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Effect of selenium enrichment on abundance of volatile and non-volatile flavour components in fruiting bodies of *Cordyceps cicadae*

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Abstract

Cordyceps cicadae has unique flavour profiles that are worth exploring to maintain and improve their flavour. A suitable concentration of Na_2SeO_3 could improve the flavour of *C. cicadae*, but there is limited research on this topic. In the present work the volatile and non-volatile flavour components of the fruiting bodies of *C. cicadae* cultured in media containing 0, 40, 80, 120, and 160 mg/kg Na_2SeO_3 were determined, and multivariate statistical analyses combined with variable importance in the projection (VIP) values further revealed the key flavour components. The results showed that selenium enrichment resulted in some improvement in the types and contents of flavour components of the fruiting bodies of *C. cicadae*, as well as an increase in the taste activity value (TAV) and equivalent umami concentration (EUC). In conclusion, the results suggested that selenium enrichment could be employed as a processing technique to improve the flavour quality of the fruiting bodies of *C. cicadae*.

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Introduction

Selenium (Se) plays a crucial role in human health, supporting important physiological processes like cellular metabolism and immune system regulation (Wang *et al.*, 2024). Selenium deficiency has been shown to predispose individuals to Keshan and Kashin-Beck diseases (Kieliszek *et al.*, 2019). Dietary Se consists of both organic and inorganic forms, with selenomethionine (SeMet), selenocysteine ($SeCys_2$), selenite (IV), and selenate (VI), making up nearly all the Se in the common human diet (Hu *et al.*, 2019). Compared with inorganic Se, organic Se is less toxic, better absorbed, more bioavailable, and more readily taken up by selenoproteins in the body (Roman *et al.*, 2013). Therefore, the conversion of inorganic to organic Se has received increasing attention in recent years. Compared with Se from plant-based sources like broccoli, Brazil nuts, rice, and wheat, as well as from animal-based sources such as meats and eggs, edible mushrooms are considered Se-enriched food materials (Kieliszek *et al.*, 2019). This is due to their rapid growth, lower calorie content, pleasant taste and flavour, and superior capacity for Se biotransformation (Ning *et al.*, 2022). Selenite and selenate particularly effective when converted to Se-methyl-selenocysteine (MeSeCys), selenomethionine (SeMet), and selenocysteine (SeCys) by *Auricularia auricular* and *Cordyceps militaris* (Hu *et al.*, 2019).

Cordyceps cicadae is a new type of edible fungus, also known as *Isaria cicadae* Miquel or *Paecilomyces cicadae*, which has been documented since the 5th century A.D. (Zeng *et al.*, 2014; Nxumalo *et al.*, 2020). The nutritional composition of *C. cicadae* is similar to *C. militaris*, and rich in amino acids, polysaccharides, adenosine, cordycepin, and mannitol (Zeng *et al.*, 2014). Modern pharmacological studies have indicated that *C. cicadae* contains many active ingredients that have

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many functions, such as resisting inflammation, relieving pain, suppressing immunity, modulating the immune system, exerting antifatigue effects, improving renal function, and exerting antibacterial effects (Xu *et al.*, 2010). Many recipes from ancient and modern folks have attracted attention for their tonic properties. As a traditional and valuable Chinese medicinal material, *C. cicadae* does not exhibit toxicity, and it is also the only Chinese medicinal material that can be given to children or even infants, demonstrating the high safety and development value of *C. cicadae* (Chen *et al.*, 2017). Previous research has examined the impact of combined Se and zinc (Zn) supplementation on enhancing several nutrients in *C. cicadae* (Zhao *et al.*, 2023). However, information about the impact of Se enrichment on the volatile and non-volatile constituents of *C. cicadae* is still unknown.

The application of Se at an appropriate concentration provides various advantages in mushroom cultivation. Several studies have reported that Se treatment can influence carbohydrate, protein, and lipid metabolisms, as well as other physiological processes, resulting in changes to mushroom biomass yield and nutritional compositions (Kieliszek *et al.*, 2019). The fruiting bodies of *Flammulina velutipes* exhibited significant increases in protein, polysaccharide, essential amino acid, and total amino acid contents after being exposed to escalating concentrations of selenite (Dong *et al.*, 2021). Furthermore, Ma *et al.* (2023) investigated the effect of Se enrichment on the volatile components of Se-hyperaccumulating vegetable *Cardamine violifolia*. The findings indicated that the edible flowers contained significantly higher Se levels compared to leaves and stems, and the flowers had more diverse range of volatile organic compounds (VOCs) than leaves and stems (Ma *et al.*, 2023). In the present work, the volatile flavour components of the fruiting bodies of *C. cicadae* cultured at different concentrations of Na_2SeO_3 were determined. Additionally, the alterations in the non-volatile flavour components, including amino acids, soluble sugars, organic acids, and 5′-nucleotides, were evaluated. In light of the findings of previous studies, it was hypothesised that Se enrichment in *C. cicadae* may improve the flavour characteristics of the fruiting bodies of *C. cicadae* by affecting the content and composition of the volatile and non-volatile flavour components.

Materials and methods

Cultivation and preparation of C. cicadae fruiting bodies

The *C. cicadae* strain (Anhui Cordyceps Source Biotechnology Co. Ltd., Huainan, China) was inoculated into media containing Na_2SeO_3 at concentrations of 0, 40, 80, 120, and 160 mg/kg, and cultured at 25°C for approximately 25 d, with triplicates for each concentration. The fruiting bodies of *C. cicadae* were harvested upon reaching maturity, dried in an electrothermal drying oven (Model DHG-9203A, Sanfa Scientific Instruments Ltd., Shanghai, China) at 50°C, and pulverised into powder using a high-speed grinder (JP-400B, Yongkang Jiupin Industry and Trade Co., Ltd., Zhejiang, China). The powders were designated CK, Se40, Se80, Se120, and Se160, and kept at 4°C for subsequent analyses.

Extraction of volatiles

Headspace solid-phase microextraction (HS-SPME) was carried out following the procedure outlined by Dong *et al.* (2017) with some modifications. In brief, 1.0 g of dried fruiting body powder from *C. cicadae* was placed into a 15 mL glass vial. The volatiles were collected *via* a polydimethylsiloxane (PDMS) fibre (100 μm; Supelco, USA). The vial was equilibrated in a 60°C water bath for 20 min, followed by fibre exposure to the headspace above the sample for an additional 30 min. Subsequently, the fibre was quickly inserted with the GC injector for desorption for 5 min at 250°C in splitless mode.

GC-MS analysis

The GC-MS analysis of volatiles was performed on an Agilent 8890AGC instrument equipped with a 5977B MS instrument (Agilent Technologies, USA). The volatiles were separated by an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies, USA). Helium was used as the carrier gas (purity > 99.999%). The flow rate was 1.0 mL/min, and the injector temperature was 250°C. The oven temperature program was as follows: held at 50°C for 2 min, increased to 110°C at 3°C/min, increased to 150°C at 2°C/min for 2 min, and finally increased to 210°C at 10°C/min for 5 min.

In addition, the mass spectrometry parameters were set as follows: ion source temperature of 230°C,

ionisation energy of 70 eV, interface temperature of 280°C, and quadrupole temperature of 150°C. Mass spectrometry was performed at a rate of one scan per second. The data acquisition rate was one scan per second, and the coverage range was from 50 to 550 *m/z*. The volatile components (VOCs) were identified by matching their mass spectra with entries in the National Institute of Standards and Technology (NIST 20) database, with retention indices (RI) calculated based on *n*-alkane values. The relative content was calculated based on the percentage of each compound's peak area to the total peak area (Dong *et al.*, 2017).

