The Effects of Different Extraction Methods on The Aroma Fingerprint, Recombination And Visualization of Clam Soup

Xu-Hui Huang [1], Yu-Ying Zhang [1], Ming Zhu [1], Da-Yong Zhou [1], Ming Du [1], Bei-Wei Zhu [1],

Xiu-Ping Dong[1], Ian Fisk [2], Lei Qin[1,*]

1 School of Food Science and Technology, National Engineering Research Center of Seafood, Collaborative Innovation Center of Seafood Deep Processing, Dalian Polytechnic University, Dalian 116034, China
2 School of Biosciences, University of Nottingham, Nottingham LE12 5RD, UK

* Correspondence: qinlei@dlpu.edu.cn (Lei Qin); Tel./ Fax: +86 411 86323262 (Lei Qin)

Abstract： Clam is a kind of nutritious and delicious economical aquatic food around the world-famous for its unique aroma. Instrumental analysis, sensory analysis, and comprehensive statistical analysis were applied to explain the relationship between aroma and odorants in clam soup. Six extraction methods combined with GC-MS and sniffing were utilized to obtain aroma fingerprints of the clam soup and to analyze the correlation with aroma perception. Solvent extractions were more effective than headspace extractions for the volatiles of clam soup. SAFE was the best method to obtain the most comprehensive volatile information of clam soup. The sequence of a combination of different extraction methods and SAFE would also affect the results of volatiles extracted from clam soup. Volatiles extracted via SDE, P&T, and SPME would add supplementary information to the result of SAFE. Totally 119 volatile compounds were obtained and identified from clam soup by summarising the results of different extraction methods. The significant effect of 14 key odorants in clam soup on aroma perception was verified by aroma recombination and odorants omitting. A neural network diagram of the aroma profile was designed to visualize the information of odor perception. Further, the results could be benefited to the aroma researches of aquatic food and the processing of clam products.

Keywords： multi-extraction methods; aroma profile; recombinant aroma; aroma perception; aroma visualization

1. Introduction

Clam (*Ruditapes philippinarum*) is a kind of bivalve filter feeder widely intensively cultivated in Asia and Europe [1]. As an important economic shellfish in the world, clam accounted for more than 25% of global shellfish production [2]. In 2017, its annual production around the world reached 4,228,206 tons, especially to China, whose annual production accounted for 98.9% of the world [3]. Due to its great flavor, low price, and rich nutrition, clams were deeply appreciated by consumers. Some studies have identified the characteristic odorants in clams [4]. A total of 41 volatile compounds have been discovered in the clam while cooked with different salinity, among which pentanal, 1-pentanol, and hexanal had a great influence on clam aroma [5]. However, the complex substance composition and the limitations of different extraction methods for volatile compounds made it difficult to obtain comprehensive and accurate aroma information of clams after cooking.

The identification of key volatile compounds is an important step to obtain the aroma information of foods. Due to the high separation efficiency and sensitivity, gas chromatography-mass spectrometry (GC-MS) technology is the best currently available tool that enables the detection of most of the important high-impact trace odorants, even at trace levels[6]. In molecular sensory research, the complete collection and correct quantification of volatile compounds are key to restructuring aroma and revealing how induvial or groups of compounds elicit sensory perception [7]. Therefore, selecting a suitable extraction method is important. Simultaneous distillation extraction (SDE) [8], direct organic solvent extraction [9], solvent-assisted flavor evaporation (SAFE) [10], solid-phase micro-extraction (SPME) [11], purge and trap (P&T) [12], etc., have been applied to extract the volatile compounds in foods. Although all of these extraction methods can be used to collect volatile information, each one has its own advantages and drawbacks. For example, direct extraction with solvents, especially using SDE, would bring in artifacts formation, lead to analyte degradation, or contaminate the instruments [13]. Furthermore, solvent properties (boiling point, solvation properties, etc.) can affect extraction efficiency and selectivity, causing losses of highly volatile components or analyte discrimination depending on their physicochemical properties [14]. Due to the solvent effect, although there were some deficiencies, SPME and P&T could be as supplementary methods without solvent [14]. In addition, the different extraction principles and efficiencies between each method are one of the main reasons for the deviation of quantitative results. Despite the fact that the stable isotope-labeled internal standard could result in better quantitative information of the key food odorants[15], due to the high cost and low availability of isotope-labeled odorants, it is not very necessary and practicable to quantify such a large quantity of key odorants in foods by isotope labeling [15]. Therefore, it is critical to compare and combine different extraction methods and identify a practical and accurate method for the analysis of odorants in foods.

In addition to obtaining the chemical composition of odorants, the sensory descriptors are also very important in explaining aroma perception. GC-olfactometry (GC-O) was developed to identify the key odorants and describe their odor characteristics using the human nose as an analytical detector [16]. Based on this method, odor-active molecules and their sensory impact ranking can be carried out by Charm analysis or aroma extract dilution analysis [16, 17]. However, as to the foods, there are enormous chemical complexities, including the large differences in concentration and volatility [18]. Therefore, it is not sufficient to only obtain the threshold information of odorants in air. It is also necessary to calculate the contribution of individual odorants to a given food aroma or their odor activity value (OAV) [19]. One study revealed that the characteristic aroma of 200 food samples was determined by 3 to 40 key odorants [20], which suggested that the information of key aroma compounds in clam could be used to reproduce the actual aroma of clam to a certain extent. By comparing the difference between the actual aroma and the recombination aroma, the contribution of each key aroma compound could be further analyzed. Besides, the relationship between the aroma loss of the recombinant aroma and the non-critical aroma compounds could also be obtained.

The odorants of clam soup have a very distinct smell. However, a comprehensive understanding of the odorants and their relative importance has not been previously achieved. In this study, various extraction methods, including liquid-liquid extraction, SAFE, SDE, P&T, and SPME, were compared and combined to obtain a more comprehensive volatile fingerprint of the clam. GC-MS, GC-O, gradient dilution, odor restructuring, and sensory analysis were used to verify the possible internal relationships between aroma and odorants. Further, a neural network diagram of the aroma profile on the statistical basis of such a relationship provided a rule for the visualization of aroma.

2. Materials and methods

2.1. Clam soup

Clams (*Ruditapes philippinarum*) used in this experiment were grown in the same area and purchased from a local market in Dalian, P. R. China. All the clams were caught from the Bohai in 2017. The total of clams used in this experiment was 50 kg. Clams were cleaned and boiled at 100 ℃ with water (1:2, w/v) for 3 min to get clam soup. The boiled liquid was vacuum-packed and stored in a freezer (Blizzard, NuAir, USA) at -80 °C.

2.2. Chemicals

Cyclohexanone, 1-pentanol, hexanal, acetic acid, butyl ester, furfural, (E)-2-hexenal, ethyl-benzene, 1,3-dimethyl-benzene, p-xylene, 1-hexanol, cyclohexanol, styrene, heptanal, methional, butyrolactone, benzaldehyde, 1-heptanol, 1-octen-3-ol, 6-methyl-5-hepten-2-one, 2-pentyl-furan, octanal, 2-ethyl-1-hexanol, benzenemethanol, 1-octanol, dimethyl-benzenemethanol, benzoic acid, methyl ester, nonanal, benzaldehyde dimethyl acetal, 1,2,4,5-tetramethyl-benzene, triethyl phosphate, naphthalene, decanal, benzothiazole, 2-(1,1-dimethylethyl)-4-methyl-phenol, eugenol, 1-dodecanol, butylated hydroxytoluene, and dibutyl phthalate were purchased from Aladdin (Shanghai, China). 2,4-Hexadienal, 3-hydroxy-butanoic acid, ethyl ester, and n-alkanes (C6 to C30) were acquired from Sigma-Aldrich (Shanghai, China). 2-Ethyl-hexanal, 2-cyclohexen-1-one, 1,2,3,5-tetramethyl-benzene, 1,3-bis(1,1-dimethylethyl)-benzene, 2,7-dimethyl-naphthalene, 1,3-dimethyl-naphthalene, acetic acid n-octadecyl ester were obtained from TCI Chemical Industry Development Co., Ltd (Shanghai, China). Toluene and phenol were purchased from Tianjin Molbase Chemical Reagent Factory (Tianjin, China). Methyl-pyrazine was obtained from Shanghai Macklin Biochemical Co., Ltd.

2.3. Extraction of volatile compounds

2.3.1. Solid-phase micro-extraction (SPME)

The extraction parameters of SPME were referred to the method of the previous study in clams [5]. The extraction volume of the sample was 3 ml. The vial with the sample was sealed and preheated at 50 °C for 20 min and extracted with a DVB/CAR/PDMS fiber. The fiber length was 2 cm. After the adsorption process, SPME fiber was immediately desorbed at 250 °C for 2 min in the GC injection port. Then the fiber was desorbed at 250 °C for an additional 10 min via a conditioning port to avoid carry-over effect.

2.3.2. Simultaneous distillation extraction (SDE)

An SDE apparatus refers to Likens-Nickerson was utilized to conduct this extraction experiment [8, 21]. For the analysis, 200 mL of sample was put into a round-bottom flask and then connected to the SDE apparatus. Each sample was extracted with 40 ml of redistilled dichloromethane. The dichloromethane was also put into one round-bottom flask and connected to the SDE apparatus. Both these two parts were heated. The sample to be extracted was held boiling state throughout the process. The solvent was kept at 50 °C. Vapors of solvent and sample were condensed by recycled water and flowed back. Each extraction time was 3 hours that start timing after the liquid sample started to boil. The sample obtained after extraction was dried over anhydrous sodium sulfate overnight and concentrated via a rotary evaporator. The solvent was further removed to 200 μL via a gentle stream of N_2. Finally, these samples were sealed and stored at -30 °C until further analysis.

2.3.3. Solvent-assisted flavor evaporation (SAFE)

The SAFE apparatus and extraction method were referred to the study that Engel etc. conducted in 1999 [10]. In this process, three different extraction combinations were examed: 1) organic solvent extraction before SAFE; 2) organic solvent extraction after SAFE; 3) dynamic headspace purge and trap after SAFE. Specifically, for organic solvent extraction before SAFE, the sample was extracted three times by 1:1 dichloromethane. During extraction, the temperature was kept at 4 °C. Sample with CH_2Cl_2 was oscillated for 1 h and centrifuged 2 min under 9190 g. Later the sample was separated by separating funnel. Before starting the extraction with SAFE, the temperature of recycled water to keep the apparatus thermostated was set to 40 °C. Besides, the SAFE apparatus had maintained a vacuum for 10^{-5} Pa. The distillation vessel (left side) was kept at a temperature of 50 °C. Meanwhile, the cooled flask (right side) and the trap were kept at a temperature of -196 °C via liquid nitrogen. And then the sample entered the SAFE device at a rate of one drop per second through the funnel. The sample obtained after extraction was dried over anhydrous sodium sulfate overnight and concentrated via a Rotary Evaporator made by Shanghai Yarong Biochemistry Instrument Factory, China. Then the solvent was further removed under a gentle stream of N_2 to 200 μL. Finally, these samples were sealed and stored at -30 °C until further analysis. For organic solvent extraction after SAFE, the volatile compounds of clam soup was extracted with the SAFE apparatus under the parameters as above. Later the sample was further extracted with dichloromethane three times (1:1, v/v) and concentrated into 200 μL as the above methods. For dynamic headspace purge and trap after SAFE, the sample obtained from SAFE was further extracted with purge and trap as the method in 2.3.4.

2.3.4. Dynamic headspace Purge and Trap (P&T)

The P&T was performed with an Atomx device made by Tekmar, USA. The extraction method was referred from Kesen [22] and optimized. The best method was used for the followed experiment. Put 20 mL samples into a headspace vial and purged with helium (40 mL/min) at 40 °C for 20 min. The extraction method of this device was the liquid mode. The dry purge flow was 100 mL/min at 50 °C for 2 min. A Teledyne Tekmar #5 trap was used to concentrate purged chemicals. The trap was composed of OV-1, Tenax, silica gel, and charcoal. The desorbed condition of volatiles separated from the trap is at 250 °C for 2 min.

