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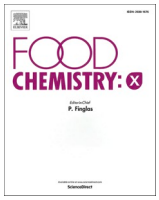

Analysis of odor compounds in Lee Kum Kee brand oyster sauce and oyster enzymatic hydrolysate: Comparison and relationship

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ABSTRACT

Oyster sauce (OS) is a highly processed oyster product. However, the significant price difference between OS and fresh oysters raises a question: Does authentic OS truly contain components from oysters or oyster enzymatic hydrolysates (OEH)? Therefore, the odor compounds of Lee Kum Kee oyster sauce (LKK), 4 OEHs, and 6 other seafood enzymatic hydrolysates (SEHs) were analyzed by using solid-phase microextraction and gas chromatography–olfactometry-mass spectrometry technology (SPME-GC-O-MS). The results of multivariate statistical analysis demonstrated the effective discrimination between LKK and OEHs from other SEHs. According to the VIP value and the differences in the composition of odor compounds among different samples, 15 essential odor compounds were screened out, which could distinguish whether the samples contained OEHs. Among them, acetic acid, 2-pentylfuran, 2-ethyl furan, 2-methylbutanal, and nonanal were only detected in LKK and OEHs, which further indicated the existence of OEH in LKK.

1. Introduction

Oyster sauce (OS) is composed of oyster enzymatic hydrolysis products as the core component, supplemented by sugar, salt, modified starch, and thickening agents. It has a bright color, delicious taste, rich nutrition, and smooth texture.

OS is especially popular with consumers in the southern provinces of China (such as Guangdong, Fujian, Hong Kong, Macao and Taiwan) and Southeast Asia (Nguyen & Wang, 2012). It has also become increasingly popular in the inland areas of China. The quality of the OS plays a crucial role in determining consumer acceptance. Although the ingredient lists of various OS brands in the market indicate that they all use oyster enzymatic hydrolysates (OEH) as raw materials, the prices of the oysters used for producing OEH and OSmay vary. Recently, consumers have become increasingly concerned about food safety due to health risks and economic losses from food adulteration. This has led to apprehension about the authenticity of OS, with many questioning whether it actually contains oysters or OEH (Esteki, Regueiro, & Simal-Gandara, 2019; Haji, Desalegn, Hassen, 2023).

Currently, there has been a growing interest in researching the OS flavors. Wang et al. (2020) found that the used of improved OEH processing technology can significantly increase the content of odor compounds in concentrated OS, especially aldehydes, thereby improving its flavor performance. This makes it the best processing technology for concentrated OS. Nguyen et al. (2012) conducted a study on 4 different commercial brands of OS and identified 75 volatile compounds, among which alcohols, furans, aldehydes, and pyrazines were the main chemical categories. The study found that the sensory classification of the 4 commercial brands of OS was precise and the difference between them was significant. In addition, changes in volatile substances that occur after the enzymatic hydrolysis of oysters have also caught the attention of many researchers. For example, Liu et al. (2010) used SPME-GC–MS to identify 62 and 60 components from OEH produced by neutral protease and papain, respectively. These components mainly including hydrocarbons, alcohols, aldehydes, ketones and sulfur-containing compounds. Yao et al. (2020) discovered that after 4 h of enzymatic hydrolysis, OEH contained the highest number of odor compounds (60), and all 4 types of OEH contained 24 volatile flavor components. Among these, aldehydes (10) emerged as the most predominant odor compounds. Su et al. (2020) utilized gas chromatography-mass spectrometry to identify 42 volatile substances in oysters and 41 in their enzymatic hydrolysates. These substances

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included aldehydes, ketones, alcohols, esters, hydrocarbons, and more. The study found that oysters exhibited mainly fruity and grassy notes before enzymatic hydrolysis, whereas post-enzymatic hydrolysis resulted in strong fishy and fragrant characteristics. However, there is limited research exploring the correlation between OS and OEH.

Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) are common multivariate analysis methods frequently employed in food research. These techniques are utilized to handle intricate omics data, enabling the analysis of distinctions and relationships between samples. Hou et al. (2016) demonstrated that the PLS-DA method can establish quality control and authenticity discrimination rules for flavoring essence. It has the capability to predict the "attribution" of new samples and identify primary ion markers, which is essential for the identification and monitoring of flavor quality. Liu et al. (2018) swiftly identified the varieties and qualities of the three prominent hops through volatile fingerprinting and multivariate statistical analysis. He et al. (2020) used PLS to study the relationship between potential aroma compounds and sensory properties in strong-aroma-type Baijiu. The study found that samples from Sichuan (China) contained higher levels of pyrazines, furans, and carbonyl compounds, which contributed to the higher intensity of wine cellar, bakery, and grain aromas. However, the higher ester and alcohol contents in samples from the Jianghuai region of China were mainly responsible for the fruity and floral aromas. Muñoz and his coworkers (2020) applied multivariate analysis to distinguish phenolic characteristics and sensory properties of green and roasted coffee brews. Green coffee brews typically exhibit floral and light flavors, whereas roasted coffee brews feature sensory characteristics of chocolate and caramel, as well as creamy and intense flavors.

