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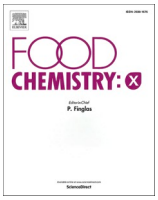

# Flavor characteristics of large yellow croaker soup served with different dried edible fungi

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ABSTRACT

The effects of different edible fungi on the flavor profiles of fish soups were analyzed by sensory evaluation, non-volatile and volatile flavor compounds. The sensory attributes of fish soups were modified by adding edible fungi, with the highest total scores obtained for AAFS and DFS. Compared with pure fish soup, the amounts of free amino acids, nucleotides, organic acids and inorganic ions were increased with fungi addition, especially AAFS. The different mushroom fish soups could be clearly distinguished by E-nose analysis, and a total of 52 flavor compounds, mainly composed of aldehydes (27), ketones (11), alcohols (8), esters (4), and others (2), were then identified by GC-IMS. Eventually, fish soup samples were classified into three groups based on OPLS-DA analysis: I (LEFS), II (BFS and BEFS) and III (ABFS, AAFS and DFS). The results showed that Agrocybe aegerita had high suitability for improving the flavor of Large yellow croaker soups.

## 1. Introduction

Soup, a traditional household food style, combines nutrition, palatability and convenience (Zou, Xu, Zou, & Yang, 2021). As a non-chewing and nutrient-rich food, it is particularly beneficial for consumers such as elderly people or those with special nutritional needs and eating difficulties (e.g., due to tooth loss, dysphagia and neuromuscular disorders) as it helps to meet their dietary needs (Mohamed et al., 2020). Flavor is an important attribute to evaluate the quality of soup, which is largely dependent on the addition of sodium, however, excessive sodium intake in the diet has potential harm to the cardiovascular system of consumers (Mattar, Gonçalves, Pereira, Faria, Souza, & Carneiro, 2018). In view of this, some flavor enhancement strategies to deal with "salt reduction" have become a hot topic.

During the cooking of the soup, flavor is generated from protein degradation, lipid oxidation and the Maillard reaction, all of which result in the dissolution of volatile and non-volatile compounds into the soup (Zhu et al., 2021). Regarding the volatile compounds, they contribute to the perception of flavor based on their amounts and composition (Mitchell, Brunton & Wilkinson, 2011). For example, alcohols and carbonyl compounds (aldehydes and ketones), usually arising from lipid oxidative degradation, influence the rich aroma of fish soup at lower thresholds (Meng et al., 2022; Qi, Liu, Zhou & Xu, 2017). Similarly, with their unique aromas, esters as well as derivatives of benzene are also commonly used to adjust the flavor profiles of soups (Qi, Liu, Zhou & Xu, 2017). On the other hand, it is often emphasized that the contribution of non-volatile compounds to the umami flavor of fish soup depends on the presence of free amino acids, flavor-contributing nucleotides, organic acids and certain inorganic ions (Yue et al., 2016; Kong et al., 2017). At the same time, the synergistic effect between MSG-like amino acids and flavor nucleotides is also important in determining the umami taste (Rotola-Pukkila, Yang, & Hopia, 2019). Therefore, the choice of raw and auxiliary materials determines the composition of the soup's flavor compounds, and thus, influences its overall flavor profile.

Appropriate ingredients play a significant role in regulating the overall flavor and enhancing the palatability of soup. Edible fungi, being a type of "medicine and food homologous" item, boast a rich nutritional profile that includes proteins, dietary fibers, vitamins as well as bioactive ingredients such as polyphenols, polysaccharides and alkaloids (Sun et al., 2019; Zhu et al., 2022). Furthermore, edible fungi are rich in umami compounds and are considered to be a good source of natural taste enhancers, which can be used as a viable alternative to salt in meat processing (Mattar et al., 2018). França et al. (2022) found that umami

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ingredients extracted from Lentinula edodes partially replacing salt had no significant effect on the texture of beef burgers, while increased the content of flavor compounds. Similarly, by soaking dry shiitake mushrooms in hot water, Dermiki et al. (2013) obtained extracts that were not only rich in umami compounds but could also be used to enhance the flavor of meat products. Therefore, it is envisaged that collocations of fish and edible fungi with a harmonious flavor may be explored to develop a novel nutrient-rich soup.

Large yellow croaker (*Larimichthys crocea*), a marine fish with a considerable aquaculture scale, represents a high-quality protein source (Ge et al., 2020). However, there are relatively few finished products prepared from this fish. In the current study, the overall flavor properties of Large yellow croaker soups containing varying edible fungi were characterized by sensory evaluation, and the effects of edible fungi on the changes of volatile and non-volatile flavor compounds in fish soup were studied. It is expected that the results will guide the development of formulations as well as the regulation of flavors for innovative and nutritious soups.

## 2. Materials and methods

### 2.1. Materials

Fresh Large yellow croaker (300 ± 50 g) were purchased from local aquatic product market (Jinzhou, Liaoning, China). Edible fungi (dry), salt and soybean oil were purchased from the local agricultural product market. Other chemical agents were of analytical grade.

### 2.2. Preparation of fish soup with different edible fungi

Large yellow croaker was pre-treated (scaled, gutted and washed), and after being fried in oil for 2 min, any excess oil was removed with oil-absorbing paper. Edible fungi were soaked for 10 min and drained for use. Then, fish, purified water and salt (0.3 %, based on water weight) were added to an electric cooker (DGD25-25GWD, Guangdong Tonze Electric Co., Ltd.) in a ratio of 1:3 (w/w), and boiled for 1 h in nutrient soup mode to obtain a blank fish soup (BFS). Mushroom fish soups, including *Boletus edulis* fish soup (BEFS), *Lentinus edodes* fish soup (LEFS), *Agaricus blazei* fish soup (ABFS), *Agrocybe aegerita* fish soup (AAFS) and *Dictyophora* fish soup (DFS), were then prepared under the same cooking conditions by adding the corresponding edible mushrooms (4 %, based on the weight of fish) to the boiling water. The resulting fish soups were eventually cooled and being passed through a 200-mesh filter cloth.

### 2.3. Sensory evaluation

The study protocol and consent procedure received ethical approval from the Research Ethics Committee of of Bohai University. In addition, all sensory team members provided written informed consent.

Sensory evaluation of the fish soup samples was performed by 20 trained panelists (10 males and 10 females). A sensory score, based on a 10-point scale, was given for each sensory attribute, and a comprehensive score was then calculated from the individual scores according to their respective weights (30 % for umami, 10 % for bitterness, 20 % for smell, 10 % for color and 30 % for richness). Each sample was evaluated three times at an interval of 30 s.