Free amino acid analysis

The free amino acids were analysed following a previous method with some modifications (Savych *et al.*, 2022). Briefly, 2 mL of 0.1 M HCl solution was added to a centrifuge tube containing 0.1 g of fruiting body powder from *C. cicadae*, and extracted by ultrasonication at 50°C for 3 h. After evaporation, the residue was reconstituted by adding 1 mL of deionised water. The suspension was subsequently centrifuged (MINI-0K Centrifuge, Mio Instruments Ltd., Hangzhou) at 8,000 rpm for 5 min. The resulting supernatant was then filtered through a 0.22 μm cellulose membrane prior to being injected into the amino acid analyser (S-433D, Sykam, Germany). Amino acids were identified and quantified by comparing the retention time of the amino acids with the relevant standard amino acids (Sykam, Germany), and the concentration of the knots and standards.

Soluble sugar and polyol analysis

The analysis of soluble sugars and polyols in the samples was performed following the method described by Li *et al*. (2015) with some modifications. A sample of 0.1 g fruiting bodies of *C. cicadae* powder was first mixed with 5 mL of deionised water, and incubated at 50°C for 30 min. The mixture was subsequently centrifuged (MINI-0K centrifuge) at 8,000 rpm for 5 min. Following centrifugation, the supernatant was filtered through a 0.22 μm cellulose membrane prior to analysis by high-performance liquid chromatography (HPLC) using an Agilent 1260 HPLC (Agilent Technology Co., Ltd., USA).

The HPLC system was equipped with a COSMOSIL Sugar-D column (4.6 × 250 mm, 5 μm; Jinpan Biotechnology Co., Ltd., Shanghai, China). The chromatographic conditions were set with a mobile phase of acetonitrile and water in a 3:1 (v/v) ratio, a flow rate of 1.0 mL/min, and an RID detector wavelength of 210 nm. The column oven was maintained at 30°C, and the injection volume was 10 μL. Sugars and polyols were identified and quantified by authentic standards of sugars and polyols obtained from Shanghai Aladdin Biotechnology Co., Ltd., China.

5′-Nucleotide analysis

The extraction and analysis of 5′-nucleotides were performed following a modified version of the procedure outlined by Li *et al*. (2015). Briefly, 0.1 g of fruiting body powder of *C. cicadae* was immersed in 5 mL of deionised water. The mixture was boiled for 5 min, and centrifuged (MINI-0K centrifuge) at 8,000 rpm for 5 min. The residue was processed *via* the same method, and the resulting mixture was filtered through a 0.22 μm cellulose membrane. Next, 20 μL of filtrate was injected into an LC-16 system (Shimadzu Corporation Management (China) Co., Japan) *via* UV detection with a Luna®-C18 column (4.6 × 250 mm, 5 μm; Phenomenex, USA), from which the 5′-nucleotides were distinguished *via* gradient elution. The temperature of the column was set at 30°C, and analyte detection occurred at 254 nm. The following solvents were used in the gradient program: solvent A, 0.5% phosphoric acid (Shanghai Aladdin Biotechnology Co., Ltd., China); and solvent B, 100% MeOH (Xilong Science Co., China). The following gradient program was used: 0 - 5 min, 0% B; 5 - 11 min, 0 - 15% B; 11 - 15.5 min, 15 - 28% B; 15.5 - 19 min, 28 - 40% B; 19 - 22 min, 40 - 0% B; and 22 - 25 min, 0% B. The flow rate was maintained at 1.0 mL/min throughout the analysis. The 5′-nucleotides were identified *via* standard 5′-nucleotides (Shanghai Aladdin Biotechnology Co., Ltd., China), and their quantification was done by comparing the peak areas of the samples to those of the external standards.

Organic acid analysis

The organic acids were analysed following a previously described method with some modifications (Li *et al.*, 2015). A suspension of fruiting body of *C. cicadae* (0.1 g in 5 mL of deionised water) was first extracted in a 40°C water bath for 5 min. Next, the suspension was centrifuged (MINI-0K centrifuge) at 8,000 rpm for 5 min. Then the supernatant was filtered through a 0.22 μm cellulose membrane, and injected into a HPLC

system. Then, 10 μL of filtrate was introduced into the Agilent 1260 HPLC system for analysis using a DAD detector, with a Luna®-C18 column (4.6 × 250 mm, 5 μm; Phenomenex, USA). The temperature of the column was maintained at 30°C, and detection occurred at 210 nm. The mobile phases were as follows: mobile phase A, 0.01 M KH_2PO_4/H_3PO_4 (pH = 2.0); mobile phase B, ultrapure water; and mobile phase C, 100% MeOH (Xilong Science Co., China). The following gradient program was used: 0 - 7 min, 75% B and 0% C; 7 - 10 min, 75 - 69% B and 0 - 6% C; 10 - 21 min, 69% B and 6% C; 21 - 23 min, 69 - 75% B and 6 - 0% C; and 23 - 30 min, 75% B and 0% C. The flow rate was maintained at 1.0 mL/min throughout the analysis. Each organic acid was identified and quantified using organic acid standards (Shanghai Aladdin Biotechnology Co., Ltd., China).

Taste activity value (TAV)

The TAV was determined by comparing the concentration of taste compounds in the fruiting bodies of *C. cicadae* with their corresponding sensory thresholds. Compound with a TAV greater than 1 have the potential to significantly contribute to the overall taste profile of the fruiting bodies of *C. cicadae* (Du *et al.*, 2024).

Equivalent umami concentration (EUC)

The EUC represents the umami intensity produced by the synergistic interaction between umami amino acids and nucleotides. The EUC is equivalent to the umami intensity produced by a single concentration of monosodium glutamate. The EUC (expressed per 100 g of a sample) reflects the MSG concentration that corresponds with the flavour intensity resulting from the interaction of MSG and 5′-nucleotides. The EUC was calculated using Eq. 1 outlined by Yamaguchi *et al.* (2006):

$$Y = \Sigma a_i b_i + 1218(\Sigma a_i b_i) \times (\Sigma a_j b_j) \quad \text{(Eq. 1)}$$

where, Y = EUC of the mixture in g MSG/100 g; a_i = concentration (g/100 g) of each umami amino acid, specifically glutamic acid (Glu) or aspartic acid (Asp); b_i = relative umami concentration (RUC) of each amino acid in relation to MSG (Glu: 1; and Asp: 0.077); a_j = concentration (g/100 g) of each umami 5′-nucleotide (5′-AMP, 5′-IMP, or 5′-GMP); b_j = RUC for each 5′-nucleotide in relation to 5′-IMP (with values of 1 for 5′-IMP, 2.3 for 5′-GMP, and 0.18 for 5′-AMP); and 1218 = synergistic constant based on the concentration of g/100 g.

Statistical analysis

The data were expressed as mean ± standard deviation (SD) of three independent experiments. Statistical analysis was performed using SPSS 26.0. The graphs were generated using Origin 2018 (Origin Lab Co., Northampton, MA, USA) and TBtools software (Du *et al.*, 2024). Multivariate statistical analysis was performed using SIMCA 14.1.

Results and discussion

Volatile compounds identified by HS-SPME-GC-MS

A total of 51 volatile compounds were identified with high confidence in five fruiting body samples of *C. cicadae via* the NIST 20.0 database and retention indices (Table 1). These compounds included one acid, two alcohols, six aldehydes, six alkanes, 17 esters, five heterocyclic compounds, six ketones, six terpenes, and two other compounds. The numbers of volatile categories of Se-enriched fruiting bodies of *C. cicadae* are shown in Figure 1B. A total of 31 compounds were detected in the control samples. In contrast, 41, 41, 34, and 36 volatile compounds were detected in the Se40, Se80, Se120, and Se160 samples, respectively. The proportions of the volatile categories in the Se-enriched fruiting bodies of *C. cicadae* are shown in Figure 1A. Compared to the CK, the proportions of aldehydes, acids, alkanes, heterocyclic compounds, and esters increased in the Se40 and Se80 samples. However, at higher Na_2SeO_3 concentrations (Se120 and Se160 samples), the relative proportions of aldehydes, ketones, alkanes, and heterocyclic compounds decreased compared with those in the CK. Moreover, the proportion of terpenes in the Se-enriched fruiting bodies of *C. cicadae* decreased compared to the CK. The proportion of esters increased from 54.92% in the CK to a maximum of 79.17% in the Se120 sample, and the number of esters increased from 11 to 15.