2.4 Volatile compounds identification

2.4.1. GC-MS(O) conditions

Identification was carried out using an Agilent 7890B GC-5977A mass selective detector (MSD). The non-polar analytical column was HP-5MS (30 m×250μm×0.25μm). Helium was used as carrier gas at a constant flow rate of 1.5 mL/min. Each sample was injected in split mode (5:1). Injector temperature was kept at 260 °C. The initial oven temperature was held at 35 °C for 3 min. And then the oven temperature was raised at 5 °C/min to 280 °C and held for 10 min. Mass spectrometer was equipped with an ion source (EI), which had 70 eV electron energy and 230 °C source temperature. The scan range was from 29 to 350 m/z. One part of the eluate was directed to the MSD, whereas the other part was directed to the sniffing port. The interface temperature of the sniffing detector was 200 ℃. During the test, moist air was injected to prevent the assessor from drying out his or her nose. During a GC run, a trained panelist placed his/her nose close to and above the top of the sniffing port, recorded the odor of the chromatographic effluent as well as the retention time. The number of perception times of each compound by panelists during sniffing was counted to calculate the detection frequency of the compound [23]. Detection frequency analyses were conducted by 13 panelists (7 females and 6 males). The average age of panelists was 25 years old. the Analyses were repeated in duplicate by each panelist.

2.4.2. Compounds Identification

The mass spectrogram information from standards or NIST14 and Wiley11 library were used for the match of the acquired mass spectrogram of volatiles from samples. The similarity of reverse match factor greater than 700 and deviation of RI value less than 5 were set to determine whether the identification was accurate. After the identification, the corresponding mass spectrogram was obtained by using the standards to verify the results further. The RI was calculated according to the retention time of n-alkanes (C_6 to C_{30}) obtain under the same conditions. The formula is as follows:

$$RI = 100n + n \times [(T_i - T_n)/ (T_{n+1} - T_n)] \qquad (1)$$

where n and (n + 1) are respectively the number of carbon in the alkanes before and after the compound, Tn and Tn+1 are the corresponding retention time, and Ti is the retention time of the compound to be identified (Tn < Ti < Tn + 1).

2.5. Quantitation analysis of aroma compounds

Forty-nine standard compounds were used as external standards for quantitative analysis. A high concentration stock standard solution contains 49 compounds were prepared with ethanol and finally diluted with ultrapure water to 10 μg/mL. The standard solution was stored at 4 °C for later use. The eight-point calibration curve range from 0.01 to 10 μg/mL of each standard compound was built and used to calculate the concentration of key compounds. Cyclohexanone was added to the sample and standard solution before extraction and invoked to calculate the different extraction and injection efficiency of methods.

2.5. Sensory comparisons of aroma

The sensory evaluation panelists were selected from 80 members of the laboratory (students and teachers) via sensitivity test [24]. The development of descriptive terminology, the final selection of judges, and the final evaluation of the samples were done before evaluation. In the end, 12 assessors (ages ranging from 23 to 35) from both genders were selected. The experiment of odor thresholds was referred to the study conducted by Esam M. Ahmed [25]. The concentrations of the standards were diluted by a gradient, such as 10 times a gradient. Orthonasal odor thresholds were determined via triangle tests with odorants dissolved in water. For aroma recombination analysis, key odorants were mixed together with seawater according to the result of quantitation. Panelists compare the recombinant solution with the original clam soup according to the pre-set standard. The respective odor intensities were ranged from 0 (not perceivable) to 5 (strong). The detail information was given in the supplement.

2.7. Statistical analysis of the data

Microsoft office 2016, TBtools, Jvenn, and R Studio were used to plot and combine figures. Analysis of significant differences and variance (ANOVA) was done by SPSS v 9.0.

3. Results

3.1. Extraction Capacity Comparison

Six typical extraction methods combined with GC-MS were used to synthesize the fingerprints of volatile compounds in clam soup (Figure 1). As shown in Figure 1, three solvent extraction methods (liquid-liquid extraction (LL), SAFE, and SDE) and two headspace extraction methods (P&T and SPME) displayed significant differences in their ability to extract volatile compounds from clam soup. As showed in Figure 1-B, four main extraction methods, SAFE, SDE, P&T, and SPME were compared. There were significantly different from each other. The total number of compounds obtained without any intersection between the four methods was more than 77. There were only 13 compounds where the three methods intersected. SAFE could extract more volatile compounds in clam soup than SDE, P&T, and SPME. Comparing the three extraction methods changed from the SAFE method, shown in Figure 1-C, the compounds obtained from these three methods had a great similarity, especially between LL-SAFE and SAFE-LL. However, the extraction capacity of the combination of two solvent extraction methods is also different due to different priorities, such as LL-SAFE and SAFE-LL. Meanwhile, the extraction capacity of combined methods was worse than that of a single extraction method sometimes. For example, the extraction capacity of combined SAFE and P&T was worse than used P&T alone. According to the yellow bar in Figure 1-A, it is obvious that the number of volatile compounds obtained by solvent extraction is relatively numerous, especially by SAFE. SPME alone extracted the least number of volatile compounds, while SAFE-LL extracted the most. According to Figure 1 and Figure 2, after removing artificial compounds introduced by the solvent effect, 92 volatile compounds could be extracted from clam soup by SAFE-LL, of which 49 could only be obtained by SAFE-LL and LL-SAFE, such as 3,4-dihydro-2H-pyran, methyl-pyrazine, methional, butyrolactone, 1-heptanol, etc. When liquid-liquid extraction was carried out either before or after SAFE, the dark green dot in Figure 1-A indicates that there were 2 distinct volatile compounds when liquid-liquid extraction was carried out first. When liquid-liquid extraction was conducted after SAFE, there were 5 distinct volatile compounds (yellow dot). Although the volatile compounds extracted by SDE after removing artificial compounds introduced by the solvent effect were few, 17 volatile compounds in clam soup could only be collected via SDE (Figure 1, dark red dot). These compounds were mostly benzenoid compounds such as 1,2,4-trimethyl-benzene, 2,6-dimethyl-naphthalene, 1-ethyl-3,5-dimethyl-benzene, 4-ethyl-1,2-dimethyl-benzene, 1-methyl-naphthalene, etc (Table S6).

The extraction of volatile compounds is the key step to identify volatile compounds and characteristic odorants. Different extraction methods will make a huge difference to the aroma profile [26]. When the order of extraction steps was changed, compared to SAFE-LL and LL-SAFE (Figure 1 and Figure 2), seven distinct compounds were found to be different. It indicates that 2-methyl-pentadecane and 3-methyl-pentadecane could be effectively collected by liquid-liquid extraction, while SAFE was more effective for the extraction of 3-methyl-1-pentene, 3-hexanone, 2-hexanone, 2-methyl-tridecane, and 3-methyl-tridecane. As shown in Figure 2, SAFE-LL and LL-SAFE could obtain more comprehensive volatiles information about clam soup. Their extraction capacities covered the vast majority of compounds with low, medium, and high retention indices. The retention index is related to the boiling point and mass of the compound. It indicated that SAFE-LL and LL-SAFE were suitable for the extraction of both low and high boiling point volatile compounds. In terms of SDE, although it could just extract a few compounds, it cannot be replaced due to a large number of unique volatiles found. This result indicated that some volatile compounds could be much easier collected via SDE. Combined with the results of SDE, more comprehensive aroma fingerprint information could be obtained. Besides, SDE was suitable for the extraction of volatile compounds with medium and high boiling point. SPME is appropriate for volatile compounds with medium RI value. Meanwhile, in the sample with high water content, the proportion of volatile components is lower than that of other samples. The volatile compounds of concentrated samples extracted with SPME did not change significantly. It indicated that the adsorption capacity of SPME had a saturation point. The change of concentration after reaching the saturation point had little effect on it. Meanwhile, SPME was not suitable for extracting volatile compounds in the liquid matrix such as clam soup. P&T also did not work well with the volatile compounds extracted from clam soup. As shown in Figure 2, volatiles extracted via P&T were concentrated at low to medium boiling points. Certainly, SPME and P&T could extract unique volatile compounds. In general, solvent extractions were more effective than headspace extractions for the volatiles of clam soup. SAFE was the best method to obtain the most comprehensive volatile information. This finding is similar to that shown for tomato [26]. However, volatiles extracted via SDE, P&T, SPME would add supplementary information to the result of SAFE. Therefore, using a range of different methods is very important to obtain a comprehensive volatile compounds profile.

3.2. Aroma profile difference obtained via different extraction methods

After comparing the results of samples and the blank of solvent, the artifacts were removed. As shown in Table 1, 49 compounds were selected from the 119 characteristic volatile compounds of clam soup identified in the previous stage via detection frequency with GC-O to further quantitative analysis, threshold value analysis, and aroma analysis. Although all 49 volatile compounds detected in clam soup appeared to play a role in the aroma, some of the compounds may have been introduced into clams due to environmental contamination, such as triethyl phosphate, dibutyl phthalate, acetic acid n-octadecyl ester, and 1,3-bis(1,1-dimethylethyl)-benzene were the common contaminants in water or food package [27]. Besides, p-xylene, toluene, 1,2,3,5-tetramethyl-benzene, 1,2,4,5-tetramethyl-benzene, etc. seemed little been as flavor compounds, but some studies had shown that they were products during amino acid degradations and the key odorants in some foodstuffs [28].

Odor activity value (OAV), as one of the parameters to determine the contribution of volatile compounds on the aroma of the food, was used to find out the aroma profile differences between different extraction methods [29]. It was mainly determined by the threshold value and content of compounds in food. The difference in extraction efficiency was one of the reasons for inaccuracies when comparing quantitative results [14]. As showed in Figure 2, the compounds in clam soup extracted by SPME were few. Therefore, SPME was not suitable for the quantitative analysis of volatile compounds in clam soup. Meanwhile, SDE has similar problems that the poor recovery rate and repeatability lead to inaccurate quantitative results. Among these six methods, LL-SAFE, SAFE-LL, SAFE-P&T, and P&T were more suitable for quantitative analysis of key aroma compounds in clam soup and calculating OAV.

As showed in Figure 3, the aroma profiles obtained via different extraction methods were different. This was due to the differences in extraction capacity, stability, and other factors among these four extraction methods. Although the aroma profiles obtained via LL-SAFE and SAFE-LL were much more similar, there were also some differences between parts of the key compounds, such as acetic acid, butyl ester, 1-dodecanol, naphthalene, ethyl-benzene, 2-pentyl-furan, etc. This suggested that the matrix may affect the efficiency of liquid-liquid extraction. The dispersion efficiency and loss of volatile compounds in the solvent were changed in different samples. These changes would lead to differences in the characteristic aroma profile. When solvent extraction and headspace extraction were combined, as SAFE-P&T, the aroma profile information cannot be improved. The aroma profiles obtained via SAFE-LL and LL-SAFE were significantly different from the aroma obtained via SAFE-P&T. However, SAFE-P&T and P&T got a more similar aroma profile. It indicated that P&T played a more dominant role in the acquired aroma profile between SAFE-P&T and P&T. Meanwhile, there were also some differences between the aroma profiles of SAFE-P&T and P&T, such as benzoic acid, methyl ester, 1,3-bis(1,1-dimethylethyl)-benzene, 1-octanol, 2-ethyl-hexanal, 2,4-hexadienal, etc. Although SAFE can effectively purify and collect volatile compounds from samples, it could also lead to a partial loss of volatile compounds and introduced a lot of solvents, resulting in aroma profile changes. The solvent would reduce the proportion of the characteristic compounds to be absorbed because the adsorption capacity of the adsorbent in the trap had a limited capacity and was largely occupied by the solvent. The advantage of P&T was to collect volatile compounds directly without other processing.

The extraction capacity and stability were two main factors affecting different quantitative data obtained by different extraction methods. Cyclohexanone was added into the samples for calculating the correction factor and normalizing the data. Quantitative data of the same compound with different extraction methods were of the same order of magnitude. The error was within acceptable limits. The compounds that could be extracted had a greater impact on the aroma profile than the precise determination of their concentration. The actual contribution of odor compounds to the aroma profile depends on their concentration and threshold. Therefore, it was necessary to find a way to combine the quantitative results detected by different extraction methods for analyzing the aroma profile of clams soup. Based on the quantitative results obtained by different extraction methods, an appropriate error point was selected to analyze the aroma contribution of the obtained volatile compounds. The feasibility of correcting and verifying the error was of certain significance for the analysis of food aroma information by multi-extraction methods.