In this study, Lee Kum Kee Brand Oyster Sauce (LKK), the best-selling product in the Chinese market, was selected as the research subject, and common seafood enzymatic hydrolysate (SEH) was used as the control. GC-O-MS combined with sensory evaluation was employed to detect the odor compounds present in LKK, the 4 types of OEH used in LKK processing, and 6 SEHs. By utilizing the advantages of this combination strategy, it is possible to examine and analyze the odor compounds, sensory components, as well as the differences between LKK, OEHs, and SEHs. The research findings are expected to provide potential for identifying indicator compounds for detecting and preventing OS adulteration.

2. Materials and methods

2.1. Materials

This experiment uses 11 samples, including OS (1), OEHs (4) and SEHs (6). Among them, LKK, the raw material for Fish Enzyme Hydrolysate (FEH) from mackerel and the raw material for SEH from hairy shrimp are purchased from Beijing YongHui Supermarket. Oyster enzymatic hydrolysates (1#, 2#, 3# and 4# oyster enzymatic hydrolysates (OEHs)), scallop meat enzymatic hydrolysates (SMEH), crab meat enzymatic hydrolysates (CMEH), spend clam meat enzymatic hydrolysates (SCMEH) and kelp enzymatic hydrolysates (KEH) were provided by Guangdong Lee Kum Kee Innovation Technology Co., Ltd. The samples were stored in a 4℃ refrigerator before analysis.

2.2. Chemicals

The chemicals, including 2-methyl-3-heptanone and *n*-alkanes (C_8-C_{26}; C_6-C_{18}), were purchased from Sigma-Aldrich, Inc. (St. Louis, United States). High-purity helium (99.999% purity) and nitrogen (99.99% purity) are produced by Beijing AP BAIF Gases Industry Co., Ltd. (Beijing, China).

2.3. Odor compounds extraction from sample

Solid phase microextraction (SPME) was used to extract odor compounds from different samples. The sample (3.0 g) was accurately weighed with a balance and put into a 40 mL headspace bottle. The 2-methyl-3-heptanone (1μL) was added to the headspace bottle as the internal standard (the concentration was 0.816 g/mL), and then the sample was placed in a constant temperature water bath at 55°C for equilibrium for 20 min. A solid-phase microextraction sampler with DVB/CAR/PDMS coating was then used to adsorb the volatiles in the top space of the headspace bottles at 55°C for 40 min. The sampler was then inserted into the GC injector for thermal desorption at 250°C for 5 min. The analysis was repeated three times for each sample.

2.4. Gas chromatography–olfactometry-mass spectrometry (GC-O-MS)

The samples were identified by GC-O-MS. The odor compounds were separated on DB-WAX and DB-5ms columns, and the two columns were verified with each other. Ultra-high purity helium gas (purity = 99.999%) was used as a carrier gas, the constant flow rate was 1.2 mL/min, and the split ratio was set at 5:1. The chromatography parameters involved an initial column temperature of 40°C, maintained for 3 min. Subsequently, the temperature was increased to 200°C at a rate of 5°C/min, followed by a further increase to 230°C at a rate of 10°C/min, held for 3 min, and finally elevated to 250°C at a rate of 10°C/min, with a 3-minute maintenance period. For mass spectrometry, the ion source temperature was set to 230°C, transmission line temperature to 250°C, and quadrupole temperature to 150°C. An electron impact (EI) ion source with an energy of 70 eV was utilized, scanning the mass range (*m/z*) from 50 to 500, with a solvent delay of 4 min. The parameters for the sniffing detector included a sniffing port temperature of 150°C. Nitrogen was employed to humidify the air by blowing it over ultrapure water to mitigate dryness in the nasal cavity of experimental personnel during detection. Three sensory evaluators conducted sniffing tests on the samples, recording the time of aroma appearance, providing detailed descriptions, and noting the intensity of the odor.

2.5. Qualitative analysis

The odor compounds were identified preliminarily by comparing the MS peaks with the fragment pattern of the 2017 edition NIST 14 libraries. The identified compounds were then reconfirmed by linear retention index (RIS) for each compound using a series of *n*-alkanes (C_8-C_{26} for DB-WAX and C_6-C_{18} for DB-5ms) (Matheis & Granvogl, 2016; Ping, Huanlu, Lijin, & Hao, 2019). Furthermore, using human sensitivity to odor compounds, qualitative analysis was carried out by odor description, and verification was carried out by standard compound (STD). Specifically, we enlisted three trained sensory panelists (two males and one female) from Beijing Technology and Business University to perform olfactory assessments under identical experimental conditions to those of the GC–MS. They were tasked with noting the peak time odor intensity and providing descriptions for the detected odor compounds through sniffing. The results are shown in Attached Table 1, where the compounds not identified by the reference compound are "preliminarily identified.".