### 2.4. Determination of amino acid nitrogen (AAN)

The AAN was analyzed refer to the method of Wang et al. (2022). The fish soup sample (5 mL) was mixed with 60 mL ultrapure water and adjusted to pH 8.2 with NaOH (0.05 mol/L). The formaldehyde (10 mL) was then poured in and the mixture was further titrated to pH 9.2 with NaOH. Finally, the volume of NaOH used in this procedure was used to calculate the AAN content.

### 2.5. Determination of free amino acid (FAA)

The FAAs of fish soup were analyzed with reference to the previous procedure (Sun et al., 2019). Each fish soup sample (4 mL) was mixed with sulfosalicylic acid solution (1 mL) and incubated at 4℃ for 12 h, then centrifuged (10000 rpm, 15 min). The resulting supernatant was passed through 0.22 μm filter and injected into amino acid analyzer (L-8900; Hitachi, Tokyo, Japan) for analysis.

### 2.6. Determination of nucleotides

The fish soup sample (5 mL) was mixed with perchloric acid (15 mL, 5 %, w/w), homogenized (9500 rpm, 15 min), and centrifuged (6000 rpm, 10 min). The pH of resulting supernatant was adjusted to 6.75 with 5 mol/L KOH and filtered through a 0.22-μm filter membrane for analysis. The determination of nucleotides in the fish soups was performed using an Agilent 1100 high performance liquid chromatography (HPLC) equipped with a C18 chromatographic column (5 μm, 4.6 mm × 250 mm). The gradient eluent program: 0 ~ 10 min (97.5 % KH2PO4: 2.5 % methanol), 10 ~ 17 min (85 % KH2PO4: 15 % methanol) and 17 ~ 20 min (97.5 % KH2PO4: 2.5 % methanol).

### 2.7. Determination of equivalent umami concentration (EUC)

EUC was calculated by the formula below based on the synergy of umami amino acids and 5′-nucleotides.

$$\mathrm{EUC}(g/100\,g) = \Sigma a_i \times b_i + 1218(\Sigma a_i \times b_i)\left(\Sigma a_j \times b_j\right)$$

$a_i$: umami amino acids (Asp or Glu) content; $a_j$: 5′-nucleotide (IMP, GMP or AMP) content; $b_i$: the relative umami concentration (RUC) for Glu (1) or Asp (0.077); $b_j$: the RUC for IMP (1), GMP (2.3) or AMP (0.18); and 1218 acted as a synergistic constant.

### 2.8. Determination of organic acid

The contents of organic acid were determined by the method of Wang et al (2022) with slightly modified. The 5 mL fish stock sample was added with 0.25 mL perchloric acid (5 %, w/w), and the mixture was homogenized and centrifuged (9600 r/min, 10 min) after equilibrium for 20 min. The resulting supernatant was filtered by a 0.22-μm filter membrane and injected into a HPLC analysis system.

### 2.9. Determination of inorganic ions

Refer to Qi et al. (2017), the concentrations of inorganic ions ($Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$ and $PO_4^{3-}$) were analyzed by an ICP-OES (Optima 8000, Perkin Elmer, USA). The fish soup samples (0.5 g) were subjected to microwave polytetrafluoroethylene digestion tank (Ethos 1, Milan, Italy) added with 5 mL $HNO_3$. The microwave digestion was performed by the following heating procedures: 100 ℃ for 3 min, 140 ℃ for 3 min, 160 ℃ for 3 min, 180 ℃ for 3 min and 190 ℃ for 15 min. The resulting digested samples were cooled to room temperature and transferred to a volumetric bottle (50 mL), then diluted with deionized water and filtered. The content of chlorine ($Cl^-$) was measured referred to Zhang et al., (2019a).

### 2.10. E-nose analysis

Aromatic information for the fish soups was obtained by a PEN3 portable e-nose. Each fish soup sample (5 g) was placed in a tube, sealed with three layers of plastic wrap, and equilibrated at room temperature for 30 min. The detection time was 120 s, and the cleaning time was 120 s. The data collected within 100–110 s were used for principal component analysis (PCA) performed with the Win Muster software provided with the e-nose.

### 2.11. GC-IMS analysis

The VOCs of fish soups were analyzed by GC-IMS (Flavorspec®, Dortmund, Germany), and the specific analysis conditions referred to Zhu et al. (2021). The soup sample (2 mL) was put into a headspace bottle (20 mL) and incubated at 60 ℃ for 15 min. Automatic sampling was adopted with a sample volume of 500 μL and a needle temperature of 65 ℃. The sample was transported through the MXT-5 column (15 m × 0.53 mm, 1 μm) by carrier gas ($N_2$), and the flow rate was as follows: 2 mL/min for 2 min, 10 mL/min for 8 min, followed by 100 mL/min for 10 min. The drift tube temperature of IMS department was 45 ℃ and the flow rate of drift gas ($N_2$) was 150 mL/min. Subsequently, the VOCs from samples were identified according to the RI and the drift time with the GC-IMS Library.

### 2.12. Statistical analysis

Statistical analysis was performed by Origin 9.0 and SPSS 26.0 software for duncan multiple comparison and ANOVA analysis. The results (mean ± standard deviation) were repeated in triplicate independently, and significance level was $P < 0.05$. The chromatograms of GC-IMS were analyzed by Laboratory Analytical Viewer and GC × IMS Library Search analysis software. Qualitative analysis of the VOCs was performed by the database of NIST and database built in GC × IMS Library. The PCA and OPLS-DA were performed using SIMCA 14.1 software.

## 3. Results and discussion

### 3.1. Sensory evaluation

The sensory properties of the different mushroom fish soups were comprehensively evaluated, with the results shown in Fig. 1. In terms of comprehensive scores (Fig. 1B), AAFS and DFS significantly outperformed ($P < 0.05$) other fish soups as a result of their relatively strong umami flavor, harmonious aroma, milky color and weak bitterness (Fig. 1A). They were followed by BEFS for which the main deficiency lay in the "color". Indeed, it can be observed from Fig. S1 that the soup body of BEFS was brown, making it unfavorable based on consumers' preferences. In addition, the composite score of LEFS was comparable to that of BFS, while that of ABFS showed that it was the least satisfactory of all samples, particularly in terms of "umami" and "smell". It could therefore be speculated that the individual flavor compounds produced by *Agaricus blazei* negatively impacted the overall flavor of the fish soup. For example, ABFS had a higher benzaldehyde and heptanal content than the other fish soup samples as shown in fingerprint (Fig. 4). However, benzaldehyde, a key flavor compound of *Agaricus blazei*, is known to confer a bitter almond flavor (Stijve et al., 2002), while heptanal yields a greasy and musty odor at high concentrations (Xun et al., 2020). Overall, it was obvious that the addition of edible fungi modified the sensory characteristics of fish soups, especially in the case of *Agrocybe aegerita* and *Dictyophora* where the umami and richness were significantly improved. The results suggest a synergistic flavor enhancement effect between Large yellow croaker and certain edible fungi.