3.3. Key odorants Selecting and Aroma Description

According to the quantitative stability and accuracy of different compounds under different extraction methods, suitable methods were selected for the quantitative analysis of 49 compounds, as shown in Table 1. The R^2 of the standard curve for the quantitative analysis of each compound was greater than 0.99. Meanwhile, the odor thresholds of odorants selected were obtained by gradient dilution. Besides, the aromas of 49 compounds were described and compared with the description results of relevant literature [30]. A total of 45 aroma descriptors of compounds were obtained. The aromas of four compounds, 2,7-dimethyl-naphthalene, 2-(1,1-dimethylethyl)-4-methyl-phenol, 1,3-bis(1,1-dimethylethyl)-benzene, and

1,2,3,5-tetramethyl-benzene, were not described as undifferentiated by the vast majority of panelists. Further, the OAV and detection frequency were also calculated with GC-O. Of the 49 volatile compounds, only 14 key odorants could be stably sensed by panelists multiple times. Hexanal, 1-hexanol, styrene, heptanal, methional, 1-heptanol, 1-octen-3-ol, octanal, 2-ethyl-1-hexanol, benzenemethanol, nonanal, decanal, eugenol, 1-dodecanol all had great aroma perception during GC-O.

The threshold of saturated straight-chain aldehyde was smaller than 10 ppb. The threshold of aldehydes with other groups or unsaturated bonds, such as (E)-2-hexenal, furfural, and 2,4-hexadienal increased significantly. Aldehydes are mainly derived from the automatic oxidation of lipids [31] with an odor like the fat, plant, citrus, etc. Alcohols have a threshold slightly higher than aldehydes. Alcohols mainly contribute to woody and fruity odor and give the product a smoother feeling [32]. 1-Octen-3-ol derived from linoleic acid was a key volatile compound in shellfish [32]. Except for naphthalene, the threshold value of aromatic compounds is relatively large. Although naphthalene could be accumulated from environmental pollution, it also affected the aroma profile [33]. OAV was introduced to evaluate the odor activity of volatile compounds. There were 14 volatile compounds in clam soup with OAV > 1. All key odorants with OAV > 1 were detected stably by panelists. At least eight out of 13 people were able to sniff these compounds. Octanal had the highest odor activity (62.84). The odor of octanal was like fat, citrus, and honey [32]. It may have a great contribution to the odor formation of clam soup. Nonanal and benzenemethanol also have a significant impact on the aroma of clam soup. They have a fruity odor when smelled alone, such as the odor of nonanal is like orange [34]. But a mixture of several volatile compounds may present a new odor [20, 35]. For methional, most of the panelists could smell the obvious aroma of cooking seafood during GC-O sniffing. Other research also has reported that it has a meat-like and soup-like odor [30], which was consistent with this result. Matching the odor description and odor intensity classification of odorants via professional sensory evaluator to the actual composition of key volatile compounds detected by the instrument, the aroma perception of the complex system can be transformed into a simple mathematical relationship.

3.4. Recombination Validation of Aroma

Regardless of the fact that the aroma profiles obtained by these extraction methods were different, their key odorants were similar. The aroma profile obtained by each method could reflect some characteristics of the sample to some extent. However, a comprehensive analysis of the results of these methods could more accurately reflect the aroma profile of the clam soup. The odor recombination solution made via the comprehensive results of these four methods was compared with the original clam soup. In Figure 4, the panelists developed five descriptors for the clam soup, fishy odor, rusty odor, roasted potato odor, earthy odor, and meaty odor. The combination of several distinct odorants will produce new aromas. Therefore, the odor perception of panelists for the original sample or reconstituted solution was different from that of a single odorant [20]. The sensory evaluation curve (Figure 4-A) of the recombined odor solution was similar to the curve of the original sample. It indicated that the aroma profile from the 49 odorants could be used to represent the aroma of clam soup.

Fourteen key odor compounds with OAV >1 were used in the odor omitting experiment to find out the aroma changing when removing one or more of these key odor compounds from the odor recombination solution. All the 14 key aroma compounds had a significant influence on the aroma profile of clam soup. Through the difference analysis, the *p*-value of each aroma omitting experiment was less than 0.05 (Figure 4). As shown in Figure 4-B, Figure 4-E, and Figure 4-G, hexanal, heptanal, and 1-octen-3-ol seemed to have the same effect on the aroma of clam soup. They all could increase the fishy and meaty odor of clam soup and make the rusty, earthy, and roasted potato odor more gentle. Except for reducing the earthy odor, hexanol, styrene, and octanal had a similar effect on clam soup to hexanal, heptanal, and 1-octen-3-ol. 1-Heptanol may be also related to the fishy odor of clam soup. It also could reduce the rusty and roasted potato odor of clam soup. Although benzenemethanol, nonanal, and 2-ethyl-1-hexanol cannot increase the characteristic aroma of clam soup, they all could make the rusty, earthy, and roasted potato odor more gentle. They were more like an accessory ingredient for the aroma strength of clam soup. Decanal was also similar to benzenemethanol, nonanal, and 2-ethyl-1-hexanol in that it only could lighten the rusty and roasted potato odor. Besides, Methional was related to the meaty odor of clam soup.

All of the 14 key compounds selected were contributed to the aroma of clam soup. The 14 key odor compounds all have their own unique odor when they exist alone. When they were mixed, however, they produced new aromas [20]. Although the odor and compound type of the key compounds may differ, their effects on the new aroma after mixing may be similar, such as hexanal and 1-octen-3-ol had the same effect on clam soup aroma, although hexanal had a fatty, grassy, and fruity odor, while the odor of 1-octen-3-ol was sweet earthy odor with a strong herbaceous and rose. The relative proportion of different odorants can be used to explain the different aromas of foods at the macro level. It relies more on OAV to determine whether an odor will be perceived and reflected. If OAV < 1, it was thought that the compound odor could not be perceived. At the micro-level, the perception of aroma relies on the activation of neurons. When a scent is delivered to the nasal cavity, all of the odorants may try to bind to olfactory receptors and act on neurons [36]. Changes in olfactory neuron signals lead to differences in odor perception [36]. That was why in the odor loss experiment when removing a key odor compound, the change in the aroma of the mixture was not consistent with the odor of the key compound left alone. Therefore, the aroma of clam soup was formed by a variety of odorants, whether the key odor compounds of OAV>1 or OAV<1 all had a certain effect on the overall aroma. However, the key odor compounds with OAV>1 has a stronger effect on clam aroma.

3.5. Visual Analysis of Aroma Profile

In the process of identifying the odor of a single volatile compound, the odor was divided into five categories, aromatic odor, fatty odor, floral odor, fruity odor, and roasted nut odor. The descriptors were different from the odor categories in Figure 4. Because there was a certain difference between the aroma of a single volatile compound and the comprehensive aroma of the mixture volatile compounds [20]. In Figure 5, the aroma profile of clams soup synthesized with the results of different extraction methods was visualized. It is easy to judge which compounds are the key odorants and what role each played in the aroma profile of the clam soup by color, size, area, distance, and solid/hollow. In clam soup, the aromatic odor was mainly provided by benzenoids compounds. Floral odor and fruity odor mainly came from alcohols and aldehydes. 1-heptanol, 1-dodecanol, eugenol, benzenemethanol, octanal, methional, nonanal, decanal, etc. were the key odorants. The contribution of the compounds to the aroma profile could be judged according to the size of the dots, the thickness of the line, and the distance from the center. For example, 1-octen-3-ol contributed more to the aroma profile of clam soup than 2-ethyl-1-hexanol because the odor threshold of 1-octen-3-ol was smaller, and subsequently, the OAV is larger. Meanwhile, in order to explain the differences in odor thresholds between different compounds, the concept of a neural network was added to Figure 5. The thicker the “nerve” (red line), the stronger the signal produced by the stimulus. For example, nonanal was believed to have a much larger influence on the aroma profile than decanal because of nonanal was a much stronger stimulus for the same amount. Based on the mathematical relationship about the threshold value, OAV, and odor, combining human perception with instrumental analysis, the neural network diagram of the aroma profile in Figure 5 could display the information of odor perception as a visual picture.

4. Conclusion

The choice of extraction method was shown to directly impact the available volatile compounds for aroma analysis. SAFE was shown to provide the most comprehensive information about volatile compounds in clam soup. SPME was not suitable for extracting volatiles in clam soup. Volatiles extracted via P&T were most at low to medium boiling points, while volatiles extracted via SDE were at medium to high boiling points. The complementarities between the P&T and SDE approaches were more obvious. However, the recovery rate and repeatability of quantitative results obtained via SDE were poor. Meanwhile, due to the unstable coatings, accurate quantitative analysis via SPME was also not very well. However, compared with precise quantification, the accurate identification of volatile compounds had more influence on the aroma profile analysis. There were 14 key odorants in clam soup that would influence the aroma profile of clam soup significantly. The odorants information obtained from different extraction methods (SAFE-LL, LL-SAFE, SAFE-P&T, and P&T) could be used to reconstruct the aroma of clam soup through chemical recombination. The neural network diagram of the aroma profile could display the information of odor perception as a visual picture.

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Reference

1. Y. Yamasaki, S. Taga, M. Kishioka and S. Kawano, A metabolic profile in Ruditapes philippinarum associated with growth-promoting effects of alginate hydrolysates, *Scientific Reports*, 2016, **6**, 32829.
2. D. Cordero, M. Delgado, B. Liu, J. Ruesink and C. Saavedraa, Population genetics of the Manila clam (*Ruditapes philippinarum*) introduced in North America and Europe, *Scientific*

Reports, 2017, **7**, 39745.

3. FAO, *The state of world fisheries and aquaculture 2018*, Food and Agriculture Organization of the United Nations, Rome, 2018.
4. Z. Ran, S. Zhang, Y. Zhu, A. Ke, J. Xu, Y. Li, K. Liao, S. Li, Y. Ran and X. Yan, Effect of salinity on volatiles in the razor clam investigated by head space-solid phase microextraction/gas chromatography-mass spectrometry, *Fisheries Science*, 2019, **85**, 137-146.
5. Z. Ran, S. Zhang, Y. Zhu, A. Ke, J. Xu, Y. Li, K. Liao, S. Li, Y. Ran and X. Yan, Effect of salinity on volatiles in the razor clam investigated by head space-solid phase microextraction/gas chromatography-mass spectrometry, *Fisheries Science*, 2018, **85**, 137-146.
6. L. Wang, P. Wang, W. Deng, J. Cai and J. Chen, Evaluation of aroma characteristics of sugarcane (Saccharum officinarum L.) juice using gas chromatography-mass spectrometry and electronic nose, *LWT-Food Science Technology*, 2019, **108**, 400-406.
7. E. Saldaña, L. Saldarriaga, J. Cabrera, R. Siche, J. H. Behrens, M. M. Selani, M. A. de Almeida, L. D. Silva, J. S. S. Pinto and C. Contreras-Castillo, Relationship between volatile compounds and consumer-based sensory characteristics of bacon smoked with different Brazilian woods, *Food Research International*, 2019, **119**, 839-849.
8. M. L. Barreiros, A. L. B. S. Barreiros, A. R. C. Ribeiro, M. Santos Leite Neta and N. Narain, Volatile constituents of cajá-umbu (Spondias sp.) fruit obtained by simultaneous distillation and extraction and solid phase microextraction, *Acta Horticulturae*, 2018, **1198**, 265-272.
9. A. L. Theis, A. J. Waldack, S. M. Hansen and M. A. Jeannot, Headspace solvent microextraction, *Analytical chemistry*, 2001, **73**, 5651-5654.
10. W. Engel, W. Bahr and P. Schieberle, Solvent assisted flavour evaporation–a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices, *European Food Research Technology*, 1999, **209**, 237-241.
11. R. M. Vivian Goh, H. Lau, S. Q. Liu, B. Lassabliere, R. Guervilly, J. Sun, Y. Bian and B. Yu, Comparative analysis of pomelo volatiles using headspace-solid phase micro-extraction and solvent assisted flavour evaporation, *LWT-Food Science Technology*, 2019, **99**, 328-345.
12. F. A. Franchina, D. Zanella, E. Lazzari, P.-H. Stefanuto and J.-F. Focant, Investigating aroma diversity combining purge-and-trap, comprehensive two-dimensional gas chromatography, and mass spectrometry, 2020, **43**, 1790-1799.
13. A. Chaintreau, Simultaneous distillation–extraction: from birth to maturity, *Flavour and Fragrance Journal*, 2001, **16**, 136-148.
14. J. Wang, J. M. Gambetta and D. W. Jeffery, Comprehensive study of volatile compounds in two Australian rose wines: Aroma extract dilution Analysis (AEDA) of extracts prepared using solvent-assisted flavor evaporation (SAFE) or headspace solid-phase extraction (HS-SPE), *Journal of agricultural and food chemistry*, 2016, **64**, 3838-3848.
15. A. M. Crump, M. A. Sefton and K. L. Wilkinson, Microwave-assisted deuterium exchange: the convenient preparation of isotopically labelled analogues for stable isotope dilution analysis of volatile wine phenols, *Food Chemistry*, 2014, **162**, 261-263.
16. A. Amanpour, G. Guclu, H. Kelebek and S. Selli, Characterization of key aroma compounds in fresh and roasted terebinth fruits using aroma extract dilution analysis and GC–MS-Olfactometry, *Microchemical Journal*, 2019, **145**, 96-104.