2.6. Quantitative analysis and odor activity value

Key odor compounds were quantitatively analyzed by GC–MS in ion monitoring (SIM) mode. The compounds being quantitative include acetic acid, propanoic acid, butanoic acid, 2-methyl pyrazine, 2-ethyl-5-methylpyrazine, 2,3,5-trimethyl pyrazine, 2-ethyl-6-methylpyrazine, 2-pentyl furan, 2-ethyl furan, 2-acetyl pyrrole, 3-methyl butanal, 2-methyl butanal, nonanal, furfuryl alcohol, 2-butanone. The corresponding standard of the quantified compound was initially dissolved in dichloromethane and then diluted to 11 different concentrations in a

Table 1
The odour Compounds with VIP value greater than 1 in LKK, OEH and SEH.

No.	compounds	VIP[a]	Perception[b]	RI[c] DB-WAX	identification method[d]
1	Benzyl alcohol	3.99	fruity	1891	RI/MS
2	acetic acid	3.05	sour	1402	RI/MS/O/STD
3	propanoic acid	3.01	–	1550	RI/MS/STD
4	Nonanoic acid	2.25	cheesy	2222	RI/MS
5	2,3,5-Trimethyl-6-ethylpyrazine	1.99	–	1501	RI/MS
6	2-Amylfuran	1.81	green bean	1263	RI/MS/O
7	5-methylfurfural	1.49	–	1577	RI/MS
8	2-propyl-Furan	1.42	green	1024	RI/MS
9	2-acetylfuran	1.37	–	1499	RI/MS/O
10	octanal	1.24	–	1262	RI/MS
11	pentanal	1.23	–	927	RI/MS
12	2,5-dimethyl-3-(3-methylbutyl)-Pyrazine	1.20	–	1205	RI/MS
13	(E)-2-Decenal	1.15	green	1591	RI/MS
14	2,5-dimethylpyrazine	1.14	roasted nut	1331	RI/MS/O
15	2-Acetyl-3-methylpyrazine	1.13	–	1655	RI/MS
16	2,6-diethyl-Pyrazine	1.12	–	1458	RI/MS
17	3-methylbutanal	1.12	nutty	913	RI/MS/STD
18	2,4-dimethyl-Phenol	1.10	–	2116	RI/MS
19	2,3-diethyl-5-methyl-Pyrazine	1.09	–	1566	RI/MS
20	2-acetylpyrrole	1.08	nutty	1244	RI/MS/O/STD
21	Thiazole	1.04	nutty	1254	RI/MS
22	benzaldehyde	1.02	nutty	1483	RI/MS/O

[a] Variable importance of projection. [b]Odor perception sensed at the sniffing port. [b]Retention indices on capillaries DB-WAX and DB-5 ms. [d]Identification methods of each aroma compound. MS, RI, O, and STD represent being identifying by mass spectra, retention indices, olfactometry, and standard agent, respectively.

multiple of 2 successively, with 2-methyl-3-heptanone (0.816 μg/μL) as an internal standard. In addition, according to their concentration range in the sample, they were divided into 3 groups (1–10 μg/μL; 11–100 μg/mL; 0.1–1 mg/mL). The standard curve was prepared by plotting the response ratio of the standard compound and internal standard compounds to their respective concentrations. All analyses were repeated three times.

Odor activity value (OAV) was calculated by the ratio of each odorant concentration to each odorant concentration. Because LKK, OEHs, and SEHs contain a large amount of water, the threshold value of the compound can be found in literature and books (Odor thresholds compilations of odor threshold values in air, water, and other media (second enlarged and revised edition)).

2.7. Statistical analysis

The tables and bar graphs were produced by Microsoft Office Excel 2019 and Origin 2018, respectively. PCA and OPLS-DA were performed using SIMCA-P 14.1 software. All experiments were repeated three times, and the data were expressed as mean ± standard deviation.

3. Results and discussion

3.1. Odor components analysis

The odor compounds in LKK, 4 OEHs, and 6 SEHs were extracted and detected using the SPME method combined with GC-O-MS technology. The composition and content of aroma compounds in the samples are detailed in Table 1.

A total of 64 odor compounds were detected in LKK, comprising 3 esters, 5 acids, 1 hydrocarbon, 21 heterocyclic compounds, 12 aldehydes, 13 alcohols, 7 ketones, and 2 other compounds. In OEH and LKK, heterocyclic compounds are the compounds with the highest content, accounting for 32% and 42% (the average in the sample), followed by alcohols, accounting for 20% and 19% (mean sample value). Compared with OEH, the proportion of heterocyclic compounds in LKK increased, while the proportion of alcohol compounds decreased. It is speculated that this change may be attributed to the inactivation of microorganisms and enzymes during the high-temperature boiling and sterilization processes of OS, leading to a decline in the production of alcohols. In addition, most of the alcohol compounds in fresh oysters evaporate during cooking, and only a few of them, such as benzaldehyde, 1-octene-3-ol, and hexanal, are transferred to OEH (Soares, Vieira, Fidler, Nandi, Monteiro, & Di Luccio, 2020). In the processing of OS, sugar will be added as an auxiliary material to increase the concentration of the substrate of the Maillard reaction. Meanwhile, the heating treatment during processing further promotes the Maillard reaction in OS (Liu, He, Xiao, Zhou, & Wang, 2021), leading to an increase in the proportion of heterocyclic compounds.