Volatile compounds, including alcohols (1,2,4-butanetriol and 1-(3-butyloxiranyl) ethenone), acids (hexanoic acid), and other compounds (such as formamide, N-methylthio, and N-formylglycine) were detected in the Se-enriched fruiting bodies of *C. cicadae* that were absent in the control. Based on the findings of previous studies, it was speculated that the production of new compounds could be linked to the

Table 1. Volatile compounds identified in the selenium-enriched fruiting bodies of *C. cicadae* using HS–SPME–GC–MS.

No.	Compounds name	RI	Relative content (%)				
			CK	Se40	Se80	Se120	Se160
	Alcohol						
1	1,2,4-Butanetriol	/	nd	nd	nd	0.27 ± 0.06^{a}	nd
2	1-(3-Butyloxiranyl) ethanone	1041.41	nd	0.16 ± 0.03^{a}	nd	nd	nd
	Aldehyde						
3	Hexanal	/	1.09 ± 0.27^{c}	3.81 ± 0.59^{a}	2.40 ± 0.17^{b}	0.82 ± 0.12^{c}	1.37 ± 0.51^{c}
4	2-n-Butylacrolein	848.50	nd	0.94 ± 0.15^{a}	0.96 ± 0.08^{a}	0.22 ± 0.03^{b}	nd
5	Nonanal	1050.22	nd	0.25 ± 0.04^{a}	0.15 ± 0.01^{b}	nd	nd
6	2-Butylhept-2-enal	1257.22	1.27 ± 0.36^{a}	1.29 ± 0.20^{a}	1.06 ± 0.17^{ab}	0.76 ± 0.04^{b}	0.70 ± 0.19^{b}
7	2-Butyl-2-octenal	1324.63	10.33 ± 2.11^{ab}	11.23 ± 2.08^{a}	9.31 ± 1.14^{abc}	7.58 ± 0.64^{bc}	6.81 ± 1.76^{c}
8	Pentadecanal-	1647.85	nd	0.20 ± 0.05^{a}	nd	nd	nd
	Acid						
9	Hexanoic acid	978.57	nd	0.68 ± 0.16^{a}	0.33 ± 0.18^{b}	nd	0.75 ± 0.29^{a}
	Ketone						
10	Methylglyoxal dimethyl acetal	/	nd	nd	nd	0.22 ± 0.03^{a}	nd
11	3-Octen-2-one	1018.19	0.64 ± 0.06^{a}	0.59 ± 0.06^{ab}	0.49 ± 0.05^{b}	0.30 ± 0.03^{c}	0.33 ± 0.11^{c}
12	(+)-2-Bornanone	1069.98	1.30 ± 0.34^{a}	0.60 ± 0.04^{b}	0.40 ± 0.05^{b}	0.38 ± 0.02^{b}	0.57 ± 0.20^{b}
13	Perilla ketone	1237.01	0.43 ± 0.09^{a}	0.32 ± 0.04^{ab}	0.21 ± 0.03^{b}	nd	0.23 ± 0.11^{b}
14	2-Undecanone	1271.48	0.99 ± 0.13^{a}	1.05 ± 0.21^{a}	0.90 ± 0.12^{ab}	0.67 ± 0.03^{bc}	0.57 ± 0.14^{c}
15	Dipivaloylmethane	1340.25	0.84 ± 0.23^{b}	1.36 ± 0.38^{a}	1.11 ± 0.12^{ab}	0.67 ± 0.03^{b}	0.74 ± 0.25^{b}
	Alkane						
16	Eucalyptol	1013.39	0.48 ± 0.11^{a}	0.29 ± 0.07^{b}	0.25 ± 0.08^{b}	0.15 ± 0.01^{b}	0.26 ± 0.11^{b}
17	Cyclopentane, butyl-	1031.47	nd	0.21 ± 0.05^{a}	0.22 ± 0.02^{a}	nd	nd
18	1-methoxy-4-methyl-bicyclo [2.2.2] octane	1307.07	0.44 ± 0.10^{c}	1.56 ± 0.24^{a}	1.14 ± 0.14^{b}	0.61 ± 0.04^{c}	0.72 ± 0.21^{c}
19	Tetradecyl chloride	1395.90	0.46 ± 0.25^{a}	0.26 ± 0.03^{a}	0.25 ± 0.02^{a}	nd	0.33 ± 0.12^{a}
20	Hexadecane	1597.71	0.42 ± 0.24^{a}	0.37 ± 0.05^{ab}	nd	nd	0.20 ± 0.02^{bc}
21	2-Bromo dodecane	1645.05	nd	0.23 ± 0.10^{a}	nd	nd	nd
	Terpene						
22	1S-α-Pinene	924.87	nd	nd	0.29 ± 0.27^{a}	nd	nd
23	Caryophyllene	1349.03	0.59 ± 0.33^{a}	0.39 ± 0.07^{a}	0.35 ± 0.02^{a}	nd	0.39 ± 0.14^{a}
24	cis-β-Farnesene	1372.98	0.99 ± 0.43^{a}	0.55 ± 0.08^{ab}	0.67 ± 0.02^{a}	0.19 ± 0.06^{b}	0.69 ± 0.27^{a}