17. W. Grosch, Evaluation of the key odorants of foods by dilution experiments, aroma models and omission, *Chemical senses*, 2001, **26**, 533-545.

18. R. B. Mastello, M. Capobiango, S. T. Chin, M. Monteiro and P. J. Marriott, Identification of odour-active compounds of pasteurised orange juice using multidimensional gas chromatography techniques, *Food Research International*, 2015, **75**, 281-288.

19. N. L. de Lerma, R. A. Peinado, A. Puig-Pujol, J. C. Mauricio, J. Moreno and T. García-Martínez, Influence of two yeast strains in free, bioimmobilized or immobilized with alginate forms on the aromatic profile of long aged sparkling wines, *Food Chemistry*, 2018, **250**, 22-29.

20. D. Andreas, S. Martin, K. Matthias, N. Bettina, K. Dietmar, S. Peter and H. Thomas, Nature's chemical signatures in human olfaction: a foodborne perspective for future biotechnology, *Angewandte Chemie*, 2014, **53**, 7124-7143.

21. S. T. Likens and G. B. Nickerson, Detection of certain hop oil constituents in brewing products, *Journal of the American Society of Brewing Chemists*, 1964, **11**, 5-13.

22. S. Kesen, Characterization of aroma and aroma-active compounds of Turkish turmeric (Curcuma longa) extract, *Journal of Raw Materials to Processed Foods*, 2020, **1**, 13-21.

23. H.-Z. Yu and S.-S. Chen, Identification of characteristic aroma-active compounds in steamed mangrove crab (Scylla serrata), *Food Research International*, 2010, **43**, 2081-2086.

24. L. zhao, X. Deng and W. Liu, *Food sensory analysis vocabulary*, China Light Industry Press, Beijing, 2015.

25. S. Daikoku, M. Mitsuda, T. Tanamura and K. Uchiyama, Measuring odor threshold using a simplified olfactory measurement method, *Journal of the human-environment system*, 2019, **21**, 1-8.

26. J. Li, Y. Fu, X. Bao, H. Li, J. Zuo, M. Zhang and J. Wang, Comparison and analysis of tomato flavor compounds using different extraction methods, *Journal of Food Measurement and Characterization*, 2020, **14**, 465-475.

27. Y. Guo, Z. Zhang, L. Liu, Y. Li, N. Ren and K. Kannan, Occurrence and profiles of phthalates in foodstuffs from China and their implications for human exposure, *Journal of agricultural and food chemistry*, 2012, **60**, 6913-6919.

28. F. J. Hidalgo and R. Zamora, Amino acid degradations produced by lipid oxidation products, *Critical Reviews in Food Science Nutrition*, 2016, **56**, 1242-1252.

29. J. Pino and S. E. Barzola-Miranda, Characterization of odor-active compounds in pechiche (Vitex cymosa Berteo ex Speng) fruit, *Journal of Raw Materials to Processed Foods*, 2020, **1**, 33-39.

30. G. A. Burdock, *Fenaroli's handbook of flavor ingredients*, CRC press, New York, 2016.

31. W. Zhang, X. Cao and S. Q. Liu, Aroma modulation of vegetable oils—A review, *Critical Reviews in Food Science Nutrition*, 2020, **60**, 1538-1551.

32. F. Bianchi, M. Careri, M. Musci and A. Mangia, Fish and food safety: Determination of formaldehyde in 12 fish species by SPME extraction and GC–MS analysis, *Food Chemistry*, 2007, **100**, 1049-1053.

33. T. Aro, R. Tahvonen, L. Koskinen and H. Kallio, Volatile compounds of Baltic herring analysed by dynamic headspace sampling–gas chromatography–mass spectrometry, *European Food Research Technology*, 2003, **216**, 483-488.

34. S. Frank, N. Wollmann, P. Schieberle and T. Hofmann, Reconstitution of the flavor signature

of Dornfelder red wine on the basis of the natural concentrations of its key aroma and taste compounds, *Journal of agricultural and food chemistry*, 2011, **59**, 8866-8874.

35. E. L. Berre, N. Béno, A. Ishii, C. Chabanet, P. Etiévant and T. Thomasdanguin, Just noticeable differences in component concentrations modify the odor quality of a blending mixture, *Chemical senses*, 2008, **33**, 389-395.

36. R. C. Araneda, A. D. Kini and S. Firestein, The molecular receptive range of an odorant receptor, *Nature neuroscience*, 2000, **3**, 1248-1255.

37. A. King, J. Readman and J. Zhou, Determination of polycyclic aromatic hydrocarbons in water by solid-phase microextraction–gas chromatography–mass spectrometry, *Analytica Chimica Acta*, 2004, **523**, 259-267.

Table 1 Thresholds, OAVs, detection frequency, and aroma descriptions of odorants in clam soup [30, 32, 34, 37].

No.	Compounds	R^2	Quantitation Ions	Extraction Methods	Content (ng/mL)	Thresholds (ng/mL)	OAVs	GC-O Detection Frequency	Aroma descriptions
1	triethyl phosphate	0.9922	99	LL-SAFE	91.05±1.85	2500	<1	1/13	mild
2	p-xylene	0.9981	91	P&T	8.29±0.35	1000	<1	1/13	sweet
3	naphthalene	0.9986	128	SAFE-P&T	3.08±0.08	6	<1	2/13	aromatic odor, odor of mothballs
4	dibutyl phthalate	0.9937	149	LL-SAFE	15.97±0.30	2500	<1	1/13	slight, aromatic odor
5	benzoic acid, methyl ester	0.9985	55	P&T	36.28±1.23	110	<1	1/13	sweet balsamic, like cresol or wintergreen oil, sweet-floral like ylang ylang, tuberose
6	2-ethyl-hexanal	0.9972	72	P&T	5.71±0.19	2500	<1	1/13	mild odor
7	1-heptanol	0.9987	56	LL-SAFE	4.83±0.27	3	1.61	8/13	fragrant
8	1,3-dimethyl-benzene	0.9953	91	SAFE-P&T	6.95±0.48	1000	<1	1/13	sweet odor
9	toluene	0.9935	65	P&T	5.65±0.07	527	<1	1/13	fragrant woody oily fatty odor and spicy
10	phenol	0.9956	65	LL-SAFE	7.84±0.52	58585.25	<1	1/13	sweet, tarry odor,somewhat sickeningly sweet and acrid
11	butyrolactone	0.9995	85	SAFE-LL	10.73±0.82	20000	<1	1/13	a faint, sweet, aromatic, slightly buttery odor
12	benzaldehyde	0.9997	51	P&T	28.74±1.57	100	<1	1/13	strong fatty harsh pungent odor and an unpleasant, fatty taste
13	1-dodecanol	0.9943	69	LL-SAFE	41.79±1.97	16	2.61	10/13	characteristic fatty odor
14	styrene	0.9988	78	P&T	9.30±0.41	3.6	2.58	11/13	characteristic, sweet, balsamic, almost floral odor that is

									extremely penetrating
15	nonanal	0.9989	98	LL-SAFE	19.19±0.94	1.1	17.45	12/13	fat, floral, green, lemon
16	furfural	0.9997	95	SAFE-LL	2.85±0.16	280	<1	1/13	sweet earthy odor with a strong herbaceous and rose
17	eugenol	0.996	131	LL-SAFE	8.21±0.39	6	1.37	8/13	odor of cloves, warm, spicy, floral
18	α,α-dimethyl-benzenemethanol	0.9963	78	LL-SAFE	8.03±0.18	1000	<1	1/13	mild green sweet earthy
19	decanal	0.9982	55	LL-SAFE	14.38±0.44	3	4.79	11/13	floral, fried, orange peel, penetrating, tallow
20	benzenemethanol	0.9988	77	SAFE-LL	20.30±0.86	1.2	16.92	9/13	mild oily sweet slightly floral
21	2-ethyl-1-hexanol	0.9987	92	P&T	156.42±2.77	100	1.56	10/13	mild, oily, sweet, slightly floral odor reminiscent of rose
22	1-octanol	0.9982	56	P&T	23.94±1.07	42	<1	3/13	bitter almond, burnt matches, fat, floral
23	1,2,4,5-tetramethyl-benzene	0.9979	119	LL-SAFE	0.75±0.030	200	<1	1/13	unpleasant at high concentrations but delicate and floral on dilution
24	octanal	0.9984	84	P&T	36.89±0.98	0.587	62.84	13/13	strong, fruity odor
25	methional	0.9949	47	LL-SAFE	20.46±1.17	4.23	4.87	13/13	pleasant fruity and sweet taste
26	hexanal	0.99	72	SAFE-P&T	52.50±1.15	5	10.5	13/13	apple, fat, fresh, green, oil
27	heptanal	0.9997	57	SAFE-P&T	15.61±0.77	2.8	5.75	8/13	citrus, fat, green, nut
28	acetic acid, butyl ester	0.9976	56	SAFE-P&T	2.68±0.15	10	<1	1/13	a strong, fruity odor; burning and then sweet taste reminiscent of pineapple
29	1-pentanol	0.9989	70	SAFE-P&T	5.68±0.24	150.2	<1	1/13	balsamic, fruit, green, pungent, yeast

30	ethyl-benzene	0.9991	91	LL-SAFE	1.85±0.11	2205.25	<1	1/13	fatty citrus honey odor
31	benzaldehyde dimethyl acetal	0.9988	77	LL-SAFE	10.86±0.32	500	<1	1/13	green, floral almond sharp fruity wine
32	2-pentyl-furan	0.9985	81	SAFE-LL	2.28±0.02	5.8	<1	1/13	orange rose citrus-like
33	2,4-hexadienal	0.993	53	P&T	31.14±1.44	60	<1	1/13	herbaceous woody fragrant, sweet, green fruity
34	1-octen-3-ol	0.9984	72	P&T	11.55±0.33	1.5	7.7	12/13	fatty, green, fruity
35	1-hexanol	0.9986	56.1	P&T	20.18±1.12	5.6	3.6	12/13	herbaceous, woody, fragrant, mild, sweet, green fruity odor and aromatic flavor
36	1,3-dimethyl-naphthalene	0.9984	141	LL-SAFE	1.97±0.09	42	<1	1/13	sweet odor fatty citrus-like
37	6-methyl-5-hepten-2-one	0.9996	55	P&T	10.35±0.62	50	<1	1/13	meat-like odor and soup-like
38	butylated hydroxytoluene	0.9962	205	LL-SAFE	19.87±1.19	10000	<1	1/13	very faint, musty, occasional cresylic-type odor
39	methyl-pyrazine	0.9999	67	SAFE-LL	4.72±0.18	60	<1	1/13	cocoa, green, hazelnut, popcorn, roasted
40	benzothiazole	0.9982	69	SAFE-LL	5.37±0.16	80	<1	4/13	odor similar to that of quinoline, nut, rubber, meaty
41	3-hydroxy-butanoic acid, ethyl ester	0.9942	60	SAFE-LL	11.81±0.14	100	<1	1/13	marshmallow, roasted nut
42	2-cyclohexen-1-one	0.9985	68	P&T	8.69±0.18	500	<1	1/13	roasted, green
43	(E)-2-hexenal	0.9987	69	P&T	17.80±0.27	398.1	<1	3/13	a sweet, fragrant, almond, fruity green, leafy, apple, plum,

									vegetable odor.
44	cyclohexanol	0.9998	82	SAFE-LL	44.36±1.72	90	<1	1/13	camphor menthol phenol
45	acetic acid n-octadecyl ester	0.995	83	SAFE-LL	184.89±2.47	5000	<1	1/13	cresol, medicinal leather
46	2,7-dimethyl-naphthalene	0.9992	141	LL-SAFE	2.32±0.06	500	<1	1/13	hard to describe, undifferentiated odor
47	2-(1,1-dimethylethyl)-4-methyl-phenol	0.9963	121	LL-SAFE	5.87±0.12	90	<1	1/13	hard to describe, undifferentiated odor
48	1,3-bis(1,1-dimethylethyl)-benzene	0.9996	57	P&T	2.48±0.03	50	<1	1/13	hard to describe, undifferentiated odor
49	1,2,3,5-tetramethyl-benzene	0.9952	91	LL-SAFE	4.08±0.18	50	<1	1/13	hard to describe, undifferentiated odor