A total of 89 odor compounds were detected in the 4 OEHs, including 11 esters, 7 acids, 2 hydrocarbons, 25 heterocyclic compounds, 13 aldehydes, 17 alcohols, 12 ketones, and 2 other compounds. In terms of compound composition, heterocyclic compounds and alcohol compounds were the most important odors in the OEHs, accounting for 34% and 19%, respectively (the mean value of the samples). In terms of compound content, heterocyclic compounds and alcohol compounds were identified as the most important odor compounds in the OEHs, both constituting 30% (the mean value of the samples). Differing from the other three OEHs, 1# OEH exhibited significantly higher levels and varieties of ester compounds. Intriguingly, in contrast to the composition traits of OEHs, the aroma of oysters predominantly originates from aldehydes, ketones, and alcohols resulting from enzymatic degradation and spontaneous oxidation of polyunsaturated fatty acids (Su, Huang, He, & Zhao, 2020), heterocyclic compounds are rarely detected in oysters (Soares et al., 2020; Van Houcke, Medina, Linssen, & Luten, 2016). Like most heterocyclic compounds, it is presumed that these substances are generated during production, processing, and storage due to the Maillard reaction (Josephson, Lindsay, & Stuiber, 1983), imparting the OEHs with a characteristic roasted flavor.

A total of 102 odor compounds were detected in 6 SEHs, including 7 esters, 6 acids, 5 hydrocarbons, 42 heterocyclic compounds, 16 aldehydes, 11 alcohols, 12 ketones, and 3 other compounds. Interestingly, more heterocyclic compounds were found in the SEHs than in other compounds, they accounted for 42% (the average in the sample). The types and proportions of heterocyclic compounds varied greatly in different SEHs. For instance, SEMH contained 23 heterocyclic compounds, constituting 52%, while FEH only contained 8 heterocyclic compounds, constituting 36%. Apart from heterocyclic compounds, aldehydes were found to be the second most abundant compounds in SEHs, comprising 19% (the mean value in the sample). Furthermore, in contrast to LKK and OEHs, significant differences were observed in the types of compounds that were most abundant in all SHEs. For example, SEMH has the highest alcohol compound content (Hao, 2016), while CEMH has the highest aldehyde compound content (Bu, 2012).

In summary, heterocyclic and alcohol compounds emerge as the most abundant volatile substances in both LKK and OEHs, with pyrazines identified as the predominant compounds. Notably, when compared to SEHs, the composition and proportion of odor compounds in LKK and OEHs are closely aligned, marking a significant departure from the profiles observed in SEHs.

3.2. Principal component analysis of samples

PCA is a mathematical method, which divides the data into 4 quadrants to reveal the relative positions and interrelationships of individual samples in the principal component space. PCA retains most of the data variation, simplifying the dataset for sample mapping, visually assessing similarities and differences, and identifying valid sample groupings. Specifically, a positive x-axis value indicates superior

performance of the sample in the corresponding principal component direction, while a negative value suggests inferior performance in that direction. Similarly, a positive y-axis value suggests superior performance of the sample in the other principal component direction, whereas a negative value suggests inferior performance in that direction (Granato, Santos, Escher, Ferreira, & Maggio, 2018; He, Liu, Qian, Yu, Xu, & Chen, 2020; Karabagias, Nikolaou, & Karabagias, 2019). Consequently, for an in-depth exploration of the relationship between LKK, OEHs, and SEHs based on the analysis of odor compounds, PCA was employed to analyze the qualitative and quantitative results of odor compounds in all samples. The scores plot and biplot diagram obtained are depicted in Fig. 1(A) and (B).

In the PCA scores plot diagram, the abscissa t[1] and the ordinate t[2], respectively represent the core values of the first two principal components. The farther away the samples are on the score map, the greater the difference and the more obvious the classification. As can be seen from Fig. 1(A), it is evident that, compared to the 6 SEHs, LKK and OEHs share certain characteristics, thus categorizing them into a similar group. Combined with Fig. 1(B), it can be seen that furfuryl propionate, ethyl phenylacetate, 2-methyl pyrazine (popcorn), propanoic acid, butanoic acid (cheese), 2,4,5-trimethyl oxazole (nutty), Pyridine, acetophenone, 2,4-dimethylbenzaldehyde (naphthyl), 2-methylbutanal (cocoa), 5-diethylpyrazine (nutty), 2-methylphenol, terpinene-4-ol, 2-methylphenol, 3-ethyl-Benzaldehyde, 2-undecanone, acetic acid (sour) and 2-octanone are the reasons of LKK and OEHs are grouped.

In addition, compared with LKK and OEHs, the 6 kinds of SEHs are similar to each other and can be categorized as another group. Referring to Fig. 1(B), it becomes evident that compounds such as butanoic acid (cheese), 2-methyl-5-(1-propenyl)-pyrazine, 2,4,5-trimethyl-thiazole, 4-methyl-benzaldehyde, tetramethyl pyrazine (nutty), 2-methyl-phenol, (Z)-2-butenal, Hexanal (fishy), 5-methylfurfural, and acetophenone are the factors contributing to the separation between SEH and LKK+OEH group. PCA analysis using the composition of sample odor compounds is more effective in distinguishing between target and discrepant samples. While PCA can reveal differences in sample status from the original data, it is somewhat less efficient in capturing the overall characteristics and regular changes in the data (Bylesjo, Rantalainen, Cloarec, Nicholson, Holmes, & Trygg, 2006). Therefore, subsequent experiments should build upon the established PCA clustering results and delve into further discussions on indicator compounds between categories.