### 3.2. Analysis of AAN

Amino acid nitrogen refers to the nitrogen within amino acids, and may occur as free amino acids or nitrogen in small molecule oligopeptides (Phat, Moon, & Lee, 2016). Changes in the AAN (Table 1) of different fish soups reflect the degree of protein decomposition. Compared with the BFS sample (0.48 mg/mL), the different mushroom fish soups showed an increase in their AAN content as follows: AAFS (0.83 mg/mL), DFS (0.76 mg/mL), LEFS (0.75 mg/mL), ABFS (0.67 mg/mL) and BEFS (0.53 mg/mL), with only the first three changes being of significance ($P < 0.05$). This could be attributed to the proteins of edible fungi which they are rich flavor precursors that can be broken down into a number of smaller peptides during heating as a result of different chemical reactions (Cho, Choi & Kim, 2006).

### 3.3. Analysis of FAAs, 5′-nucleotides and EUC values

The FAAs content of the different mushroom fish soups is shown in Table 1. Overall, the addition of the fungi significantly improved the amount of FAAs in the soups, with AAFS (348.63 mg/100 g) showing the greatest increase. It was followed by ABFS (330.15 mg/100 g), LEFS (296.65 mg/100 g), DFS (232.10 mg/100 g), BEFS (191.46 mg/100 g) and BFS (150.67 mg/100 g). The FAAs accumulated in mushroom fish soup was partly from the hydrolysis of fish protein, and the other part was related to the degradation of proteins and polypeptides in mushrooms. Sun et al. (2019) identified large amounts of FAAs in domestic-cooking mushroom soup, including Glu, Asp, Thr, Gly, Pro, Ser and Ala. Of these, MSG-like amino acids (Glu and Asp) are known to the key compounds responsible for mushrooms' umami flavor (Yang, Lv, Liu, Bi, & Zhang, 2022; França et al., 2022). Therefore, the amount of umami amino acids in the different edible fungi fish soups was consistently higher than that of BFS, especially in the case of AAFS. It has been suggested that interactions between sweet amino acids (represented by Ser, Gly and Ala) and IMP could represent another pathway through which umami flavors are enhanced (Yue et al., 2016), with such amino acids being present in relatively higher amounts in ABFS and AAFS. Moreover, a number of bitter amino acids were also detected in the soup

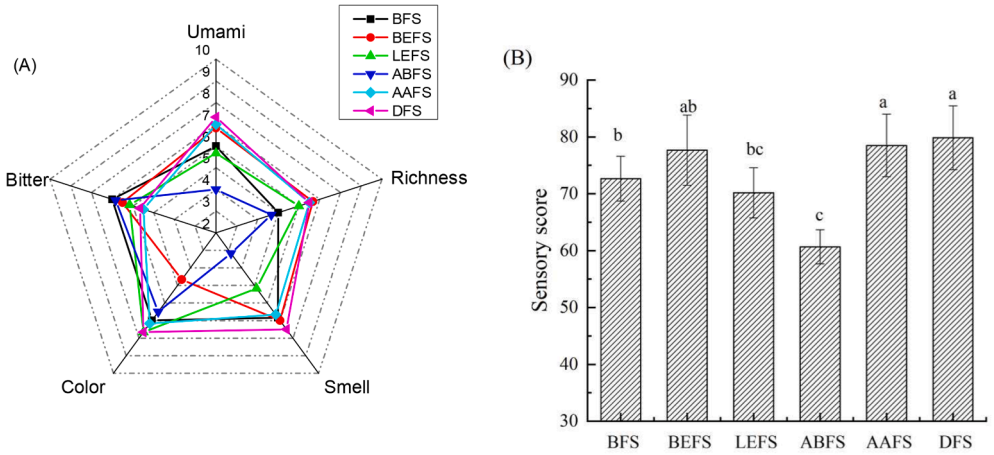


**Fig. 1.** Radar chart (A) and comprehensive score (B) for sensory evaluation of fish soups added with varying edible fungi ($P < 0.05$).

**Table 1**
The amounts of 5′-nucleotide, FAA, amino nitrogen and EUC evaluation of fish soups added with varying edible fungi.