Figure

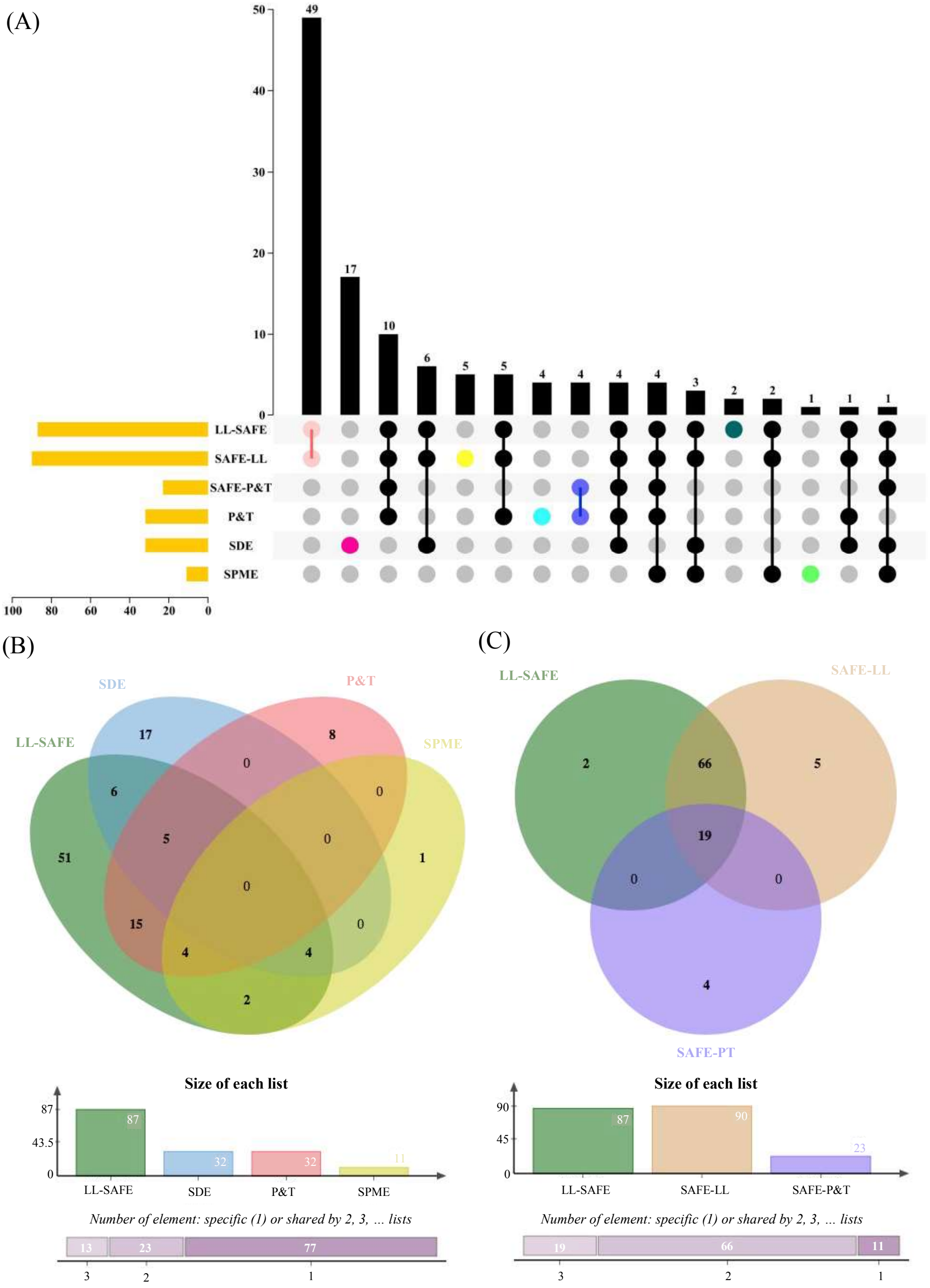


Figure 1 The extraction ability of different extraction methods for odorants in clam soup. (A) was the upset plot about all of the six extraction methods for odorants in clam soup; The yellow bars represent the number of volatile compounds that can be extracted; The gray dots represent nonexistence, while the other colored dots represent the existence of unique compounds; If more than one method had the same unique compounds, they were connected by lines; The number of the unique compounds owned individually or jointly was represented by black bars. (B) was the Venn about four kinds of different extraction methods for odorants in clam soup. (C) was the Venn about three methods evolved from SAFE for odorants in clam soup. SAFE, solvent-assisted flavor evaporation; L-L, liquid-liquid extraction; P&T, purge and trap; SDE, Simultaneous distillation extraction; SPME, solid-phase micro-extraction.

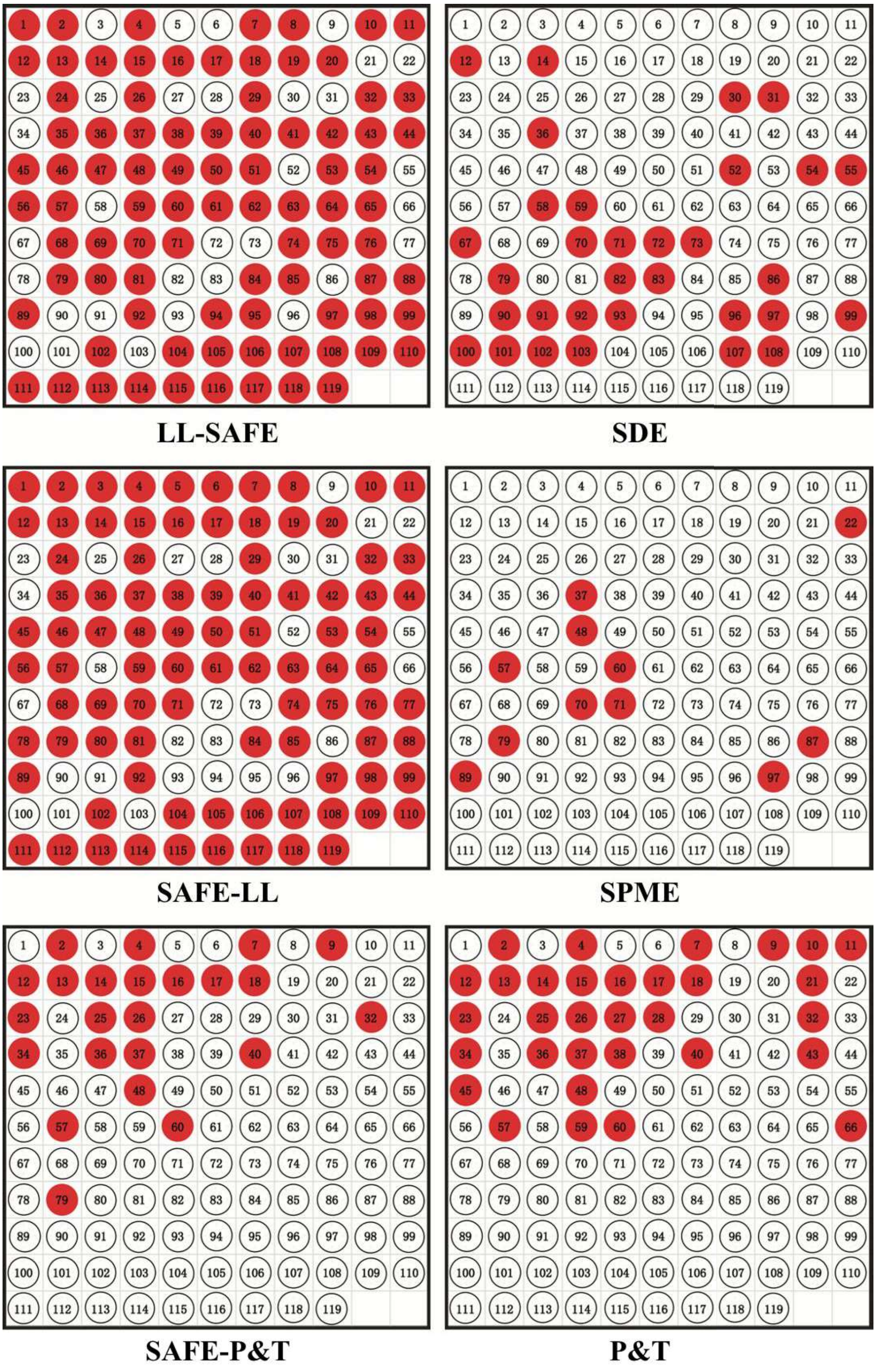
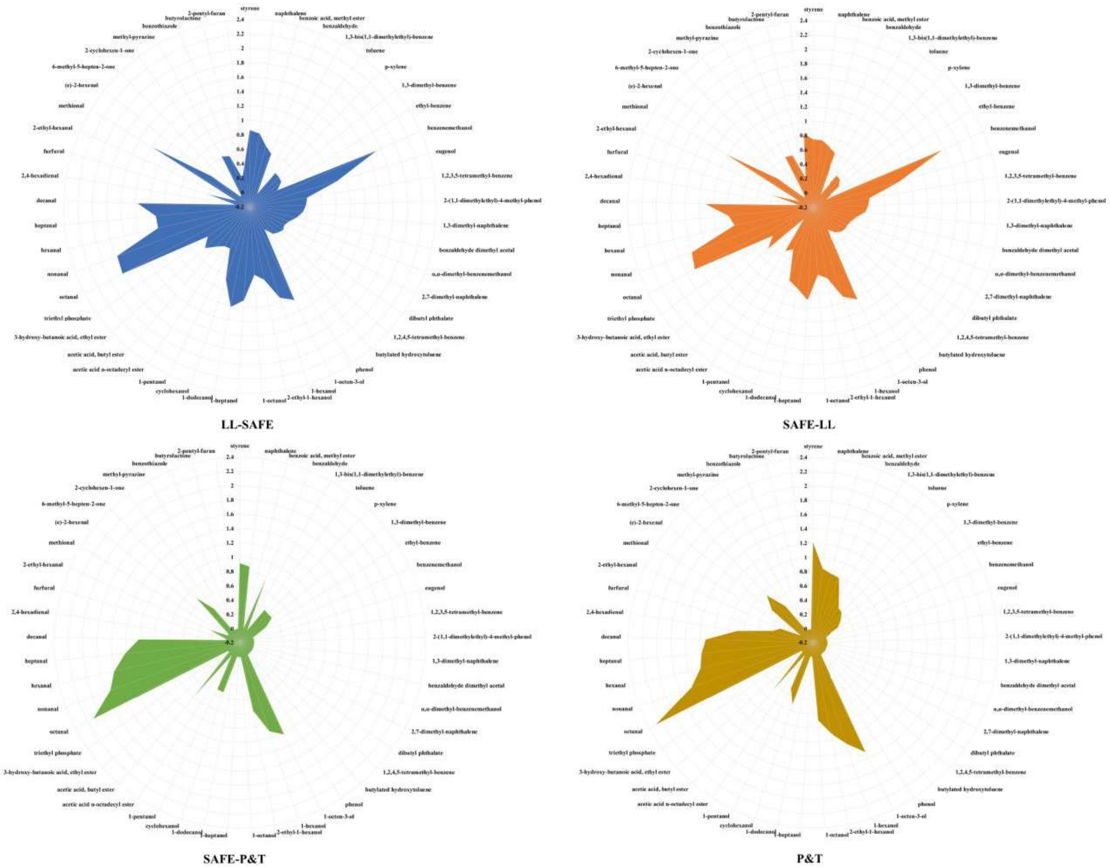


Figure 2 Information of odorants in clam soup obtained by different extraction methods. Each dot represents a compound. Red dots indicate that the compound can be extracted, while white dots indicate that it cannot be extracted. Compounds are arranged in order of RI from smallest to largest. Details of the compounds were shown in Table S6.