3.3. Orthogonal partial least squares discriminant analysis

To better identify which odor compounds were responsible for the differences between the samples containing OEH or not, the supervised

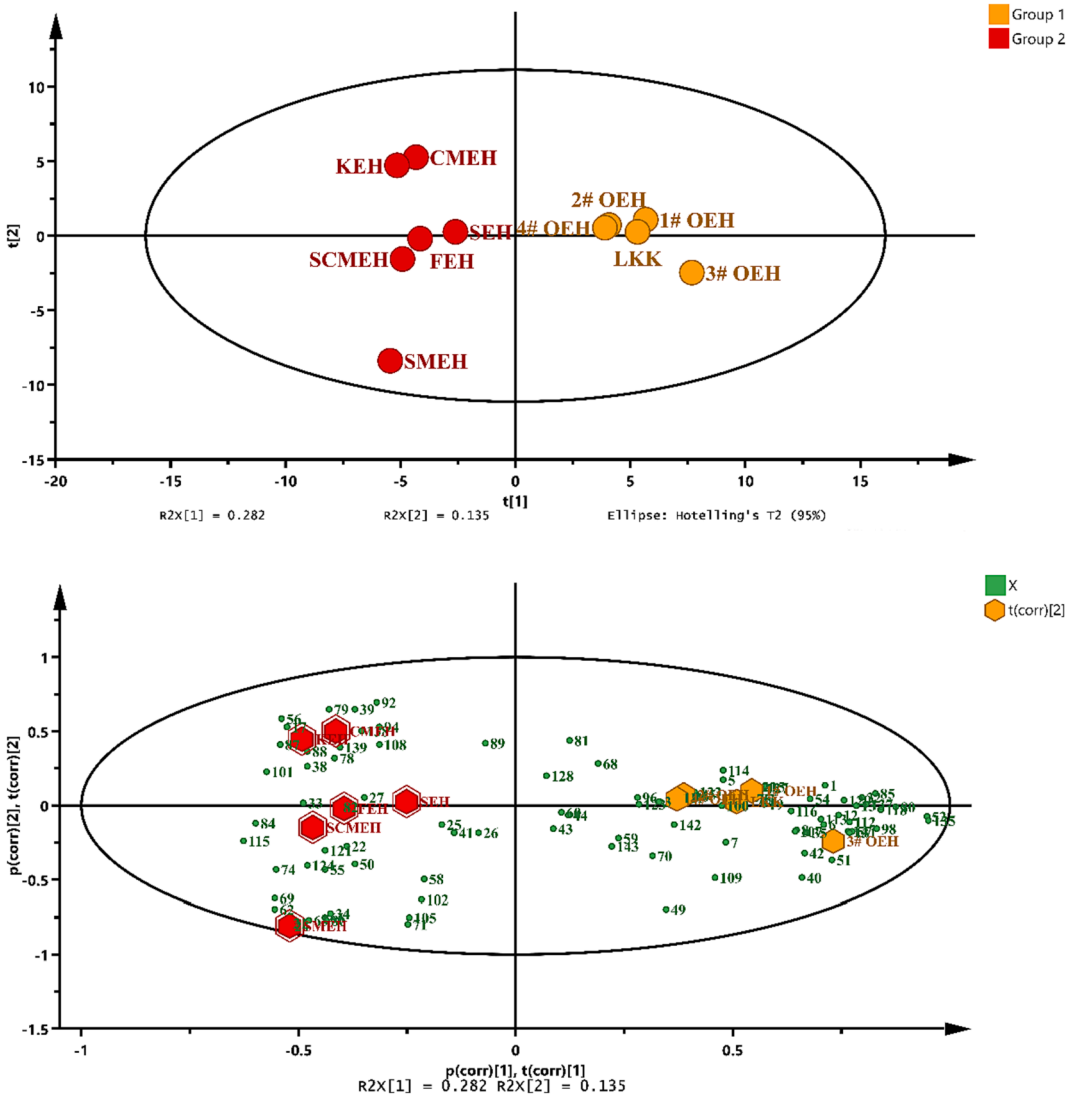

Fig. 1. The PCA scores plot (A) and biplot diagram (B) of LKK, OEH and SEH.

OPLS-DA multivariate statistical analysis method was employed to distinguish between SHE, OEH, and LKK, the results were displayed in Fig. 2.