| Content | BFS | BEFS | LEFS | ABFS | AAFS | DFS |
|---|---|---|---|---|---|---|
| 5′-nucleotide (mg/100 g) | | | | | | |
| GMP | 2.88 ± 0.07$^{c}$ | 2.79 ± 0.02$^{c}$ | 2.84 ± 0.04$^{c}$ | 2.27 ± 0.01$^{d}$ | 3.18 ± 0.09$^{a}$ | 3.08 ± 0.03$^{b}$ |
| IMP | 4.81 ± 0.01$^{d}$ | 5.18 ± 0.01$^{c}$ | 6.43 ± 0.02$^{bc}$ | 6.72 ± 0.00$^{b}$ | 7.60 ± 0.01$^{a}$ | 6.26 ± 0.00$^{bc}$ |
| AMP | 1.16 ± 0.05$^{e}$ | 1.35 ± 0.01$^{bc}$ | 1.38 ± 0.00$^{b}$ | 1.33 ± 0.00$^{c}$ | 1.46 ± 0.06$^{a}$ | 1.25 ± 0.06$^{d}$ |
| Total nucleotides | 8.85 ± 0.11$^{e}$ | 9.32 ± 0.01$^{d}$ | 10.65 ± 0.03$^{b}$ | 10.32 ± 0.01$^{c}$ | 12.24 ± 0.03$^{a}$ | 10.59 ± 0.04$^{b}$ |
| Umami amino acid (mg/100 g) | | | | | | |
| Asp | 2.44 ± 0.12$^{c}$ | 3.92 ± 0.33$^{b}$ | 3.92 ± 0.27$^{b}$ | 5.68 ± 0.40$^{ab}$ | 5.92 ± 0.62$^{a}$ | 2.64 ± 0.22$^{c}$ |
| Glu | 19.24 ± 1.28$^{d}$ | 30.44 ± 1.14$^{c}$ | 37.08 ± 1.07$^{b}$ | 39.48 ± 1.36$^{b}$ | 44.00 ± 1.47$^{a}$ | 22.52 ± 1.20$^{d}$ |
| EUC(g MSG/100 g) | 0.30 ± 0.02$^{d}$ | 0.50 ± 0.09$^{bc}$ | 0.64 ± 0.02$^{b}$ | 0.63 ± 0.04$^{b}$ | 0.87 ± 0.00$^{a}$ | 0.40 ± 0.01$^{c}$ |
| Sweet amino acid (mg/100 g) | | | | | | |
| Thr | 9.48 ± 0.23$^{d}$ | 9.56 ± 0.55$^{c}$ | 28.16 ± 1.04$^{a}$ | 14.76 ± 0.68$^{c}$ | 21.56 ± 0.33$^{b}$ | 16.88 ± 1.01$^{bc}$ |
| Ser | 12.08 ± 1.42$^{d}$ | 16.04 ± 1.28 $^{cd}$ | 22.6 ± 1.55$^{c}$ | 44.00 ± 1.63$^{a}$ | 34.32 ± 1.37$^{b}$ | 21.2 ± 1.22$^{c}$ |
| Pro | 16.4 ± 1.22$^{d}$ | 22.96 ± 1.14 $^{cd}$ | 47.2 ± 1.66$^{b}$ | 32.92 ± 1.04$^{c}$ | 68.00 ± 1.59$^{a}$ | 42.00 ± 1.28$^{b}$ |
| Gly | 24.12 ± 1.17$^{d}$ | 27.00 ± 1.44$^{d}$ | 49.60 ± 1.33$^{c}$ | 92.00 ± 2.42$^{a}$ | 59.60 ± 1.03$^{b}$ | 40.00 ± 1.25$^{c}$ |
| Ala | 18.64 ± 1.33$^{c}$ | 21.92 ± 1.01$^{c}$ | 32.28 ± 0.99$^{b}$ | 40.4 ± 1.58$^{a}$ | 33.68 ± 1.05$^{b}$ | 19.8 ± 1.22$^{c}$ |
| Bitter amino acid (mg/100 g) | | | | | | |
| Val | 2.80 ± 0.28$^{c}$ | 4.28 ± 0.76$^{b}$ | 7.32 ± 0.58$^{ab}$ | 7.32 ± 0.66$^{ab}$ | 8.24 ± 0.49$^{a}$ | 3.68 ± 0.41$^{bc}$ |
| Met | 1.53 ± 0.37$^{c}$ | 2.22 ± 0.33$^{a}$ | 1.48 ± 0.31$^{c}$ | 1.87 ± 0.22$^{b}$ | 2.11 ± 0.12$^{ab}$ | 2.02 ± 0.08$^{ab}$ |
| Phe | 1.86 ± 0.11$^{d}$ | 3.28 ± 0.32$^{b}$ | 3.37 ± 0.26$^{b}$ | 5.00 ± 0.21$^{a}$ | 4.96 ± 0.17$^{a}$ | 2.48 ± 0.14$^{c}$ |
| Iso | 1.48 ± 0.13$^{d}$ | 2.36 ± 0.21$^{c}$ | 3.92 ± 0.22$^{ab}$ | 3.56 ± 0.17$^{b}$ | 4.68 ± 0.43$^{a}$ | 1.64 ± 0.13 $^{cd}$ |
| Leu | 1.72 ± 0.29$^{c}$ | 3.92 ± 0.13$^{b}$ | 3.84 ± 0.17$^{b}$ | 7.04 ± 0.15$^{a}$ | 7.16 ± 0.33$^{a}$ | 2.68 ± 0.24$^{bc}$ |
| Lys | 25.68 ± 0.56$^{bc}$ | 29.00 ± 0.78$^{b}$ | 37.92 ± 0.86$^{a}$ | 20.76 ± 0.45$^{c}$ | 36.44 ± 0.53$^{a}$ | 37.00 ± 0.67$^{a}$ |
| His | 12.88 ± 0.23$^{c}$ | 13.52 ± 0.18$^{bc}$ | 14.96 ± 0.11$^{b}$ | 13.32 ± 0.22$^{bc}$ | 14.36 ± 0.21$^{b}$ | 17.12 ± 0.33$^{a}$ |
| Arg | 0.32 ± 0.06 $^{cd}$ | 1.04 ± 0.20$^{c}$ | 3.00 ± 0.19$^{ab}$ | 2.04 ± 0.43$^{b}$ | 3.6 ± 0.55$^{a}$ | 0.44 ± 0.16 $^{cd}$ |
| Total FAAs | 150.67 ± 2.15$^{c}$ | 191.46 ± 2.43$^{c}$ | 296.65 ± 1.37$^{b}$ | 330.15 ± 2.58$^{a}$ | 348.63 ± 2.29$^{a}$ | 232.10 ± 2.08$^{bc}$ |
| amino nitrogen (mg/mL) | 0.48 ± 0.01$^{b}$ | 0.53 ± 0.01$^{b}$ | 0.75 ± 0.00$^{a}$ | 0.67 ± 0.02$^{ab}$ | 0.83 ± 0.01$^{a}$ | 0.76 ± 0.00$^{a}$ |

BFS: blank fish soup, BEFS: *Boletus edulis* fish soup, LEFS: *Lentinus edodes* fish soup, ABFS: *Agaricus blazei* fish soup, AAFS: *Agrocybe aegerita* fish soup, DFS: *Dictyophora* fish soup; Asp: aspartic acid, Glu: glutamic acid, Ser: serine, His: histidine, Gly: glycine, Thr: threonine, Arg: arginine, Ala: alanine, Tyr: tyrosine, Cys: cysteine, Val: valine, Met: methionine, Phe: phenylalanine, Ile: isoleucine, Leu: leucine, Lys: lysine, Pro: proline; FAAs: Free amino acids; IMP: Inosine-5′-monophosphate, AMP: Adenosine-5′-monophosphate, GMP: Guanosine-5′-monophosphate; EUC: Equivalent umami concentration; MSG: Monosodium glutamate.

samples, and although their content was higher in the mushroom-containing ones compared with BFS, it was still much below the threshold. Interestingly, a previous study (Lioe et al., 2005) confirmed that subthreshold levels of bitter amino acids could actually enhance the umami taste.

The amount of flavor-enhancing 5′-nucleotides in the fish soups are presented in Table 1. In general, the samples differed significantly ($P < 0.05$) in terms of their total nucleotide content. The contribution of individual nucleotides to the flavor being as follows: GMP > IMP > AMP (Manninen, Rotola-Pukkila, Aisala, Hopia, & Laaksonen, 2018). Of these, GMP confers a meaty flavor and its taste intensity tends to be roughly equivalent to 2.3 times that of IMP, or even more in comparison with MSG (Meng et al., 2022; Pei et al., 2014). Regarding IMP, which promotes a sweet and pleasant taste, the current study found that it was present in higher amounts (ranging from 4.81 to 7.60 mg/100 g) compared with other nucleotides (Yue et al., 2016). Finally, AMP and its sweet taste is considered to be effective for suppressing bitterness (Pei et al., 2014). It should be noted that the highest amounts of the three nucleotides were present in AAFS, and this could have contributed to the high performance of the sample during sensory evaluation (Fig. 1). Similarly, the remaining mushroom fish soups also had a higher total 5′-nucleotides content compared with BFS. This was likely due to the dissolution of the flavor nucleotides, present in edible fungi, into the fish soup during boiling, thereby increasing their overall content in the soups (Abd El-Aleem, Taher, Lotfy, El-Massry, & Fadel, 2017).