No.	Compounds name	RI	Relative content (%)				
			CK	Se40	Se80	Se120	Se160
25	Germacrene D	1385.29	nd	nd	nd	nd	0.25 ± 0.09a
26	γ-Gurjunene	1388.03	21.27 ± 8.00^{a}	11.25 ± 2.02^{bc}	15.14 ± 0.51^{ab}	5.57 ± 1.60^{c}	17.23 ± 5.61^{ab}
27	β-Sesquiphellandrene	1519.85	1.75 ± 0.78^{a}	0.87 ± 0.13^{ab}	1.42 ± 0.09^{a}	0.29 ± 0.11^{b}	1.66 ± 0.67^{a}
	Heterocyclic compound						
28	Pyrazine, 2,5-dimethyl-	907.41	0.43 ± 0.04^{c}	0.89 ± 0.09^{a}	0.62 ± 0.05^{b}	0.32 ± 0.03^{cd}	0.29 ± 0.07^{d}
29	Praxadine	973.28	0.66 ± 0.24^{ab}	0.96 ± 0.25^{a}	0.53 ± 0.11^{b}	0.49 ± 0.04^{b}	0.46 ± 0.06^{b}
30	2-Pentylfurane	986.51	nd	0.62 ± 0.12^{a}	0.40 ± 0.07^{b}	0.34 ± 0.02^{b}	0.47 ± 0.11^{b}
31	2-Ethyl-3,6-dimethylpyrazine	1038.62	0.43 ± 0.04^{c}	1.81 ± 0.30^{a}	0.99 ± 0.09^{b}	0.19 ± 0.02^{c}	0.30 ± 0.08^{c}
32	Pyrazine, tetramethyl-	1042.63	0.26 ± 0.01^{a}	0.20 ± 0.03^{ab}	0.16 ± 0.03^{b}	nd	0.20 ± 0.07^{ab}
	Ester						
33	Methyl acetate	/	nd	nd	nd	nd	0.31 ± 0.07^{a}
34	Methyl valerate	809.40	nd	0.53 ± 0.08^{a}	0.30 ± 0.06^{b}	0.22 ± 0.00^{b}	0.29 ± 0.07^{b}
35	Methyl caproate	918.78	2.63 ± 1.01^{d}	10.84 ± 1.19^{a}	8.54 ± 0.29^{b}	4.29 ± 0.08^{c}	7.69 ± 0.69^{b}
36	Methyl heptanoate	1002.01	nd	0.34 ± 0.01^{ab}	0.16 ± 0.06^{ab}	0.42 ± 0.43^{a}	0.30 ± 0.05^{ab}
37	Methyl caprylate	1062.05	0.59 ± 0.08^{b}	0.81 ± 0.10^{a}	0.57 ± 0.12^{b}	0.24 ± 0.01^{c}	0.35 ± 0.04^{c}
38	Methyl nonanoate	1218.51	0.41 ± 0.12^{b}	0.67 ± 0.06^{a}	0.66 ± 0.04^{a}	0.26 ± 0.01^{b}	0.40 ± 0.14^{b}
39	Bornyl formate	1264.52	0.42 ± 0.17^{a}	0.27 ± 0.03^{ab}	0.17 ± 0.01^{b}	nd	0.20 ± 0.07^{b}
40	Hexanoic acid, pentyl ester	1267.40	nd	0.17 ± 0.02^{a}	nd	nd	nd
41	Methyl dodecanoate	1523.09	nd	nd	0.18 ± 0.01^{a}	0.19 ± 0.02^{a}	nd
42	Methyl tetradecanoate	1731.15	1.02 ± 0.35^{b}	1.40 ± 0.13^{b}	1.49 ± 0.08^{b}	2.50 ± 0.04^{a}	1.49 ± 0.52^{b}
43	Methyl pentadecanoate	1827.42	0.54 ± 0.18^{b}	0.96 ± 0.18^{a}	0.99 ± 0.05^{a}	0.93 ± 0.08^{a}	0.99 ± 0.29^{a}
44	Methyl palmitoleate	1902.47	1.31 ± 0.32^{c}	1.68 ± 0.32^{bc}	2.05 ± 0.15^{ab}	2.28 ± 0.21^{a}	1.55 ± 0.38^{bc}
45	Methyl palmitate	1923.46	34.41 ± 10.17^{b}	32.13 ± 3.12^{b}	34.96 ± 1.80^{b}	51.4 ± 1.43^{a}	41.43 ± 5.86^{ab}
46	Methyl linoleate	/	5.37 ± 0.75^{a}	1.47 ± 0.57^{b}	2.39 ± 0.28^{b}	4.27 ± 0.32^{a}	2.53 ± 1.17^{b}
47	Methyl petroselinate	/	7.89 ± 1.01^{b}	5.75 ± 1.92^{b}	6.90 ± 0.46^{b}	11.26 ± 0.62^{a}	6.95 ± 2.42^{b}
48	Methyl 11-octadecenoate	/	nd	nd	0.39 ± 0.04^{b}	0.55 ± 0.04^{a}	nd
49	Methyl stearate	/	0.33 ± 0.05^{a}	nd	0.33 ± 0.07^{a}	0.36 ± 0.03^{a}	nd
	Other						
50	Formamide, N-methylthio	/	nd	nd	nd	0.80 ± 0.21^{a}	nd
51	N-Formylglycine	/	nd	nd	0.19 ± 0.04^{a}	nd	nd

Note: nd, not detected. /: no RI value has been calculated for the substance. Means followed by different lowercase superscripts in similar column are significantly different ($p < 0.05$).

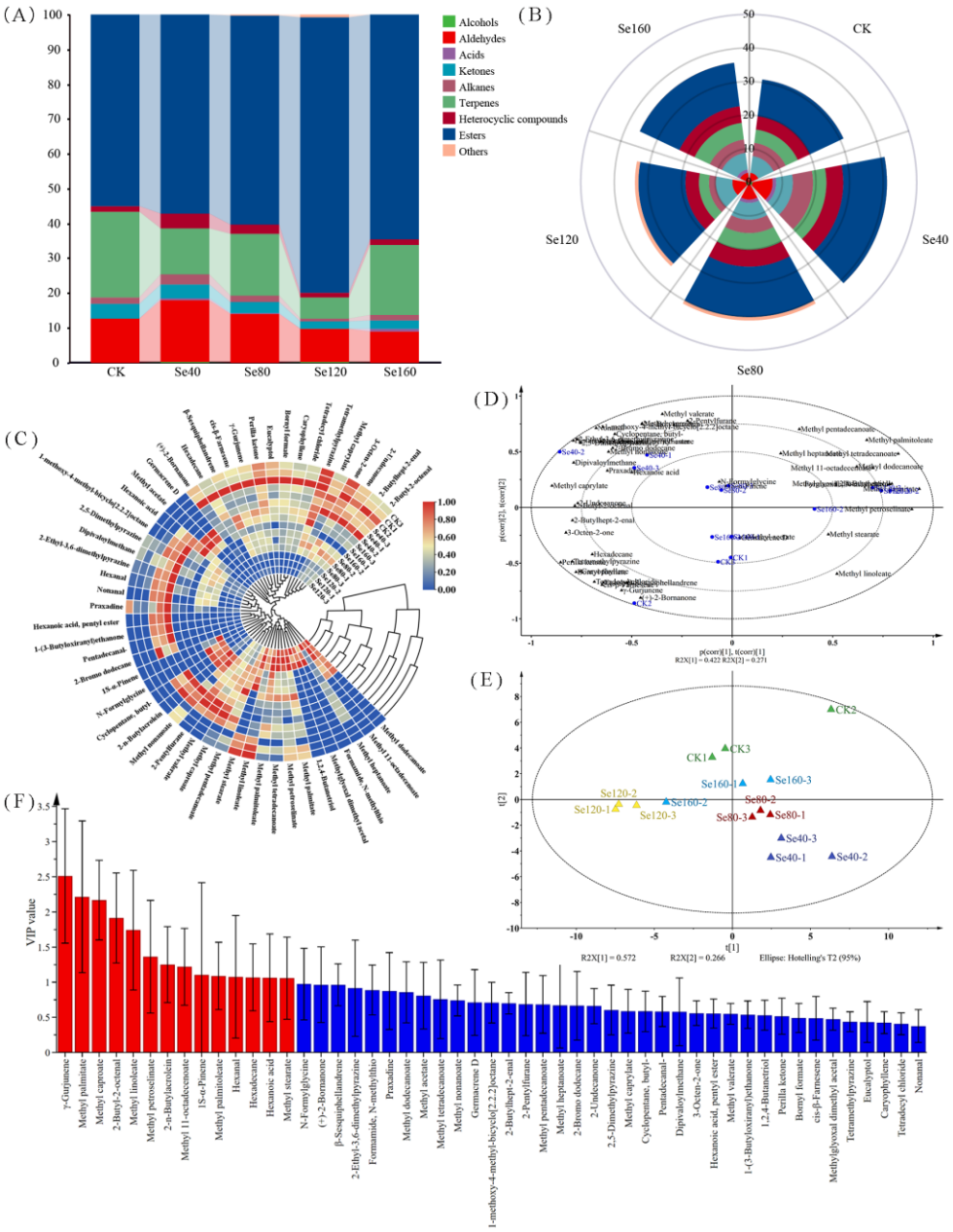

Figure 1. Volatile profiles of selenium-enriched fruiting bodies of *C. cicadae*: **(A)** proportion of the volatile categories, **(B)** number of volatile categories, **(C)** clustering heatmap of volatile compounds, **(D)** PCA score plot, **(E)** PLS-DA score plot, and **(F)** volatile compounds with VIP > 1.

mechanisms of Se biosynthesis and metabolism (Roman *et al.*, 2013). Selenium is a vital component of numerous selenoproteins. Selenium may enhance the stability and activity of enzymes involved in the biosynthesis of volatile compounds through the regulation of oxidative stress (Wang *et al.*, 2021). It was postulated that Se may lead to the formation of reactive Se-containing metabolites that can participate in various biochemical reactions, potentially altering the pathways leading to the synthesis of volatile compounds (Li *et al.*, 2022). Furthermore, Se may result in the up- or down-regulation of enzymes related to volatile compounds by influencing the expression of specific genes associated with metabolic pathways (Zhang *et al.*, 2017). However, these hypotheses require further investigation to elucidate the precise mechanisms and linkages between these pathways.