In Fig. 2, LKK and the 4 types of OEHs were grouped into one category (Group I), while the 6 types of SEHs were classified into another category (Group II). The samples in these two groups demonstrated apparent clustering on the OPLS-DA score chart. A VIP map was generated based on the OPLS-DA model to further explore the odor compounds that exerted the most significant influence on Group I and Group II. VIP indicates the importance of the variable in causing differences between groups. When VIP $>$ 1.0, variable X is significantly different between the groups (Huang, Chen, Xiao, Zha, Luo, Wang, et al., 2017). Table 1 revealed that 22 odor compounds contributed to the distinction between Group I and Group II. Among these, heterocyclic compounds were the primary constituents, comprising 12 species. Heterocyclic compounds, primarily Maillard products, impart aromas of baked potatoes, nuttiness, and toasting in Group I. Aldehydes and acids also contribute to the distinction between Group I and Group II, imparting a sour taste and a fishy aroma, respectively. In addition, furfuryl alcohol (burnt) played a significant role in distinguishing Group I and Group II, with a VIP value of 3.99. Currently, several research studies have proposed different hypotheses regarding the formation of furfuryl alcohol in food substrates. Furfuryl alcohol may be formed by glucose oxidation, decarboxylation, further dehydration and cyclization (Yaylayan & Keyhani, 2000). It can also be created from 1, 2-enediol by β-elimination and α-dicarbonyl cleavage (Brands & van Boekel, 2001). In addition, Moon and Shibamoto pointed out that the formation pathway of furfuryl alcohol may involve processes such as dehydration, formic acid elimination, homozygous bonding, and free radical reactions (Joon-Kwan & Shibamoto, 2010). Considering the processing characteristics of LKK and OEH, it is speculated that aldose and cysteine undergo Amadori rearrangement, 1,2-enolation, and other chemical reactions, leading to the production of 3-deoxyglucoside. On this basis, the 3-deoxyglucoside further undergoes reverse aldol condensation, dealcoholization, dehydration, and reduction reaction to produce furfuryl alcohol (Liu, 2020).

3.4. Quantitative and OAVs analysis of key odor combination

To confirm the contribution of key odor compounds that differentiate group I from group II, the standard addition method was employed to quantitatively analyze 15 specific odor compounds (those with VIP≥1 present in both LKK and OEHs) based on the OPLS-DA analysis results. Subsequently, the OAVs were calculated, and the corresponding results are presented in Table 2.

Among the 15 key odor compounds, heterocyclic compounds exhibited the most diversity, comprising a total of 7 different kinds. These compounds had OAVs higher than 1, indicating their significant role in imparting the roast and burnt flavor to group I. Most heterocyclic compounds possess a robust roast flavor and an exceedingly low aroma threshold. The formation of roast flavor is primarily attributed to the Maillard reaction induced by high temperatures, representing one of the fundamental mechanisms. This reaction is complex and produces a lot of important flavor compounds, including furan, pyrazine, pyrrole, etc. (Josephson, Lindsay, & Stuiber, 1983). 2-pentylfuran and 2-ethylfuran are newly generated substances after enzymatic hydrolysis of oysters (Su, Huang, He, & Zhao, 2020), in which 2-pentylfuran has a green fragrance, and 2-ethylfuran mainly presents an intense caramel aroma and sweet taste (Chen, Chen, Chen, Cai, Wan, Zhu, et al., 2016). Meanwhile, the threshold values of these two substances are relatively low, especially the threshold value of 2-pentylfuran is only 0.0058 mg/kg in water. Therefore, its OAVs are more than 100 in all samples in group I. It contributes to the aroma of LKK and OEHs. Moreover, pyrazines and pyrroles are generated through the condensation of α-amino ketones resulting from amino acid degradation, typically emanating pleasant odors. Compounds such as 2-methylpyrazine, 2-ethyl-5-methylpyrazine, 2,3,5-trimethylpyrazine, 2-acetylpyrrole, and 2-ethyl-6-methylpyrazine, all possessing low odor thresholds, are key aromatic active compound in medium (MRC) and deep-roasted coffee (DRC) brewing samples (Turan Ayseli, Kelebek, & Selli, 2021), commercial high-salt liquid-state soy sauce (Wang, Guo, Song, Meng, & Guan, 2021) and non-sophisticated cane sugar (Maria Garcia, Cesar Narvaez, Jose Heredia, Orjuela, & Osorio, 2017). Among them, the OAVs of 2-ethyl-6-methylpyrazine are more than 100 in all samples in group I. It contributes to the roasted aroma of group I.

Aldehydes play a pivotal role in various food odors and have a very low threshold. Consequently, even though the concentration of key aldehyde compounds in the enzymatic hydrolysates of group I is not high, the OAV value is still high. Aldehydes in oyster meat may be generated through the oxidative degradation of polyunsaturated fatty acids under the influence of enzymes and microorganisms (Josephson, Lindsay, & Stuiber, 1985). They might be closely associated with the fishy aroma of enzymatically hydrolyzed oysters (Pino, 2014), thus contributing to the fishy smell of OS. Moreover, although the aldehyde content in group I is not high, aldehydes exert a potent additive effect and even trace amounts significantly contribute to the overall flavor