Umami FAAs and nucleotides exhibited a synergistic effect in enhancing the umami, which could be reflected by the increase in the EUC value (Yang, Lv, Liu, Bi, & Zhang, 2022; Wang et al., 2022). In general, higher concentrations of MSG-like compounds correspond to higher levels of EUC (Phat, Moon, & Lee, 2016). Table 1 shows the different EUC values for the fish soups, and from these, the soups could be ordered as follows according to descending EUC values: AAFS > LEFS > ABFS > BEFS > DFS > BFS. At the same time, the EUC values of fish soups containing edible fungi were significantly different from that of BFS ($P < 0.05$). A previous study (Hajšlová et al., 2002) concurred that edible fungi with high levels of Glu and Asp generally exhibit elevated EUC values, while simultaneously contributing to a superior umami taste as well as overall palatability.

### 3.4. Analysis of organic acid and inorganic ions

The effects of edible fungi on the organic acid content of fish soups are shown in Table 2. Compared with pure fish soup sample (BFS), the mushroom ones had significantly higher amounts of total organic acids, except in the case of DFS for which the increase was not significant. In particular, the greatest increase was noted for LEFS (55.34 mg/100 g), followed by AAFS (49.68 mg/100 g). Succinic acid (SA) has been identified as a major flavor component in seafoods, and it usually confers both a sour and an umami taste. Although the SA content of the fish soups was relatively low (0.11 ~ 0.49 mg/100 g), it still exceeded the reported threshold (0.11 mg/100 g), and thus, had a non-negligible contribution to the overall flavor (Liu, Zhang, & Chen, 2013). Lactic acid (LA), an important flavor compound found in aquatic products and fermented foods, imparts a distinct sour taste (Wang et al., 2022). The levels of LA and Malic acid (MA) were significantly higher, accounting for more than 90 % of the total content, indicating their substantial contribution to the taste of the fish soup samples. Furthermore, trace amounts of citric acid (CA) were also detected, which could provide a mild and refreshing taste to fish soups (Zhu et al., 2021).

The inorganic ion content of the fish soup samples increased significantly ($P < 0.05$) in comparison with BFS, except in the case of BEFS. The contents of inorganic ions in different fish soup also showed variability, with $Na^+$ and $Cl^-$ being the predominant ions. Hayashi et al (1981) discovered that $Na^+$ and $Cl^-$ were the main inorganic ions in fish, with freshwater fish usually exhibiting lower levels of these ions compared to seafood. The presence of $Na^+$ could synergistically enhance the umami taste of meat by interacting with 5′-nucleotides and FAAs. On the other hand, the presence of $Cl^-$ could reduce the perception of sour taste, while simultaneously enhancing the sweetness and umami sensation (Schlichtherle-Cerny & Grosch, 1998). Overall, for the

**Table 2**
The amounts of organic acid and inorganic ion of fish soups added with varying edible fungi.

| Content | BFS | BEFS | LEFS | ABFS | AAFS | DFS |
|---|---|---|---|---|---|---|
| Organic acid (mg/100 g) | | | | | | |
| MA | 20.15 ± 1.33 $^{cd}$ | 20.76 ± 1.79$^{c}$ | 28.09 ± 0.06$^{a}$ | 22.78 ± 0.01$^{b}$ | 23.88 ± 0.12$^{b}$ | 20.98 ± 0.08$^{c}$ |
| LA | 20.64 ± 0.54$^{e}$ | 22.45 ± 0.21$^{c}$ | 24.96 ± 0.09$^{a}$ | 22.60 ± 0.11$^{c}$ | 23.34 ± 0.07$^{b}$ | 21.39 ± 0.21$^{d}$ |
| CA | 1.27 ± 0.03$^{e}$ | 1.50 ± 0.02$^{c}$ | 1.81 ± 0.01$^{b}$ | 1.94 ± 0.01$^{a}$ | 1.75 ± 0.01$^{b}$ | 1.46 ± 0.00$^{d}$ |
| SA | 0.11 ± 0.01$^{e}$ | 0.32 ± 0.09$^{c}$ | 0.49 ± 0.02$^{a}$ | 0.48 ± 0.01$^{a}$ | 0.43 ± 0.01$^{b}$ | 0.21 ± 0.00$^{d}$ |
| Total organic acid | 42.17 ± 1.58$^{e}$ | 45.03 ± 1.96$^{d}$ | 55.34 ± 0.14$^{a}$ | 47.80 ± 0.11$^{c}$ | 49.68 ± 0.20$^{b}$ | 44.04 ± 0.28$^{e}$ |
| Inorganic ion (mg/100 g) | | | | | | |
| $Na^{+}$ | 282.00 ± 1.22$^{d}$ | 284.00 ± 0.99$^{d}$ | 313.00 ± 1.21$^{c}$ | 341.00 ± 1.27$^{a}$ | 327.00 ± 1.09$^{b}$ | 304.00 ± 1.01 $^{cd}$ |
| $K^{+}$ | 64.40 ± 0.88$^{d}$ | 73.60 ± 0.84$^{c}$ | 83.00 ± 0.66$^{b}$ | 101.00 ± 1.12$^{a}$ | 80.50 ± 1.02$^{bc}$ | 82.00 ± 0.74$^{b}$ |
| $Mg^{2+}$ | 62.40 ± 0.28$^{b}$ | 69.80 ± 0.40$^{b}$ | 80.10 ± 0.17$^{a}$ | 79.40 ± 0.11$^{a}$ | 80.00 ± 0.28$^{a}$ | 79.40 ± 0.17$^{a}$ |
| $Ca^{2+}$ | 103.00 ± 0.33$^{b}$ | 97.80 ± 0.76$^{c}$ | 129.00 ± 0.63$^{a}$ | 107.00 ± 0.43$^{b}$ | 119.00 ± 0.22$^{ab}$ | 93.20 ± 0.47$^{c}$ |
| $PO_4^{3-}$ | 205.00 ± 0.76$^{d}$ | 259.00 ± 0.83$^{c}$ | 288.00 ± 0.67$^{b}$ | 334.00 ± 0.70$^{a}$ | 271.00 ± 0.74$^{bc}$ | 271.00 ± 0.59$^{bc}$ |
| $Cl^{-}$ | 460.00 ± 1.59 $^{cd}$ | 440.00 ± 1.68$^{d}$ | 470.00 ± 2.05$^{c}$ | 520.00 ± 1.76$^{a}$ | 500.00 ± 1.45$^{b}$ | 500.00 ± 1.37$^{b}$ |

BFS: blank fish soup; BEFS: *Boletus edulis* fish soup; LEFS: *Lentinus edodes* fish soup; ABFS: *Agaricus blazei* fish soup; AAFS: *Agrocybe aegerita* fish soup; DFS: *Dictyophora* fish soup; MA: Malic acid; LA: Lactic acid; CA: Citric acid; SA: Succinic acid.

different fish soup samples, ABFS exhibited the highest concentrations of $Na^{+}$ and $Cl^{-}$ ions, followed by AAFS. The results supported the fact that adding edible fungi was an effective way to increase the amount of inorganic ions, and hence, influence the taste of fish soups.