Among all five samples, esters were the dominant volatile compounds, followed by terpenes and aldehydes, contributing to a more complex flavour profile. Among the esters, methyl palmitate was the most abundant, whereas γ-gurjunene was the most prominent among the terpenes. Additionally, 2-butyl-2-octenal, with a characteristic fatty aroma, was the most notable aldehyde. Lai *et al.* (2022) reported

that Se-enriched shiitake mushrooms possessed a relatively higher number of volatile flavour compounds, suggesting that Se content may influence both the quantity and diversity of these substances. Similarly, Zhang *et al.* (2024) found that Se sprayed on tomato plants increased the concentration of flavour compounds, thereby enhancing the flavour quality of the tomatoes. Chen *et al.* (2019) reported that the application of 0.1 mg/L Se affected the composition of flavour compounds in lettuce leaf, and significantly increased the total content of flavour substances. Additionally, it has also been found that Se-enriched soybeans can enhance the flavour compound content in soy sauce (Gao *et al.*, 2022). These results corroborated the present work, and further confirmed that Se enrichment could improve the composition and content of volatile compounds in the fruiting bodies of *C. cicadae*.

Cluster analysis of volatile compounds

To compare the volatile compounds of Se-enriched fruiting bodies of *C. cicadae*, a hierarchical clustering analysis (HCA) was performed *via* a clustered heatmap. As shown in Figure 1C, the heatmap visually represents the overall profile of each volatile compound *via* coloured boxes. The HCA results revealed that the five samples were distinctly grouped into two clusters as follows: Se80 and Se120 formed one cluster, whereas CK, Se40, and Se160 formed the other cluster. The results suggested that the types and proportions of volatile compounds varied when *C. cicadae* were cultivated in media with different concentrations of Na_2SeO_3.

Differential volatile compounds

As an unsupervised clustering method, PCA provides an overview of changes in volatile compounds without prior knowledge of the data (Yang *et al.*, 2018). PCA was performed to analyse the differences in the Se-enriched fruiting bodies of *C. cicadae*, with 51 volatile compounds set as the variables (Figure 1D). The cumulative percentage of variation explained by the first two principal components was 69.3%, with 42.2% from the first component and 27.1% from the second, respectively. The five samples were not distinctly separated in the PCA plot. The Se40, Se80, and Se120 samples were positioned on the positive semiaxis of the first principal component, whereas the remaining samples were positioned on the negative semiaxis. Moreover, the CK and Se160 samples were closely positioned together. The PCA results revealed some differences in the aroma compounds of the fruiting bodies of *C. cicadae* at various Na_2SeO_3 concentrations.

The PLS-DA was employed to further analyse the differences in volatile compounds. PLS-DA is commonly used to distinguish between multiple groups of samples by establishing a relationship between two data matrices, namely, the predictor dataset (X) and response dataset (Y) (Yang *et al.*, 2018). As shown in Figure 1E, the independent variable fitting index (R^2X) was 0.984, the dependent variable fitting index (R^2Y) was 0.990, and the predictive performance index (Q^2) was 0.959. Both the R^2 and the Q^2 values were greater than 0.5, thus the model demonstrated a satisfactory fit. Additionally, the analysis showed good reproducibility and predictive ability.

The VIP scores measure the contribution and explanatory power of each metabolite in distinguishing between sample groups, facilitating the identification of marker metabolites. Typically, a threshold value of 1 is used to screen for significant metabolites (Song *et al.*, 2020). On the basis of VIP values greater than 1 as illustrated in Figure 1F, a total of 14 key compounds, such as γ-gurjunene, methyl palmitate, and methyl caproate were identified as differential volatile compounds in the five samples. These compounds contribute to the differentiation of the volatile profiles of the fruiting bodies of *C. cicadae* cultured in media with different concentrations of Na_2SeO_3, and are considered distinctive volatile compounds.

Analysis of free amino acids

Free amino acids (FAAs) are key umami-active compounds in edible mushrooms. Owing to their distinct taste profiles, FAAs can impart umami, sweet, or bitterness. Specifically, the umami amino acids consist of Glu and Asp, while the sweet amino acids consist of Gly, Thr, Ala, Pro, and Ser. In addition, the bitter amino acids consist of Met, His, Ile, Leu, Val, Arg, and Phe. Cys, Tyr, and Lys are considered tasteless (Goh *et al.*, 2017). Although the umami intensity of sodium aspartate is approximately 10% lower than that of monosodium glutamate (MSG) (Dermiki *et al.*, 2013), it still provides a distinct umami flavour. Both Asp and Glu are generally considered as umami amino acids.

The contents of FAAs in the Se-enriched fruiting bodies of *C. cicadae* are shown in Figure 2A and Table 2. Compared with that in the CK, the total

