일링을 통해 한식형 가정간편식의 휘발성 특성 향상에 유용한 기초자료를 제공한다.