### 3.5. E-nose analysis

E-nose is a sensitive technique that was used for characterizing the aroma profile of the different mushroom fish soups, with Fig. 2(A) showing the response intensity curves for flavor sensors obtained for each sample. Overall, the fish soups showed higher response values for sensors R(2) (sensitive to nitrogen oxide), R(5) (sensitive to alkanes and aromatic), R(8) (sensitive to ethanol), R(9) (sensitive to aromatic and sulphur-organic) and R(10) (sensitive to alkane), and this could be attributed to the large amounts of flavor compounds, such as alcohols, aldehydes and ketones, present in fish meat (Zhu et al., 2021). The response values also varied across the different fish soup samples, likely due to differences between the edible fungi that changed the flavor profile of the soups. In addition, the curves for BEFS and LEFS at sensors R(1) (sensitive to aromatic), R(2) (sensitive to nitrogen oxide) and R(6) (sensitive to methane) showed significant overlapping, with the response values being superior to those of other groups. Finally, the responses of ABFS, AAFS and DFS greatly outperformed those of other groups for sensor R(7), which was sensitive to inorganic sulfides.

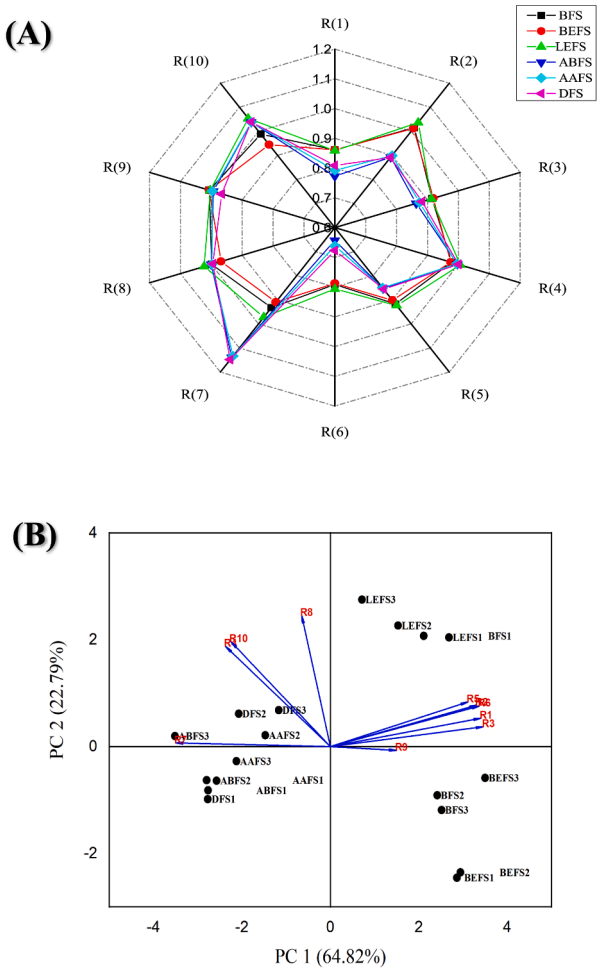


**Fig. 2.** Radar chart (A) and PCA analysis (B) for E-nose of fish soups added with varying edible fungi.

In order to explore the correlation between the fish soups and the sensors, a PCA analysis plot, accounting for 87.61 % of the total variance, was generated (Fig. 2(B)). The soups containing different edible fungi were then distributed within four quadrants, with no overlap between the samples. All fish soup samples could be divided into three clusters (the LEFS sample, the BFS, BEFS sample or the ABFS, AAFS, DFS sample) according to their distribution. In particular, LEFS was significantly correlated with most of the sensors, hence indicating that it was rich in aromatic compounds.

### 3.6. GC-IMS analysis

#### 3.6.1. Vocs of different soup samples

The flavor profiles of fish soup containing different edible fungi were explored using GC-IMS, with the results, presented in the form of topographic plots (Fig. 3(A)), showing the retention time (RT-vertical axis), drift time (DT-horizontal axis) and reaction ion peak (RIP-vertical line) (Gerhardt, Birkenmeier, Sanders, Rohn, & Weller, 2017). All samples contained large amounts of VOCs as inferred from the color depth and peak intensity of the plots. In order to examine differences in the VOCs of the different fish soups, a 2D spectrum (Fig. 3(B)) of the flavor compounds in edible fungi-fish soup was obtained after subtracting that of the pure fish soup (BFS). The amount of flavor compounds contained in the red area (X) was higher for ABFS than for BFS, and conversely, the amount of flavor compounds in the blue area (Y) was lower in AAFS compared with the BFS sample. Furthermore, qualitative analyses of the soups' VOCs were also performed using the ion drift time and ion peak strength. In this case, each point of the RIP peak represented a VOC, which was identified via the GC-IMS Library before being summarized in Table S1. A total of 52 flavor compounds, which included aldehydes (27), ketones (11), alcohols (8), esters (4), and others (2), were identified from the fish soups.