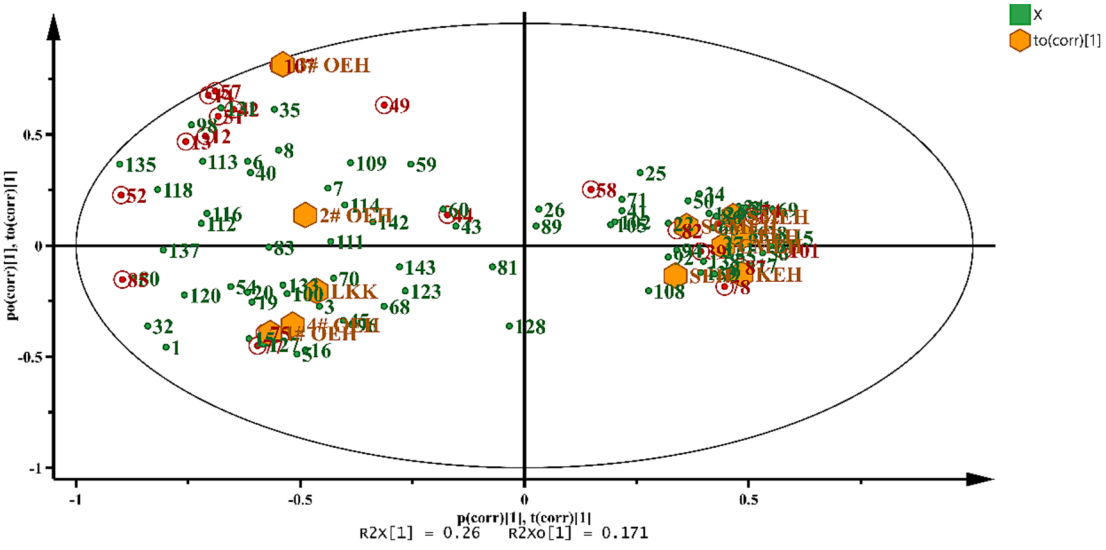

Fig. 2. The OPLS-DA of LKK, OEH and SEH.

Table 2
Concentrations of key odor compounds in LKK, OEHs, SEHs.

No.	key odor compounds[a.]	linear equations.	$R^{2.}$	odor threshold[b.] (mg/kg)	quota selected ion[c] (m/z)	concentration(mg/kg) /(OAV)[d.]				
						LKK.	1# OEH.	2# OEH.	3# OEH.	4# OEH.
1.	acetic acid.	y = 232.5x + 0.1129.	0.9943.	22.	60, 45, 43.	43.01 ± 0.16 (1)	141.84 ± 2.32 (6)	237.65 ± 13.12 (10)	151.43 ± 8.39 (6)	98.93 ± 3.82 (4)
2.	furfuryl alcohol.	y = 4.149x − 0.0273.	0.9836.	15.	98, 97, 81, 70.	143.25 ± 8.40 (9)	557.38 ± 15.92 (37)	228.92 ± 12.65 (15)	427.43 ± 13.74 (28)	520.74 ± 18.97 (34)
3.	propanoic acid.	y = 0.126x − 0.0217.	0.9874.	2.	74, 57, 45.	0.56 ± 0.05 (0)	3.72 ± 2.72 (1)	4.63 ± 6.93 (2)	1.64 ± 9.94 (0)	2.53 ± 3.86 (1)
4.	2-ethyl-6-methylpyrazine.	y = 1.596x − 0.0514.	0.9962.	0.04.	122, 121,94.	12.65 ± 1.25 (316)	59.29 ± 8.83 (1482)	34.74 ± 1.92 (868)	76.37 ± 4.24 (1909)	66.48 ± 3.66 (1662)
5.	2-methylbutanal.	y = 4.765x − 0.0209.	0.976.	0.001.	86, 58, 57, 41.	1.04 ± 0.16 (1040)	14.13 ± 1.01 (14130)	5.25 ± 0.29 (5250)	4.56 ± 0.25 (4560)	2.63 ± 0.14 (2630)
6.	butanoic acid.	y = 42.37x − 0.0155.	0.9689.	0.27.	88, 73, 60.	2.54 ± 0.31 (9)	7.78 ± 0.43 (28)	16.93 ± 1.16 (62)	12.89 ± 1.26 (47)	11.57 ± 0.08 (42)
7.	2-acetylpyrrole.	y = 5.299x − 0.1061.	0.9943.	>2.	109, 94, 66.	11.34 ± 0.63 (5)	113.49 ± 6.28 (56)	60.26 ± 3.34 (30)	9.61 ± 0.53 (4)	77.23 ± 4.29 (38)
8.	2-methylpyrazine.	y = 0.1421x + 0.039.	0.9937.	27.	94, 67, 53.	57.58 ± 3.97 (2)	203.34 ± 11.23 (7)	178.43 ± 14.35 (6)	196.62 ± 9.33 (7)	185.64 ± 5.83 (6)
9.	nonanal.	y = 0.4495x − 0.0131.	0.9963.	0.0011.	142, 98, 57.	0.67 ± 0.03 (609)	2.93 ± 0.31 (2663)	5.32 ± 0.18 (4836)	4.95 ± 0.27 (4500)	3.24 ± 0.23 (2945)
10.	2-ethyl-5-methylpyrazine.	y = 102.8x + 0.1030.	0.989.	0.036.	122, 121, 94.	3.07 ± 1.28 (85)	17.26 ± 3.90 (479)	21.07 ± 1.16 (585)	24.14 ± 2.45 (670)	31.39 ± 2.27 (871)
11.	3-methylbutanal.	y = 0.0499x + 0.0211.	0.9886.	0.002.	86, 71, 58, 57.	1.67 ± 0.20 (835)	17.81 ± 1.76 (8905)	5.88 ± 0.32 (2940)	7.71 ± 0.42 (3855)	6.34 ± 0.46 (3170)
12.	2,3,5-trimethylpyrazine.	y = 10.01x − 0.045.	0.9778.	0.19.	122, 81, 42.	4.19 ± 0.28 (22)	6.74 ± 0.37 (35)	34.93 ± 1.88 (183)	51.53 ± 2.86 (271)	17.86 ± 0.98 (94)
13.	2-pentylfuran.	y = 0.1015x + 0.035.	0.9812.	0.0058.	138, 82, 81.	3.47 ± 0.47 (598)	14.98 ± 1.93 (2582)	5.34 ± 0.29 (920)	7.72 ± 0.42 (1331)	6.87 ± 0.83 (1184)
14.	2-ethyl furan.	y = 1.084x + 0.0837.	0.9704.	8.	96, 81, 53.	26.16 ± 0.34 (3)	76.13 ± 4.22 (9)	95.83 ± 7.87 (11)	128.69 ± 7.55 (16)	113.45 ± 5.24 (14)
15.	2-butanone.	y = 6.260x + 0.0389.	0.9755.	1.3.	72, 57, 43.	6.74 ± 0.37 (5)	12.84 ± 1.77 (9)	5.61 ± 0.31 (4)	8.06 ± 0.44 (6)	8.71 ± 0.48 (6)