LL-SAFE SAFE-LL

SAFE-P&T P&T

Figure 3 Aroma profile obtained via different extraction methods.

The size of the coordinate axis is normalized by the OAV. The OAV of each compound was calculated with the odor threshold value obtained via gradient dilution and the quantification via different extraction methods. The quantification results of different extraction methods were shown in Figure S7.

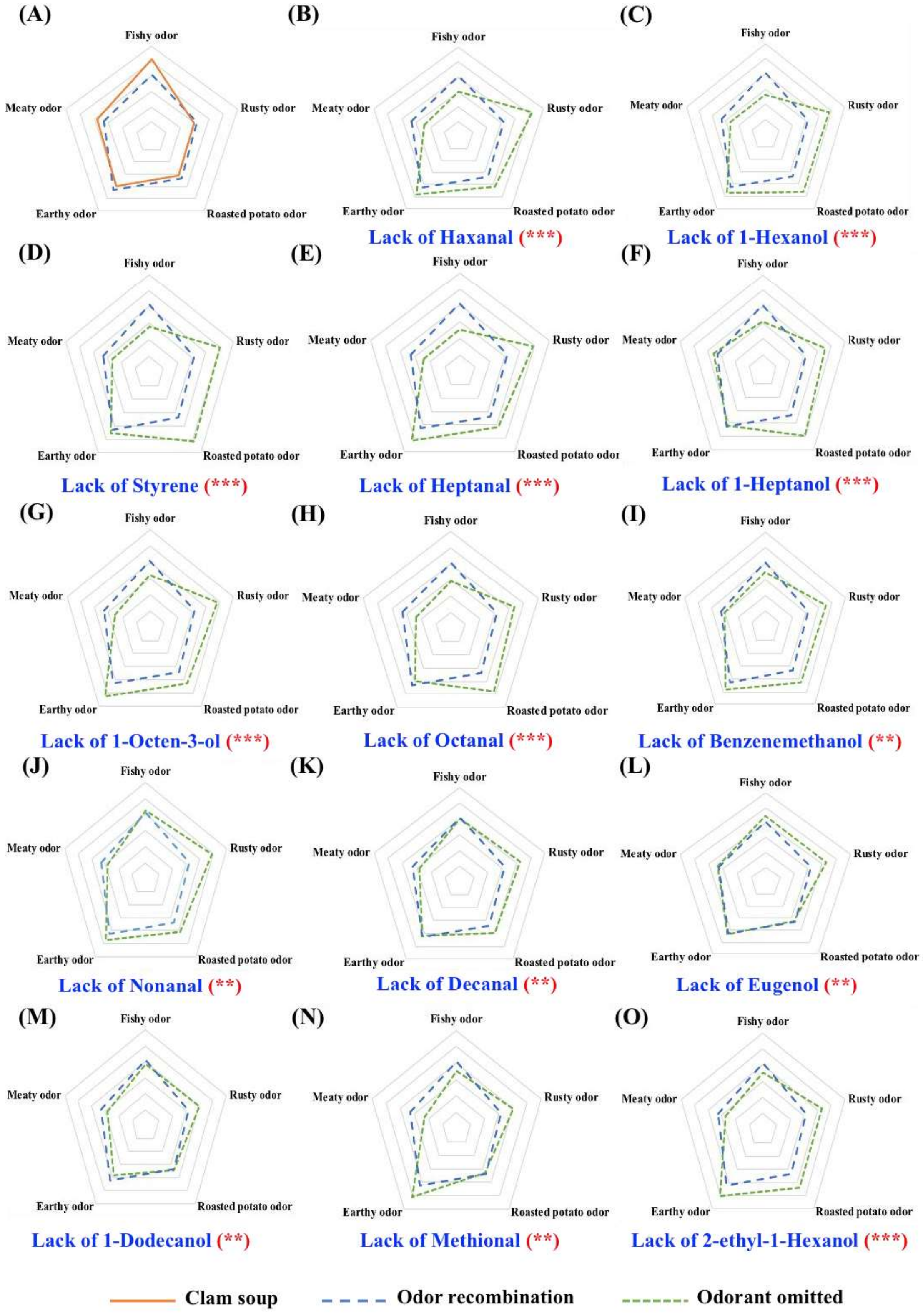


Figure 4 radar chart of e-Nose response and sensory score.

(A) was the aroma comparison of clam soup and odor recombination solution; (B)-(O) were the aroma change of odor recombination solution lacked different key odor compound. ** indicated a significant difference ($p \leq 0.05$); *** indicated a highly significant difference ($p \leq 0.01$).

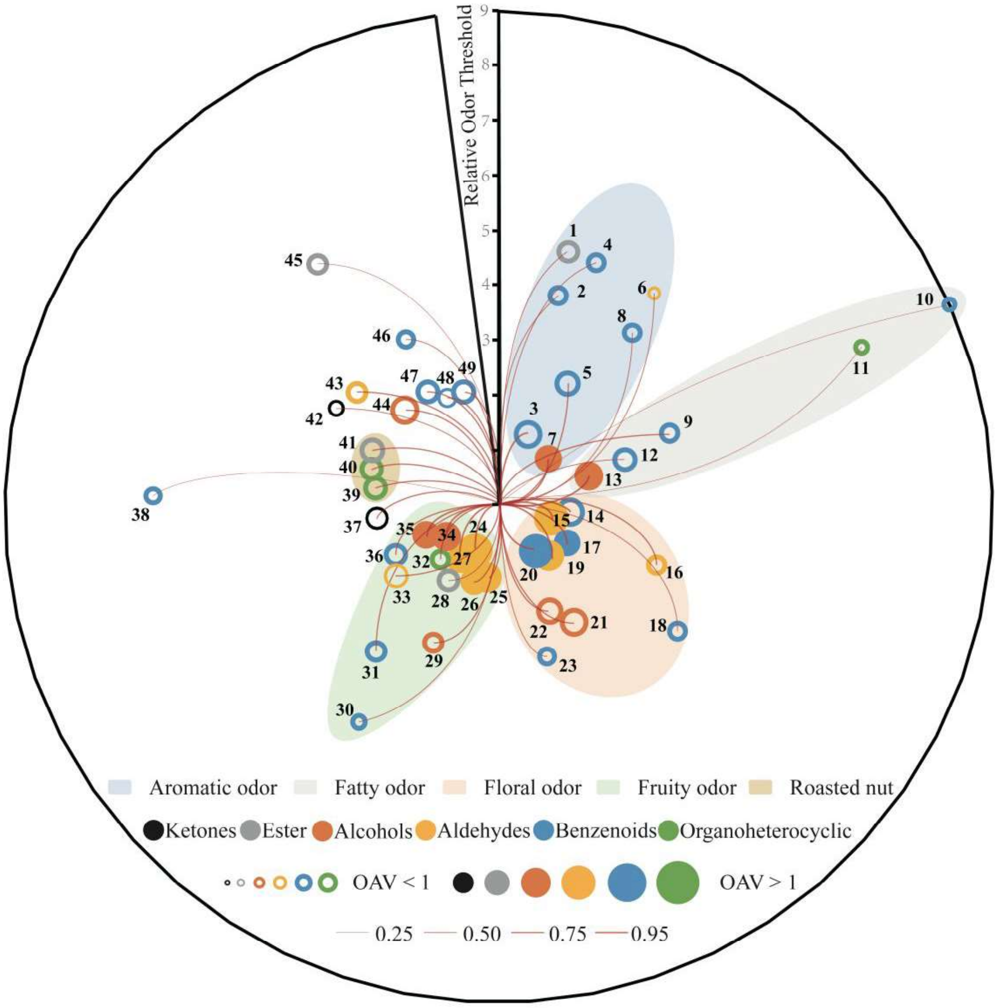


Figure 5 Neural network diagram of the aroma profile.

The size of the coordinate axis is normalized by the odor threshold. The red lines representing nerves were divided into four levels. The larger the odor threshold is, the less thickness it is. The dots were represented odorants. Arabic numerals were the No. in Table 1. The size of the dots was plotted according to the normalized OAV. Dots with OAV > 1 were solid, while with OAV < 1 was hollow. Different colors of dots indicated different types of compounds. Five color areas represented the five main aroma categories. The dots that fall in these color areas represent the contribution of the corresponding compounds to the aroma.

Multi-Extraction Combined with GC-MS(O) and Aroma Recombination for Aroma Profile and Aroma Visualization of Clam Soup

Sensory assessment

The 32 sensory evaluation panelists were selected from 80 members of the laboratory (students and teachers) via sensitivity test of odor and taste (zhao, Deng, & Liu, 2015), include basic flavor test, olfactory match test, three-point test, and ranking order test. Sensory evaluation was performed with clam soup and odorants solution. The odor and taste were evaluated by Quantitative Descriptive Analysis (QDA) method (Silva, Estévez, Ferreira, Silva, Lemos, Ida, et al., 2018). The development of descriptive terminology, final selection of judges and final evaluation of the samples were done before evaluation. At the end, 13 assessors (age ranging from 23 to 35) from both genders were selected. The amplitude of attributes was rated in four kinds of non-structured linear scales according to different factors contributed to the final production, with terms of descriptor anchored at the extremes. Evaluations took place in individual booths under white fluorescence light. The samples were labeled with three-digit random numbers, presented in a monadic sequential way in a balanced complete block design. Two samples were presented to the panelists in each session, with the serving order of the samples randomized according to the Latin Square design.

Table S1 Questionnaire

Name：	Sex：male and female	Age range：23-35	
Question		Tick the choice	
Whether sensory analysis is necessary?		Yes ☐ (80)	No ☐ (0)
Are you interested in sensory analysis?		Yes ☐ (52)	No ☐ (28)
Experience in sensory analysis?		Yes ☐ (48)	No ☐ (32)
Are there any foods you don't eat?		Yes ☐ (31)	No ☐ (49)
Are you familiar with sensory evaluation methods？		Yes ☐ (48)	No ☐ (32)
Have you had any allergies？		Yes ☐ (80)	No ☐ (0)
Whether there is any nasal disease？		No nasal disease (68) Have nasal disease (12)	
Food preferences (sour, sweet, bitter, spicy, etc.)		Sweet (45), Spicy (35)	
Favorite and least favorite foods		Favorite foods (apple, chocolate, strawberry, biscuits, cake, cherry, etc.) Dislike foods (coriander, garlic, onion, preserved egg, durian, Spirrali piain, etc)	
Describe your favorite foods. (at least 3 feature)		sweet, succulent, fragrant, savoury, tasty, etc.	
The appropriate words to describe the flavor of carp meat (at least 2)		umami, fishy, meaty, earthy, fatty, etc.	
Which odor words are associated with seafood? (at least 2)		umami, fishy, meaty	
Which odor are associated with “fresh” and “clean”? (at least 2)		umami, meaty	
Describe the flavor and texture of apples. (at least 3)		sweet, sour, crisp, hard	
Qualification evaluation results			

Table S2-1 Preparation of substance for the paired comparison test

	Descriptor	Compounds	Concentration
Taste	Sweet	sugar	16 g/L
	Sour	citric acid	1 g/L
	Bitter	caffeine	0.5 g/L
	Salty	sodium chloride	5 g/L
	Astringent	aluminium potassium sulfate	0.5 g/L
	Metallic	ferrous sulfate	0.01 g/L
Odor	Citric Odor	citral	0.001M/L
	Vanilla Odor	vanillina	0.001M/L
	Thymic Odor	thymol	0.001M/L
	Jasmine Odor	benzyl acetate	0.001M/L

Table S2-2 Answer sheet of the paired comparison test

No.: Name: Date:

	Taste						Odor			
References	187	265	557	147	248	352	631	982	741	659
Samples										
Descriptor										

Note： Remember the characteristics of references firstly, and then matching the samples to the references. Write down the descriptor.