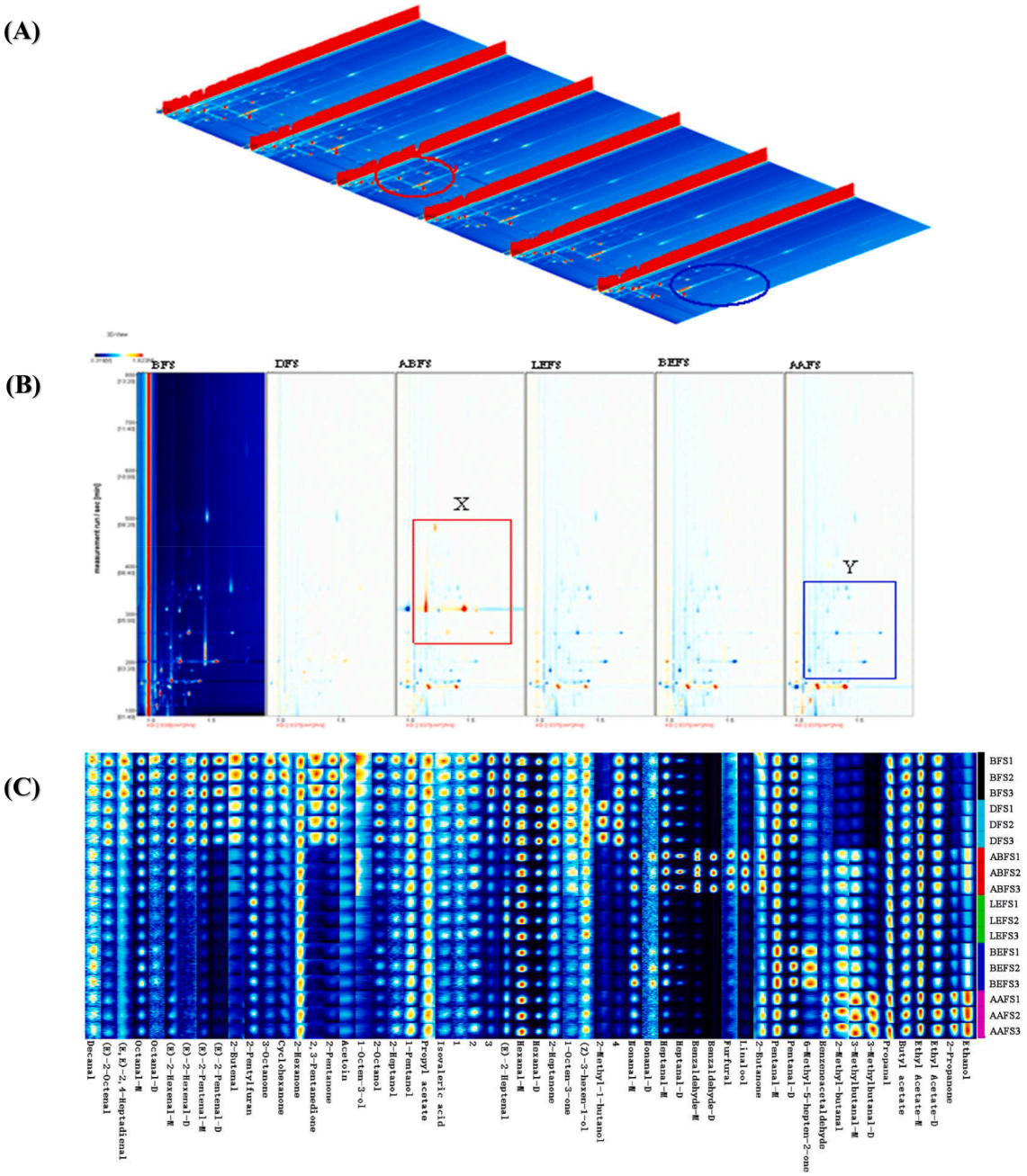


**Fig. 3.** Topography (A), topographic plots (B) and gallery plots (C) of flavor compounds in fish soups added with varying edible fungi.

*3.6.2. Gallery plots analysis of different soup samples*

In order to highlight the difference of VOCs in fish soups containing different edible fungi, the VOCs of each soup sample were tested three times in parallel, and the GC-IMS fingerprint was obtained. As shown in fingerprint plot (Fig. 3C), aldehydes, ketones and alcohols were the major flavor compounds with low threshold and strong odor, which contributed significantly to the overall flavor of fish soup (Zhang et al., 2019b).

Aldehydes are mainly generated from amino acids degradation, Strecker degradation and fatty acids oxidation (Feng et al., 2017). In this study, a total of 18 odor-active aldehydes, with carbon chain lengths mostly between C4 and C10, were identified in the fish soup samples. The BFS sample contained abundant aldehydes, including E-2-octenal, (E, E)-2,4-heptadienal, octanal, E-2-hexenal, E-2-pentenal and E-2-heptenal. Generally, aldehydes such as hexanal, octanal, E-2-octenal, E-2-heptenal, (E, E)-2,4-heptadienal, heptanal and nonanal are characterized by fatty, fishy, grassy and earthy-musty odors, and have been regarded as the main flavor compounds involved in off-flavor of fish and fish products (Podduturi, Petersen, Mahmud, Rahman, & Niels, 2017; Zhou, Chong, Ding, Gu, & Liu, 2016). The addition of different edible fungi altered the aldehyde composition of the fish soups. As far as DFS was concerned, hexanal, E-2-hexenal and E-2-heptenal were the main aldehydes, while 2-butenal, E-2-pentenal and E-2-octenal were detected in slightly higher amounts compared with the other mushroom fish soups. On the other hand, large amounts of furfural, benzaldehyde, heptanal and nonanal were found in the case of ABFS, with the levels largely exceeding those present in other edible fungi fish soups. Benzaldehyde, with its bitter almond flavor, is a characteristic flavor compound of *Agaricus blazei* (Stijve, Amazonas, & Giller, 2002), while heptanal exerts a coffee and chocolate aroma at low concentrations, but a greasy, spicy and musty odor at high concentrations (Xun et al., 2020). These could explain the lower "Smell" score received by the ABFS

sample (Fig. 1(A)). BEFS had the highest pentanal content of all fish soups, and benzeneacetaldehyde, 3-methylbutanal, 2-methylbutanal and propanal were the main aldehydes for AAFS. Of these, benzeneacetaldehyde and 3-methylbutanal were produced from the Strecker degradation (Meng et al., 2022; Feng et al., 2017), with 3-methylbutanal and 2-methylbutanal also resulting from lipid degradation during heating (Chang et al., 2021). In general, compared with pure fish soup, the addition of edible fungi reduced the contents of some odorous aldehydes. For example, the contents of (E, E)-2,4-heptadienal, octanal and E-2-pentenal in all mushroom-fish soups were lower than that of BFS. In addition, the contents of E-2-octenal, E-2-hexenal and E-2-heptenal in ABFS, LEFS, BEFS and AAFS were reduced compared with BFS. It was speculated that the improvement of sensory scores of fish soup after adding mushroom could be related to the decline of these off-flavor aldehydes.