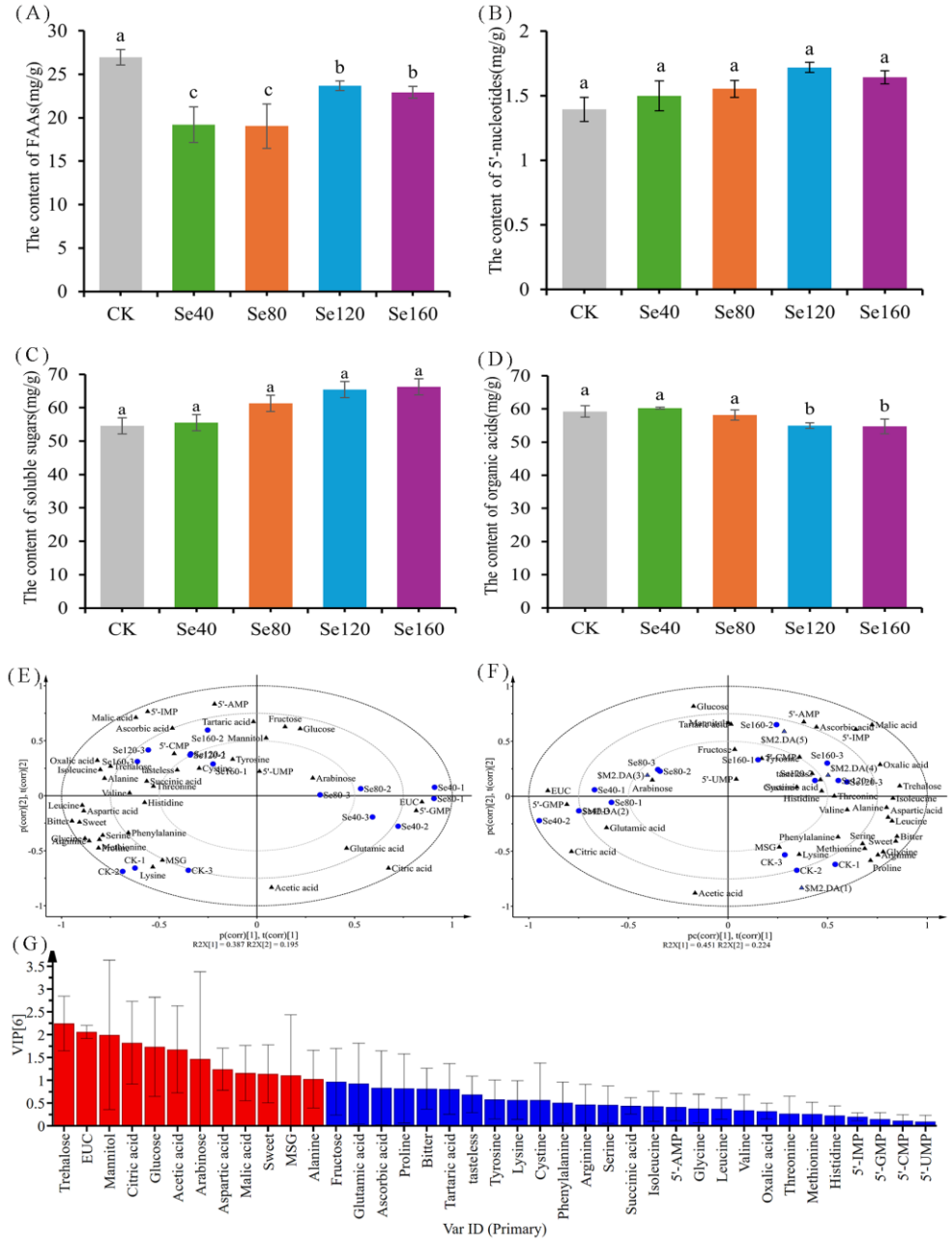

Figure 2. Non-volatile compounds analysis of selenium-enriched fruiting bodies of *C. cicadae*: **(A)** content of free amino acids, **(B)** content of 5′-nucleotides, **(C)** content of soluble sugars, **(D)** content of organic acids, **(E)** PCA score plot, **(F)** PLS-DA score plot, and **(G)** non-volatile compounds with VIP > 1.

Table 2. Non-volatile compounds in selenium-enriched fruiting bodies of *C. cicadae*.

		Content (mg/g dry weight)				
		CK	**Se40**	**Se80**	**Se120**	**Se160**
Free amino acids						
MSG	Aspartic acid	3.35 ± 0.17^{a}	1.00 ± 0.11^{c}	0.97 ± 0.11^{c}	2.17 ± 0.35^{b}	3.14 ± 0.10^{a}
	Glutamic acid	7.18 ± 0.55^{ab}	7.68 ± 1.09^{a}	7.86 ± 1.11^{a}	6.38 ± 0.37^{ab}	6.14 ± 0.23^{b}
	Total	10.53 ± 0.72^{a}	8.69 ± 1.20^{c}	8.84 ± 1.21^{c}	8.55 ± 0.10^{c}	9.27 ± 0.33^{ab}
Sweet	Threonine	1.01 ± 0.04^{a}	0.78 ± 0.06^{a}	0.88 ± 0.13^{a}	1.10 ± 0.38^{a}	0.94 ± 0.12^{a}
	Serine	2.41 ± 0.09^{a}	1.65 ± 0.13^{b}	1.67 ± 0.21^{b}	2.03 ± 0.39^{ab}	1.97 ± 0.31^{ab}
	Glycine	0.82 ± 0.04^{a}	0.45 ± 0.04^{c}	0.41 ± 0.04^{c}	0.64 ± 0.00^{b}	0.61 ± 0.06^{b}
	Alanine	2.50 ± 0.11^{b}	1.82 ± 0.27^{c}	1.75 ± 0.21^{c}	3.18 ± 0.08^{a}	2.19 ± 0.16^{b}
	Proline	3.00 ± 0.44^{a}	1.54 ± 0.04^{c}	1.26 ± 0.12^{c}	2.07 ± 0.14^{b}	2.03 ± 0.36^{b}
	Total	9.73 ± 0.50^{a}	6.24 ± 0.52^{c}	5.97 ± 0.69^{c}	9.03 ± 0.80^{a}	7.75 ± 0.85^{b}
Bitter	Valine	0.90 ± 0.04^{ab}	0.68 ± 0.11^{ab}	0.66 ± 0.09^{b}	0.99 ± 0.33^{a}	0.81 ± 0.06^{ab}
	Methionine	0.36 ± 0.02^{a}	0.18 ± 0.02^{b}	0.19 ± 0.03^{b}	0.26 ± 0.10^{b}	0.27 ± 0.06^{ab}
	Isoleucine	0.35 ± 0.03^{b}	0.20 ± 0.05^{c}	0.21 ± 0.03^{c}	0.52 ± 0.07^{a}	0.32 ± 0.06^{b}
	Leucine	0.78 ± 0.06^{a}	0.37 ± 0.07^{b}	0.38 ± 0.09^{b}	0.71 ± 0.17^{a}	0.70 ± 0.22^{a}
	Phenylalanine	1.54 ± 0.07^{a}	0.83 ± 0.46^{b}	1.01 ± 0.13^{ab}	1.21 ± 0.51^{ab}	1.18 ± 0.03^{ab}
	Histidine	0.25 ± 0.01^{a}	0.19 ± 0.03^{b}	0.21 ± 0.05^{ab}	0.21 ± 0.02^{ab}	0.26 ± 0.01^{a}
	Arginine	1.02 ± 0.05^{a}	0.57 ± 0.01^{c}	0.47 ± 0.04^{c}	0.77 ± 0.05^{b}	0.75 ± 0.13^{b}
	Total	5.20 ± 0.27^{a}	3.03 ± 0.70^{b}	3.14 ± 0.45^{b}	4.66 ± 0.43^{a}	4.30 ± 0.44^{a}
Tasteless	Cysteine	0.15 ± 0.04^{a}	0.17 ± 0.12^{a}	0.11 ± 0.05^{a}	0.40 ± 0.35^{a}	0.17 ± 0.01^{a}
	Tyrosine	0.57 ± 0.03^{a}	0.59 ± 0.51^{a}	0.43 ± 0.14^{a}	0.59 ± 0.39^{a}	0.81 ± 0.66^{a}
	Lysine	0.75 ± 0.04^{a}	0.49 ± 0.04^{c}	0.54 ± 0.07^{bc}	0.45 ± 0.02^{c}	0.61 ± 0.07^{b}
	Total	1.47 ± 0.09^{a}	1.25 ± 0.35^{a}	1.08 ± 0.24^{a}	1.43 ± 0.52^{a}	1.59 ± 0.62^{a}
Grand total		**26.93 ± 0.89^{a}**	**19.2 ± 2.06^{c}**	**19.03 ± 2.55^{c}**	**23.67 ± 0.57^{b}**	**22.91 ± 0.67^{b}**
5′-nucleotide						
5'-CMP		0.068 ± 0.013^{a}	0.069 ± 0.046^{a}	0.064 ± 0.009^{a}	0.076 ± 0.001^{a}	0.087 ± 0.006^{a}
5'-AMP		0.527 ± 0.042^{c}	0.578 ± 0.065^{c}	0.642 ± 0.048^{b}	0.787 ± 0.013^{a}	0.680 ± 0.024^{b}
5'-UMP		0.685 ± 0.053^{a}	0.689 ± 0.094^{a}	0.698 ± 0.044^{a}	0.703 ± 0.026^{a}	0.706 ± 0.022^{a}
5'-GMP		0.113 ± 0.002^{b}	0.162 ± 0.011^{a}	0.150 ± 0.053^{ab}	0.104 ± 0.004^{b}	0.120 ± 0.006^{ab}
5'-IMP		nd	nd	nd	0.049 ± 0.002^{a}	0.050 ± 0.008^{a}
Total		**1.393 ± 0.093^{a}**	**1.498 ± 0.116^{a}**	**1.553 ± 0.066^{a}**	**1.719 ± 0.04^{a}**	**1.643 ± 0.051^{a}**
EUC (g MSG/100 g)		**32.87 ± 2.88^{b}**	**46.28 ± 6.40^{a}**	**44.25 ± 4.2^{a}**	**34.99 ± 2.37^{b}**	**35.57 ± 2.62^{b}**
Sugar/polyol						
Arabinose		3.83 ± 0.14^{a}	6.10 ± 1.48^{a}	4.34 ± 0.14^{a}	5.15 ± 1.67^{a}	5.02 ± 1.58^{a}
Fructose		4.03 ± 0.23^{c}	4.41 ± 0.15^{b}	4.35 ± 0.14^{b}	4.83 ± 0.10^{a}	4.27 ± 0.11^{bc}
Mannitol		18.89 ± 1.19^{a}	21.97 ± 3.77^{a}	21.34 ± 5.99^{a}	21.22 ± 2.00^{a}	24.39 ± 3.49^{a}
Glucose		7.42 ± 1.02^{b}	10.54 ± 1.40^{ab}	12.44 ± 3.95^{a}	9.71 ± 0.61^{ab}	13.33 ± 3.10^{a}
Trehalose		20.39 ± 0.62^{b}	12.49 ± 1.42^{d}	18.83 ± 0.72^{c}	24.44 ± 0.22^{a}	19.19 ± 0.51^{bc}
Total		**54.57 ± 2.50^{a}**	**55.51 ± 4.27^{a}**	**61.30 ± 1.02^{a}**	**65.36 ± 1.31^{a}**	**66.19 ± 6.30^{a}**
Organic acid						
Oxalic acid		1.09 ± 0.09^{ab}	0.95 ± 0.06^{c}	1.01 ± 0.06^{bc}	1.10 ± 0.04^{ab}	1.20 ± 0.07^{a}
Tartaric acid		7.15 ± 0.58^{b}	8.08 ± 0.41^{ab}	8.10 ± 0.42^{ab}	8.37 ± 0.46^{a}	8.43 ± 0.81^{a}
Malic acid		5.07 ± 0.31^{b}	3.78 ± 0.28^{c}	5.49 ± 0.50^{b}	7.07 ± 0.31^{a}	7.54 ± 0.71^{a}
Ascorbic acid		1.12 ± 0.07^{c}	1.08 ± 0.01^{c}	1.08 ± 0.06^{c}	1.31 ± 0.09^{b}	1.87 ± 0.07^{a}
Acetic acid		23.65 ± 0.34^{a}	22.52 ± 0.07^{b}	21.45 ± 0.22^{c}	21.44 ± 0.20^{c}	19.36 ± 0.30^{d}
Citric acid		19.60 ± 1.00^{b}	22.41 ± 0.26^{a}	19.57 ± 0.40^{b}	14.19 ± 0.10^{c}	14.65 ± 1.13^{c}
Succinic acid		1.56 ± 0.23^{ab}	1.39 ± 0.03^{b}	1.45 ± 0.03^{ab}	1.46 ± 0.14^{ab}	1.68 ± 0.08^{a}
Total		**59.24 ± 1.75^{a}**	**60.21 ± 0.32^{a}**	**58.14 ± 1.56^{a}**	**54.95 ± 0.86^{b}**	**54.72 ± 2.21^{b}**