Table S2-3 Results of the paired comparison test

The case of correctly	Number of panelists	
	Taste	Odor
Absolutely right	42	38
accuracy rate between 90%-100%	9	11
accuracy rate between 80%-90%	12	8
accuracy rate between 70%-80%	5	7
accuracy rate between 60%-70%	9	11
Accuracy rate less 60%	3	5

Table S3-1 Preparation of substance for the triangle test

compounds	Concentration
citric acid	1 g/L
sugar	16 g/L
benzyl acetate	0.001M/L

Table S3-2 Answer sheet of the triangle test

No.:	Name:		Date:
	1	2	3
Sample	768 □	126 □	213 □
	328 □	356 □	653 □
	578 □	986 □	553 □

Note: Feel samples successively according to the serial number. Two samples are the same and one is different. Select different samples and mark “×” in the corresponding box and descript the sample.

Table S3-3 Results of the triangle test

The case of correctly	Number of panelists
Absolutely right	72
accuracy rate between 90%-100%	8
accuracy rate between 80%-90%	0
accuracy rate between 70%-80%	0
accuracy rate between 60%-70%	0
Accuracy rate less 60%	0

Table S4-1 Preparation of substance for the ranking test

	Compounds	Concentration
Taste	citric acid (g/L)	0.1, 0.15, 0.22, 0.34
Odor	benzyl acetate (mg/kg)	5, 10, 20, 40

Table S4-2 Answer sheet of the ranking test

No.: Name: Date:

	compounds	Sequence (from weak to strong)
Taste	citric acid	
Odor	benzyl acetate	

Table S4-3 Results of the ranking test

The case of correctly	Number of panelists	
	Taste	Odor
Absolutely right	35	38
accuracy rate between 90%-100%	13	7
accuracy rate between 80%-90%	14	11
accuracy rate between 70%-80%	9	12
accuracy rate between 60%-70%	5	6
Accuracy rate less 60%	4	6

Table S5-1 Preparation of substance for the descriptive ability test

Compounds	Descriptor
benzaldehyde	bitter almonds, cherries
1-Octen-3-ol	mushroom
ionone	Violets, raspberries
menthol	mint
vanillina	vanilla
benzyl acetate	jasmine

Table S5-2 Answer sheet of the descriptive ability test

No.： Name: Date:

Compounds	Odor
A	
B	
C	
D	
E	
F	

Table S5-3 Results of the triangle test

The case of correctly	Number of panelists
Absolutely right	54
accuracy rate between 90%-100%	10
accuracy rate between 80%-90%	11
accuracy rate between 70%-80%	5
accuracy rate between 60%-70%	0
Accuracy rate less 60%	0

Odorants information

Table S6 Results of volatile compounds in clam soup identified by different extraction methods.

No.	compounds	RI	Identification	SPME	SDE	LL-SAFE	SAFE-LL	SAFE-P&T	P&T
1	2H-Pyran, 3,4-dihydro-	716	MS,RI.			+	+		
2	Toluene	754	MS,RI,Std.			+	+	+	+
3	1-Pentene, 3-methyl-	758	MS,RI.				+		
4	1-Pentanol (CAS)	771	MS,RI,Std.			+	+	+	+
5	3-Hexanone	783	MS,RI.				+		
6	2-Hexanone	789	MS,RI.				+		
7	Hexanal	802	MS,RIStd.			+	+	+	+
8	Pyrazine, methyl-	822	MS,RI,Std.			+	+		
9	Acetic acid, butyl ester	824	MS,RI,Std.					+	+
10	Furfural	834	MS,RI,Std.			+	+		+
11	2-Hexenal, (E)-	854	MS,RI,Std.			+	+		+
12	Benzene, ethyl-	860	MS,RI,Std.		+	+	+	+	+
13	Benzene, 1,3-dimethyl-	862	MS,RI,Std.		+	+	+	+	+
14	p-Xylene	868	MS,RI,Std.		+	+	+	+	+
15	1-Hexanol (CAS)	871	MS,RI,Std.			+	+	+	+
16	Cyclohexanol	883	MS,RI,Std.			+	+	+	+
17	Styrene	890	MS,RI,Std.			+	+	+	+
18	Heptanal	903	MS,RI,Std.			+	+	+	+
19	Methional	907	MS,RI,Std.			+	+		
20	Butyrolactone	913	MS,RI,Std.			+	+		
21	2,4-Hexadienal	914	MS,RI,Std.						+

No.	compounds	RI	Identification	SPME	SDE	LL-SAFE	SAFE-LL	SAFE-P&T	P&T
22	Oxime-, methoxy-phenyl-_	928	MS,RI.	+					
23	2-Cyclohexen-1-one	934	MS,RI,Std.					+	+
24	Butanoic acid, 3-hydroxy-, ethyl ester	936	MS,RI,Std.			+	+		
25	Hexanal, 2-ethyl-	958	MS,RI,Std.					+	+
26	Benzaldehyde	959	MS,RI,Std.			+	+	+	+
27	Nonane, 4-methyl-	964	MS,RI.						+
28	Nonane, 2-methyl-	967	MS,RI.						+
29	1-Heptanol	971	MS,RI,Std.			+	+		
30	Octane, 3-ethyl-	974	MS,RI.		+				
31	Benzene, 1,2,4-trimethyl-	976	MS,RI.		+				
32	1-Octen-3-ol	982	MS,RI,Std.			+	+	+	+
33	Phenol	983	MS,RI,Std.			+	+		
34	5-Hepten-2-one, 6-methyl-	990	MS,RI,Std.					+	+
35	Furan, 2-pentyl-	994	MS,RI,Std.			+	+		
36	Decane	1000	MS,RI.		+	+	+	+	+
37	Octanal	1005	MS,RI,Std.	+		+	+	+	+
38	Nonane, 2,5-dimethyl-	1015	MS,RI.			+	+		+
39	Nonane, 2,6-dimethyl-	1023	MS,RI.			+	+		
40	1-Hexanol, 2-ethyl-	1030	MS,RI,Std.			+	+	+	+
41	Benzenemethanol	1033	MS,RI,Std.			+	+		
42	Decane, 4-methyl-	1059	MS,RI.			+	+		
43	1-Octanol	1071	MS,RI,Std.			+	+		+
44	Benzenemethanol, .alpha.,.alpha.-dimethyl-	1085	MS,RI,Std.			+	+		

No.	compounds	RI	Identification	SPME	SDE	LL-SAFE	SAFE-LL	SAFE-P&T	P&T
45	Benzoic acid, methyl ester	1095	MS,RI,Std.			+	+		+
46	Undecane	1100	MS,RI.			+	+		
47	Decane, 2,4-dimethyl-	1103	MS,RI.			+	+		
48	Nonanal	1105	MS,RI,Std.	+		+	+	+	+
49	Benzaldehyde dimethyl acetal	1111	MS,RI,Std.			+	+		
50	Benzene, 1,2,3,5-tetramethyl-	1115	MS,RI,Std.			+	+		
51	Benzene, 1,2,4,5-tetramethyl-	1120	MS,RI,Std.			+	+		
52	Benzene, 1-ethyl-3,5-dimethyl-	1123	MS,RI.		+				
53	triethyl phosphate	1126	MS,RI,Std.			+	+		
54	Benzene, 1,2,3,4-tetramethyl-	1152	MS,RI.		+	+	+		
55	Benzene, 4-ethyl-1,2-dimethyl-	1160	MS,RI.		+				
56	Undecane, 2-methyl-	1165	MS,RI.			+	+		
57	Naphthalene	1183	MS,RI,Std.	+		+	+	+	+
58	3-Dodecene, (Z)-	1193	MS,RI.		+				
59	Dodecane	1200	MS,RI.		+	+	+		+
60	Decanal	1206	MS,RI,Std.	+		+	+	+	+
61	Undecane, 2,4-dimethyl-	1211	MS,RI.			+	+		
62	Undecane, 2,6-dimethyl-	1215	MS,RI.			+	+		
63	Benzothiazole	1223	MS,RI,Std.			+	+		
64	Undecane, 4-ethyl-	1245	MS,RI.			+	+		
65	Dodecane, 5-methyl-	1251	MS,RI.			+	+		
66	Benzene, 1,3-bis(1,1-dimethylethyl)-	1256	MS,RI,Std.						+
67	Dodecane, 2,6,11-trimethyl-	1257	MS,RI.		+				
68	Dodecane, 4-methyl-	1260	MS,RI.			+	+		

No.	compounds	RI	Identification	SPME	SDE	LL-SAFE	SAFE-LL	SAFE-P&T	P&T
69	Dodecane, 2-methyl-	1265	MS,RI.			+	+		
70	Dodecane, 2,6,11-trimethyl-	1281	MS,RI.	+	+	+	+		
71	Tridecane	1300	MS,RI.	+	+	+	+		
72	Naphthalene, 1-methyl-	1313	MS,RI.		+				
73	Naphthalene, 2-methyl-	1332	MS,RI.		+				
74	Dodecane, 4,6-methyl-	1327	MS,RI.			+	+		
75	Phenol, 2-(1,1-dimethylethyl)-4-methyl-	1354	MS,RI,Std.			+	+		
76	Eugenol	1359	MS,RI,Std.			+	+		
77	Tridecane, 2-methyl-	1364	MS,RI.				+		
78	Tridecane, 3-methyl-	1371	MS,RI.				+		
79	Tetradecane	1400	MS,RI.	+	+	+	+	+	
80	Naphthalene, 2,7-dimethyl-	1404	MS,RI,Std.			+	+		
81	Naphthalene, 1,3-dimethyl-	1420	MS,RI,Std.			+	+		
82	Naphthalene, 2,6-dimethyl-	1427	MS,RI.		+				
83	Naphthalene, 1,6-dimethyl-	1444	MS,RI.		+				
84	Tetradecane, 4,11-dimethyl-	1463	MS,RI.			+	+		
85	1-Dodecanol	1474	MS,RI,Std.			+	+		
86	Naphthalene, 1,5-dimethyl-	1484	MS,RI.		+				
87	Pentadecane	1500	MS,RI.	+		+	+		
88	Phenol, 2,5-bis(1,1-dimethylethyl)-	1513	MS,RI.			+	+		
89	Butylated Hydroxytoluene	1516	MS,RI,Std.	+		+	+		
90	Phenol,2,4-bis(1,1-imethylethyl)-	1523	MS,RI.		+				
91	Phenol, 3,5-bis(1,1-imethylethyl)-	1526	MS,RI.		+				

No.	compounds	RI	Identification	SPME	SDE	LL-SAFE	SAFE-LL	SAFE-P&T	P&T
92	Tetradecane, 2,6,10-trimethyl-	1533	MS,RI.		+	+	+		
93	Naphthalene, 1,6,7-trimethyl-	1558	MS,RI.		+				
94	Pentadecane, 2-methyl-	1564	MS,RI.			+			
95	Pentadecane, 3-methyl-	1571	MS,RI.			+			
96	Naphthalene, 1,4,5-trimethyl-	1575	MS,RI.		+				
97	Hexadecane	1600	MS,RI.	+	+	+	+		
98	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis-	1608	MS,RI.			+	+		
99	Heptadecane	1700	MS,RI.		+	+	+		
100	Heptadecane, 2-methyl-	1714	MS,RI.		+				
101	Hexadecane, 2,6,10,14-tetramethyl-	1758	MS,RI.		+				
102	Octadecane	1800	MS,RI.		+	+	+		
103	Octadecane, 2-methyl-	1822	MS,RI.		+				
104	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	1871	MS,RI.			+	+		
105	Nonadecane	1900	MS,RI.			+	+		
106	Dibutyl phthalate	1965	MS,RI,Std.			+	+		
107	Eicosane	2000	MS,RI.		+	+	+		
108	Heneicosane	2100	MS,RI.		+	+	+		
109	Docosane	2200	MS,RI.			+	+		
110	Acetic acid n-octadecyl ester	2210	MS,RI,Std.			+	+		
111	Tricosane	2300	MS,RI.			+	+		

No.	compounds	RI	Identification	SPME	SDE	LL-SAFE	SAFE-LL	SAFE-P&T	P&T
112	Hexanedioic acid, bis(2-ethylhexyl) ester	2302	MS,RI,Std.			+	+		
113	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	2328	MS,RI,Std.			+	+		
114	Pentacosane	2500	MS,RI.			+	+		
115	Hexacosane	2600	MS,RI.			+	+		
116	Heptacosane	2700	MS,RI.			+	+		
117	Octacosane	2800	MS,RI.			+	+		
118	Nonacocane	2900	MS,RI			+	+		
119	Triacontane	3000	MS,RI.			+	+		

MS, identified by mass spectral data.