Compared with aldehydes, ketones, produced from fatty acid oxidation, amino acid degradation and the Maillard reaction, have higher thresholds (Xun et al., 2020). High concentrations of a number of ketones, including 2-hexanone, 2,3-pentanedione, 2-pentanone, 3-octanone and acetoin, were identified in the BFS sample. For DFS, the amount of 2-heptanone, 1-octen-3-one, 2-pentanone and 2,3-pentanedione was comparable with that of BFS, but it significantly exceeded that of other edible fungi-fish soups. Of these, 2-heptanone, produced by linoleic acid oxidation, imparted a cinnamon-like and fruity flavor (Yang et al., 2021), while 1-octen-3-one brought a unique fungal flavor to the sample. As far as the ABFS and BEFS samples were concerned, 2-butanone and 6-methyl-5-heptene-2-one were respectively the most dominant flavor compound. Finally, the concentration of 2-propanone was particularly prominent in AAFS, but was almost absent in the other soup samples, including BFS. Therefore, it could be inferred that 2-propanone was mainly obtained from *Agrocybe aegerita*.

Alcohols can be divided into saturated and unsaturated ones, both of which are considered to be involved in imparting fatty meat flavor (Chang et al., 2021). A total of eight alcohols, including 1-octen-3-ol, 2-octanol, 2-heptanol, 1-pentanol, linalool, ethanol, Z-3-hexen-1-ol and 2-methyl-1-butanol, were detected in the fish soup samples. 1-octen-3-ol, with its sweet, earthy odor, is a common compound found in fish products (Peinado, Miles & Koutsidis, 2016; Dermiki et al., 2013). Regarding the soup samples, the amounts of Z-3-hexen-1-ol and 2-methylbutanol were higher in DFS, while for ABFS, a high concentration of linalool with its strong fresh sweet, woody and floral fragrance was obtained. In addition, the concentration of ethanol in the AAFS sample was also of concern.

Esters, which emit pleasant fruit flavor, are generally formed by esterification of carboxylic acids and alcohols (Liu, Chien & Kuo, 2013). In the current results, esters were detected only in BFS and AAFS samples. Propyl acetate was higher in BFS sample, and butyl acetate and ethyl acetate were higher in AAFS sample. Ethyl acetate imparted a pleasant fruity ethereal aroma, which provided a pleasant aroma for AAFS sample and contributed to the high sensory acceptability (Fig. 1) of AAFS sample.

Others. Furans imparts a caramel, sweet and baked flavor. 2-pentylfuran, a type of non-carbonyl oxidation product derived from linoleic acid and other n-6 PUFAs, can also impart a pleasant flavor to meat products (Li et al., 2011). Finally, isovaleric acid could be found in the BFS sample.

### 3.6.3. *Clustering analysis of different soup samples*

PCA analysis of the fish soup samples was performed based on the relative content of the volatile compounds. As presented at Fig. 4(A), the different samples could be clearly distinguished from each other and clustering trend.

In order to further analyze the differences in volatile compounds between the different fish soups, OPLS-DA analysis was undertaken. In this case, the model values for R2X, R2Y and Q2 were 0.934, 0.979 and 0.954 respectively, and hence, the model was a good fit with suitable

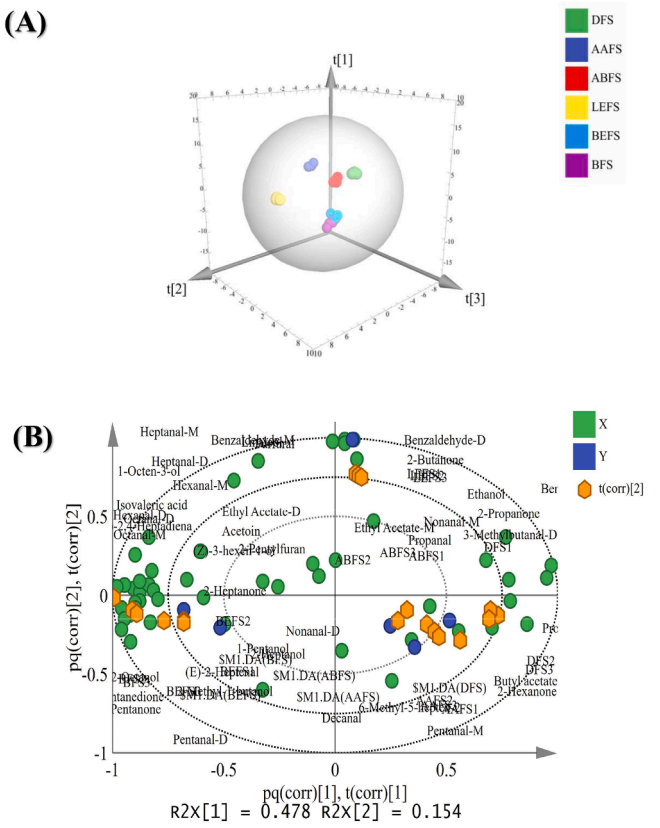


**Fig. 4.** PCA (A) and OPLS-DA analysis (B) of flavor compounds in fish soups added with varying edible fungi.

predictive value. As shown in Fig. 4(B), the fish soup samples containing different edible fungi were concentrated in the first, third and fourth quadrants, with most characteristic VOCs being distributed in ellipses between 50 % and 100 %. The flavor of the BFS sample was close to that of BEFS, and while in terms of flavor, ABFS, DFS and AAFS were similar. However, LEFS was significantly different, particularly with the presence of benzaldehyde, furfural, linalool and 2-butanone in the sample. Based on the results, the fish soup samples could be divided into three parts (Category I: BFS and BEFS, Category II: ABFS, DFS and AAFS, Category III: LEFS).

## 4. Conclusion

This study explored the flavor profile of fish soups containing different edible fungi. The sensory acceptance of the fish soup was optimized by adding the edible fungi, with AAFS and DFS exhibiting the most harmonious flavor. There were also marked increases in the amounts of non-volatile compounds, including free amino acids, nucleotides, organic acids and inorganic ions in the mushroom fish soups. The EUC value of AAFS was 2.9 times higher than that of BFS, with the results reflecting its more harmonious taste. The overall odor characteristics of fish soups containing different edible fungi could be well distinguished using E-nose. A total of 52 flavor compounds, including aldehydes, ketones, alcohols and esters, were identified by GC-IMS. The OPLS-DA results further showed that the fish soups could be divided into three categories (I: BFS and BEFS, II: ABFS, DFS and AAFS, III: LEFS) based on similarities in their flavor characteristics. The results support that edible fungi as a natural component can improve the flavor profile of fish soup, and this cooperate has potential advantages in the development of novel soup under reduced salt conditions.

## CRediT authorship contribution statement

**Yanan Lv:** Writing – original draft. **Xuting Bai:** Data curation. **Honglei Zhao:** Writing – review & editing. **Yongxia Xu:** Conceptualization, Writing – review & editing. **Jianrong Li:** Project administration. **Xuepeng Li:** Supervision, Project administration.

## 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

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

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.101059.

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