[a] The VIP value is greater than 1, and it is contained in both OS and 4 kinds of oyster hydrolysates. [b]Odor thresholds were referenced from a book named Odor thresholds compilations of odor threshold values in air, water and other media (second enlarged and revised edition). [c] The ions selected for quantitative analysis. The ion with the largest molecular weigLKK was the mother ion. [d]Mean values of triplicates with standard deviations (SDs).

(Varlet, Knockaert, Prost, & Serot, 2006).

Among the 15 key odor compounds, one alcohol and one ketone, furfuryl alcohol and 2-butanone, were also included. In LKK, furfuryl alcohol has the highest content (143.25 ± 8.40 mg/kg) among 15 key odor compounds. At the same time, owing to its lower threshold (15 mg/kg), it possesses a higher OAV value, making a significant contribution to the burnt flavor of LKK. Among ketones, furanones have a pleasant smell (Xu, Yibin, Shen, Nana, Baoqing, Ying, et al., 2020), often exhibiting caramel and sweet (Wencan, Rui, Yonghong, Tiankui, & Shaoquan, 2016). 1-hydroxy-2-propanone has a burnt flavor and is a key aroma compound in roasted coffee (Qiaoxuan, Wintersteen, & Cadwallader, 2002).

In addition, only 5 key odor compounds were found in group I: acetic acid, 2-pentylfuran, 2-ethylfuran, 2-methylbutanal, and nonanal, indicating the presence of oysters in LKK. Simultaneously, the OAVs of these 5 key odor compounds are all significantly greater than 1, making them easily detectable. Among them, although the content of the two aldehyde substances is not high due to their shallow threshold values, their OAVs are very high, contributing significantly to the fishy taste of LKK, particularly OEH.

4. Conclusion

In this study, molecular sensory analysis was employed to examine the odor compounds in LKK, 4 types of OEHs, and 6 varieties of SEHs. The results revealed that heterocyclic compounds were the most prevalent volatile substances in both LKK and OEHs, with similar proportions, while a significant disparity existed in SEHs. The statistical analyses conducted through PCA and OPLS-DA further substantiate that OEHs are more akin to LKK and can be effectively distinguished from SEHs. In conjunction with the VIP values and the composition analysis of odor compounds in each sample, a total of 15 key odor compounds were identified. Subsequent quantitative analysis and calculation of OAVs were performed to serve as an indicator for the presence of oysters or OEHs in the sample. Among these, furfuryl alcohol (burnt) emerged as the most significant contributor to identifying the company of oysters or OEHs in the sample. Furthermore, 5 key odor compounds (2-methylbutanal, 2-pentylfuran, 2-ethylfuran, acetic acid, nonanal) found exclusively in LKK were identified in OEH. These compounds not only impart the cocoa, green, sweet, burnt, sour, and fishy tastes characteristic of LKK and OEHs but also offer further insights into the presence of oysters or OEHs in LKK. The findings of this study could serve as a reference for determining the presence of oysters or OEHs in commercially available OS.

CRediT authorship contribution statement

Liang Zhuang: Data curation, Validation, Writing – original draft. **Qian Luo:** Methodology. **Mingming Zhang:** Data curation, Formal analysis, Investigation. **Xuzeng Wang:** Data curation, Software, Visualization. **Shan He:** Data curation, Methodology, Software, Visualization. **Guiju Zhang:** Project administration, Resources, Writing – review & editing. **Xuchun Zhu:** Funding acquisition, Project administration, Resources, Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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