RI, identified by retention indices.

Std., identified by standards.

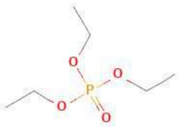
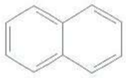
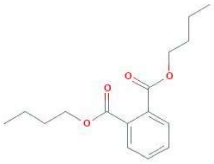
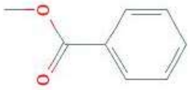
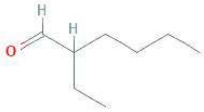
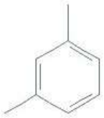
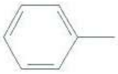
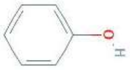
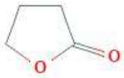
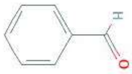
Table S7 Quantification of 49 volatile compounds in clam soup via different extraction methods.

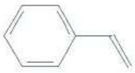
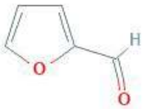
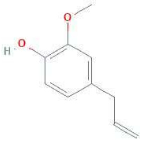
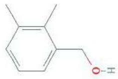
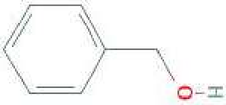
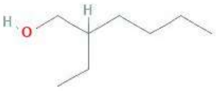
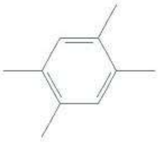
No.	Compounds	LL-SAFE (ng/mL)	SAFE-LL (ng/mL)	SAFE-P&T (ng/mL)	P&T (ng/mL)
1	triethyl phosphate	91.05±1.85	70.13±1.66	n/a	n/a
2	p-xylene	7.65±0.99	4.26±0.95	6.37±0.89	8.29±0.35
3	naphthalene	2.31±0.92	1.34±0.22	3.08±0.08	2.64±0.61
4	dibutyl phthalate	15.97±0.30	10.81±0.84	n/a	n/a
5	benzoic acid, methyl ester	15.28±0.68	14.98±0.83	n/a	36.28±1.23
6	2-ethyl-hexanal	n/a	n/a	2.75±0.41	5.71±0.19
7	1-heptanol	4.83±0.27	4.66±0.55	n/a	n/a
8	1,3-dimethyl-benzene	5.63±0.41	1.19±0.06	6.95±0.18	2.48±0.64
9	toluene	4.51±0.79	2.58±0.14	4.16±0.30	5.65±0.07
10	phenol	7.84±0.12	7.31±0.23	n/a	n/a
11	butyrolactone	9.21±0.74	10.73±0.22	n/a	n/a
12	benzaldehyde	7.34±0.58	8.22±0.43	25.63±1.20	28.74±1.57
13	1-dodecanol	41.79±1.97	12.63±0.86	n/a	n/a
14	styrene	1.74±0.09	0.82±0.28	2.32±0.63	9.30±0.41
15	nonanal	19.19±0.94	11.84±0.49	16.85±0.25	12.23±0.68
16	furfural	2.71±0.46	2.85±0.16	n/a	2.59±0.33
17	eugenol	8.21±0.39	7.61±0.37	n/a	n/a
18	dimethyl-benzenemethanol	8.03±0.18	6.57±0.49	n/a	n/a
19	decanal	14.38±0.44	11.33±0.32	7.73±0.87	11.46±0.97
20	benzenemethanol	18.19±1.25	20.30±0.86	n/a	n/a
21	2-ethyl-1-hexanol	38.97±1.06	33.72±0.76	27.73±1.24	156.42±2.77
22	1-octanol	9.65±0.80	9.37±0.86	n/a	23.94±1.07
23	1,2,4,5-tetramethyl-benzene	0.75±0.03	0.60±0.05	n/a	n/a

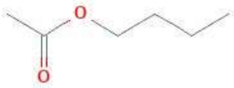
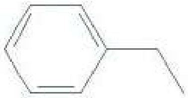
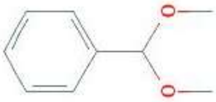
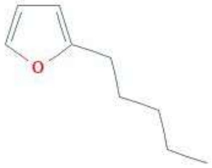
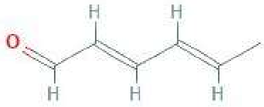
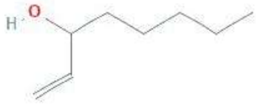
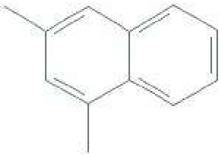
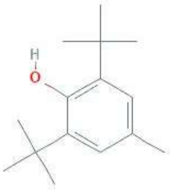
No.	Compounds	LL-SAFE (ng/mL)	SAFE-LL (ng/mL)	SAFE-P&T (ng/mL)	P&T (ng/mL)
24	octanal	11.11±0.66	7.61±0.65	24.56±1.00	36.89±0.98
25	methional	20.46±1.17	10.61±0.44	n/a	n/a
26	hexanal	8.49±0.30	3.46±0.80	52.50±1.15	29.16±0.79
27	heptanal	4.86±0.72	2.70±0.31	15.61±0.77	13.65±0.80
28	acetic acid, butyl ester	0.39±0.39	n/a	2.68±0.15	1.16±0.12
29	1-pentanol	2.48±0.45	2.61±0.31	5.68±0.24	5.11±0.81
30	ethyl-benzene	1.85±0.11	n/a	1.10±0.64	0.90±0.68
31	benzaldehyde dimethyl acetal	10.86±0.32	6.51±0.71	n/a	n/a
32	2-pentyl-furan	0.05±0.01	2.28±0.02	n/a	n/a
33	2,4-hexadienal	n/a	n/a	n/a	31.14±1.44
34	1-octen-3-ol	4.22±0.20	4.17±0.57	4.18±0.57	11.55±0.33
35	1-hexanol	10.08±0.60	9.72±0.28	9.46±0.74	20.18±1.12
36	1,3-dimethyl-naphthalene	1.97±0.09	1.79±0.10	n/a	n/a
37	6-methyl-5-hepten-2-one	n/a	n/a	6.44±0.61	10.35±0.62
38	butylated hydroxytoluene	19.87±1.19	12.01±0.51	n/a	n/a
39	methyl-pyrazine	4.61±0.32	4.72±0.18	n/a	n/a
40	benzothiazole	4.33±0.81	5.37±0.16	n/a	n/a
41	3-hydroxy-butanoic acid, ethyl ester	11.29±0.82	11.81±0.14	n/a	n/a
42	2-cyclohexen-1-one	n/a	n/a	4.73±0.10	8.69±0.18
43	(e)-2-hexenal	9.74±0.86	6.03±0.58	n/a	17.80±0.27
44	cyclohexanol	41.79±2.30	44.36±1.72	3.78±0.73	16.64±0.47
45	acetic acid n-octadecyl ester	94.43±4.23	184.89±2.47	n/a	n/a

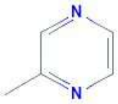
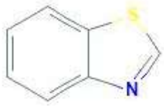
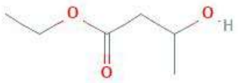
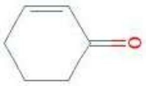
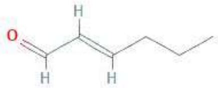
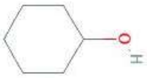
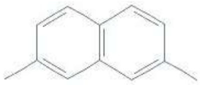
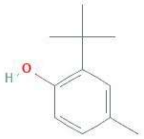
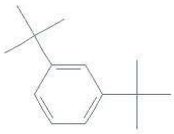
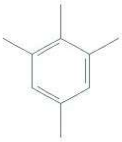
No.	Compounds	LL-SAFE (ng/mL)	SAFE-LL (ng/mL)	SAFE-P&T (ng/mL)	P&T (ng/mL)
46	2,7-dimethyl-naphthalene	2.32±0.06	2.19±0.03	n/a	n/a
47	2-(1,1-dimethylethyl)-4-methyl-phenol	5.87±0.12	5.37±0.85	n/a	n/a
48	1,3-bis(1,1-dimethylethyl)-benzene	n/a	n/a	n/a	2.48±0.03
49	1,2,3,5-tetramethyl-benzene	4.08±0.18	3.82±0.11	n/a	n/a

Table S8 List of 49 volatiles compounds selected.

No.	Key odorants	CAS no.	structure
1	triethyl phosphate	78-40-0	
2	p-xylene	106-42-3	
3	naphthalene	91-20-3	
4	dibutyl phthalate	84-74-2	
5	benzoic acid, methyl ester	93-58-3	
6	2-ethyl-hexanal	123-05-7	
7	1-heptanol	111-70-6	
8	1,3-dimethyl-benzene	108-38-3	
9	toluene	108-88-3	
10	phenol	108-95-2	
11	butyrolactone	96-48-0	
12	benzaldehyde	100-52-7	
13	1-dodecanol	112-53-8	

14	styrene	100-42-5	
15	nonanal	124-19-6	
16	furfural	98-01-1	
17	eugenol	97-53-0	
18	α,α-dimethyl-benzenemethanol	13651-14-4	
19	decanal	112-31-2	
20	benzenemethanol	100-51-6	
21	2-ethyl-1-hexanol	104-76-7	
22	1-octanol	111-87-5	
23	1,2,4,5-tetramethyl-benzene	95-93-2	
24	octanal	124-13-0	
25	methional	3268-49-3	
26	hexanal	66-25-1	
27	heptanal	111-71-7	

28	acetic acid, butyl ester	123-86-4	
29	1-pentanol	71-41-0	
30	ethyl-benzene	100-41-4	
31	benzaldehyde dimethyl acetal	1125-88-8	
32	2-pentyl-furan	3777-69-3	
33	2,4-hexadienal	142-83-6	
34	1-octen-3-ol	3391-86-4	
35	1-hexanol	111-27-3	
36	1,3-dimethyl-naphthalene	575-41-7	
37	6-methyl-5-hepten-2-one	110-93-0	
38	butylated hydroxytoluene	128-37-0	

39	methyl-pyrazine	109-08-0	
40	benzothiazole	95-16-9	
41	3-hydroxy-butanoic acid, ethyl ester	5405-41-4	
42	2-cyclohexen-1-one	930-68-7	
43	(E)-2-hexenal	6728-26-3	
44	cyclohexanol	108-93-0	
45	acetic acid n-octadecyl ester	822-23-1	
46	2,7-dimethyl-naphthalene	582-16-1	
47	2-(1,1-dimethylethyl)-4-methyl-phenol	2409-55-4	
48	1,3-bis(1,1-dimethylethyl)-benzene	1014-60-4	
49	1,2,3,5-tetramethyl-benzene	527-53-7	

Reference

Silva, F. A., Estévez, M., Ferreira, V. C., Silva, S. A., Lemos, L. T., Ida, E. I., Shimokomaki, M., & Madruga, M. S. (2018). Protein and lipid oxidations in jerky chicken and consequences on sensory quality. *LWT, 97*, 341-348.

zhao, L., Deng, X., & Liu, W. (2015). *Food sensory analysis vocabulary*: China Light Industry Press.