nd: not detected. Means followed by different lowercase superscripts in similar column are significantly different ($p < 0.05$).

FAAs content in the Se-enriched samples significantly decreased ($p < 0.05$). In the Se80 samples, the content of FAAs reached its lowest point. This decrease could have been due to a large amount of Se binding to amino acids, forming polypeptides in which the free α-amino or αcarboxyl groups were absent, leading to a lower response in the ninhydrin colorimetric method (Shi *et al.*, 2017). The total FAAs content of Se120 and Se160 samples was greater than that in the Se80 samples, but remained lower than that in the CK. This could have been due to accelerated metabolism, which enhanced the absorption and assimilation of amino acids (Shi *et al.*, 2017). The content of FAAs with different taste characteristics also varied. The total content of MSG amino acids in the Se-enriched fruiting bodies of *C. cicadae* was notably lower ($p < 0.05$) compared with the control, ranging from 8.55 - 9.27 mg/g, with Glu as the major component. The high Glu content may be attributed to its central role in the transamination process, where it serves as a direct donor for the synthesis of other amino acids. As the most abundant amino acid in the central nervous system, Glu also regulates the metabolism of various substances in living organisms. Additionally, Glu can combine with blood ammonia to form glutamine, which detoxifies certain tissues and organs in the human body (Slominski *et al.*, 2012). The sweet amino acid contents ranged from 5.97 to 9.73 mg/g, with the highest content in the CK. The bitter amino acid contents were significantly lower in the Se40 and Se80 samples than in the CK ($p < 0.05$), while the Se120 and Se160 samples did not differ significantly ($p > 0.05$) from CK. As the concentration of Na_2SeO_3 increased, the bitterness increased slightly but remained lower than that of the control, suggesting that Se enrichment may contribute to the decrease in bitterness in the fruiting bodies of *C. cicadae*. Decreasing the level of bitter amino acids may increase the sweetness and umami of other amino acids (Lioe *et al.*, 2005). Tasteless amino acids, primarily Lys and Phe, have a minimal effect on the overall flavour (Dajanta *et al.*, 2011).

Analysis of 5′-nucleotides

The unique umami taste of edible mushrooms is influenced not only by free amino acids, but also by flavour nucleotides. These flavour nucleotides include 5′-IMP, 5′-GMP, 5′-XMP, and 5′-AMP, with 5′-XMP being generated through the oxidation of 5′-IMP. The 5′-nucleotide contents in the Se-enriched fruiting bodies of *C. cicadae* are shown in Figure 2B and Table 2. The contents of 5′-nucleotides increased with increasing Na_2SeO_3 concentration, with the total content of Se120 reaching a maximum, and the content of Se160 beginning to decrease, and overall being higher than that of CK. As shown in Table 2, the 5′-AMP and 5′-UMP contents in the Se-enriched samples increased compared to CK. Except for Se120, the 5′-GMP content was higher than CK in all three groups. Since the nucleotide samples were subjected to hot water extraction prior to analysis, the content of 5′-XMP produced by oxidation was minimal, and therefore not analysed. The results showed that the Se enrichment resulted in an enhancement of the 5′-nucleotide contents in the fruiting bodies of *C. cicadae.*

Analysis of soluble sugars and polyols

Small-molecule soluble sugars are the primary source of sweetness in edible mushrooms, but their unique flavour profile is not defined. Seven soluble sugars and polyols in the Se-enriched fruiting bodies of *C. cicadae* were analysed (Figure 2C and Table 2). The contents of total soluble sugars increased with increasing Se concentration, and reached its highest point at Se160. As can be seen in Table 2, both mannitol and glucose contents of Se160 sample were the highest among the five samples. There are previous studies with similar results to the present study. Huang *et al.* (2023) reported that the application of bionanoselenium significantly increased the soluble sugars content of radish by 12.82 - 41.54% compared to the CK. As shown in Table 2, among the total soluble sugars, mannitol (18.89 - 24.39 mg/g) had the highest concentration, accounting for more than 32.47%, followed by trehalose (12.49 - 24.44 mg/g), and glucose (7.42 - 13.33 mg/g). Mannitol, the main sugar alcohol in the Se-enriched fruiting bodies of *C. cicadae*, provides half the energy of regular sugar, and is not easily absorbed by the body, thus having a lower impact on insulin levels, and a reduced risk of causing tooth decay (Balagiannis *et al.*, 2009). Trehalose, a unique sugar found in edible mushroom, is a low-cariogenic sweetener, and has gained popularity in food and beverages in recent years due to its mild sweetness, stability, and moisturising properties (Galmarini *et al.*, 2011).

Analysis of organic acids

Organic acids are present in living organisms, and are a general term for organic compounds with carboxyl groups (-COOH). In the present work, seven organic acids were analysed (Figure 2D and Table 2). The total organic acid content in the CK was 59.24 mg/g, while in the Se-enriched samples, it ranged from 54.72 to 60.21 mg/g, and the total content of Se120 and Se160 samples was significantly lower than that of the CK, which was in agreement with the finding of exogenous Se on organic acids in lettuce (Chen *et al.*, 2019). Selenium enrichment had little effect on oxalic and succinic acids. Tartaric, malic, and ascorbic acids were significantly increased in the Se120 and Se160 samples compared to the CK; citric acid was significantly higher in the Se40 samples than that in CK, and the content began to decrease with the increase in the concentration of Na_2SeO_3, which was significantly lower in the Se120 and Se160 samples than that in CK. The results suggested that moderate concentration Se enrichment may help to improve the compositional content of organic acids in the fruiting bodies of *C. cicadae*.

Analysis of TAV and EUC value

The TAV values were applied to assess the role of various non-volatile flavour substances (Du *et al.*, 2024), and the results are shown in Table 3. The addition of appropriate amounts of Na_2SeO_3 enhanced the TAV of the fruiting bodies of *C. cicadae*, influencing their flavour profiles. Several FAAs had TAV greater than 1, indicating that the unique umami taste of the fruiting bodies of *C. cicadae* was a synergistic effect of different FAAs. Among these, Glu presented the highest TAV (20.47 - 23.93 mg/g), suggesting that it was the primary contributor to the umami flavour, which was consistent with the findings of Ismail *et al.* (2020). It can be seen that the TAV value of Glu in the Se80 sample was the highest. The TAV of Thr, Gly, Pro, Ile, and Leu were less than 1, and these amino acids did not contribute to the flavour. Among the sweet amino acids, Ser and Ala in the five samples were greater than 1. These sweet amino acids help mask the bitterness, and work in concert with the umami amino acids to enhance the overall flavour (Luo *et al.*, 2021). For bitter amino acids, His, Phe, Val, and Arg all had TAV values greater than 1, with Val reaching a TAV of 1.65 - 2.48 mg/g, and being the major contributor to bitter taste. But bitterness is often overshadowed by the stronger sweet and umami flavours, reducing its taste impact (Gao *et al.*, 2021). Additionally, 5′-AMP had the highest TAV value, and was consistently greater than 1, suggesting that it may play a key role in the prominent umami flavour of the fruiting bodies of *C. cicadae* (Du *et al.*, 2024). The EUC value pointed to a direct synergistic interaction between nucleotides and free amino acids (Zhang *et al.*, 2019). Table 2 shows a comparative analysis of the EUC values in the Se-enriched fruiting bodies of *C. cicadae*. Among the five samples, the Se40 sample had the highest EUC value, indicating that it had the strongest fresh flavour intensity, followed by the Se80 sample, and the CK had the lowest EUC value. Based on these results, it can be concluded that Se enrichment enhanced the release of umami compounds in the fruiting bodies of *C. cicadae*.

Multivariate analysis for samples

Multivariate analysis techniques, including PCA and PLS-DA, were used to visually compare the differences in the non-volatile flavour compounds. As shown in Figure 2E, PC1 and PC2 explained 38.70 and 19.50% of the total variance, respectively, with a combined total of 58.20%. The five samples were separated based on concentration with Na_2SeO_3 in the third quadrant, Se40 and Se80 clustered together, mainly in the first and fourth quadrants, and Se120 and Se160 clustered together, mainly in the second quadrant. The PLS-DA plot is shown in Figure 2F. The R^2X was 0.914, the R^2Y was 0.979, and the Q^2 was 0.814. Both the R^2 and the Q^2 values were greater than 0.5, thus the model demonstrated a satisfactory fit. Similar to the results of PCA analyses, the five samples were distributed by concentration in different quadrants. Multivariate statistical analyses showed that various non-volatile compounds were the main factors contributing to the differences in the distribution of the five fruiting bodies of *C. cicadae* samples. These findings confirmed that Se enrichment had significant effect on non-volatile compounds. The integration of the biplot and VIP plot from the PLS-DA revealed that 12 variables were identified as key markers (VIP > 1, $p < 0.05$), including trehalose, the EUC value, mannitol, citric acid, glucose, acetic acid, arabinose, aspartic acid, malic acid, sweet amino acids, MSG, and alanine (Figure 2G). Among these, succinic acid presented the highest VIP score of 2.24, whereas the other markers presented VIP scores ranging from 1.03 to 2.06.

Table 3. TAVs of non-volatile compounds in selenium-enriched fruiting bodies of *C. cicadae*.

		Taste threshold	TAV				
		(mg/g)	CK	Se40	Se80	Se120	Se160
Free amino acid							
MSG	Aspartic acid	1.00	3.35	1.00	0.97	2.17	3.14
	Glutamic acid	0.30	23.93	25.60	26.20	21.27	20.47
Sweet	Threonine	2.60	0.39	0.30	0.34	0.42	0.36
	Serine	1.50	1.61	1.10	1.11	1.35	1.31
	Glycine	1.30	0.63	0.35	0.32	0.49	0.47
	Alanine	0.60	4.17	3.03	2.92	5.30	3.65
	Proline	3.00	1.00	0.51	0.42	0.69	0.68
Bitter	Valine	0.40	2.25	1.70	1.65	2.48	2.03
	Methionine	0.30	1.20	0.60	0.63	0.87	0.90
	Isoleucine	0.90	0.39	0.22	0.23	0.58	0.36
	Leucine	1.90	0.41	0.19	0.20	0.37	0.37
	Phenylalanine	0.90	1.71	0.92	1.12	1.34	1.31
	Histidine	0.20	1.25	0.95	1.05	1.05	1.30
	Arginine	0.50	2.04	1.14	0.94	1.54	1.50
tasteless	Cysteine	/	/	/	/	/	/
	Tyrosine	/	/	/	/	/	/
	Lysine	0.50	1.50	0.98	1.08	0.90	1.22
5′-nucleotide							
	5'-CMP	/	/	/	/	/	/
	5'-AMP	0.25	2.11	2.31	2.57	3.15	2.72
	5'-UMP	/	/	/	/	/	/
	5'-GMP	0.125	0.90	1.30	1.20	0.83	0.96
	5'-IMP	0.50	nd	nd	nd	0.10	0.10

nd: not detected. /: no threshold for the substance.

Conclusion

In the present work, the volatile and non-volatile flavour components of the fruiting bodies of *C. cicadae* cultured in different concentrations of Na_2SeO_3 were analysed, and the concentrations of Na_2SeO_3 on the overall flavour was evaluated by chemometric methods. Multivariate statistical analysis demonstrated differences in the distribution of compounds across the five samples. Analysis of volatile and non-volatile flavour compounds using VIP values identified 14 and 12 potential markers, respectively. In the analysis of volatile components, new compounds which did not present in the control were identified in the Se-enriched samples. In the analysis of non-volatile components, the Se160 sample had the highest content of soluble sugars, and the Se120 sample had the highest content of 5'-nucleotides. While CK had the highest free amino acids content, the Se80 sample had the highest TAV of Glu, and the Se40 sample had the highest organic acid content and EUC value. Based on the results, it can be concluded that Se enrichment at suitable concentrations could improve the flavour profile of the fruiting bodies of *C. cicadae*. The findings of the present work would provide valuable theoretical insights for the potential use of Se in *C. cicadae*, as well as for the development and enhancement of *C. cicadae*-derived products.

The development of advanced cultivation methods, quality control strategies, and continued research are driving the growing applications of Se-enriched *C. cicadae*, especially in functional foods, dietary supplements, and therapeutic products. Due to the abundant nutrients in *C. cicadae*, selenium-enriched *C. cicadae* could be utilised in the preparation of nutrient-rich soups, functional beverages, and other health-focused products. Furthermore, Se-enriched *C. cicadae* can be formulated into capsules, tablets, or powders to be

marketed as dietary supplements. These products may support immune health, boost antioxidant capacity, regulate gut microbiota balance, and offer other potential health benefits (Chen *et al.*, 2023). To enhance the production efficiency and scale of Se-enriched *C. cicadae*, it is crucial to address challenges like low bioavailability and environmental contamination. Additionally, the development of Se-enriched foods focuses on further investigation into their physiological roles, and the optimal amount of exogenous Se to be added through both clinical and preclinical research, to enhance the safety and effectiveness of selenium supplementation. This will not only help to promote the widespread application of Se-enriched *C. cicadae*, but also guarantee the provision of high-quality health products for consumers.

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