



Effects of Dielectric Barrier Discharge Cold Atmospheric Plasma on Volatile Components of *Cordyceps chanhua* Fruiting Bodies

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Abstract: The effects of dielectric barrier discharge cold atmospheric plasma (DBD-CAP) on the volatile profiles of *C. chanhua* fruiting bodies were investigated. Powdered samples were treated at 80 or 120 kV for 2 or 4 min and volatile compounds were analyzed using headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) and chemometrics. In total, 82 volatile compounds were identified and the number of compounds increased from 42 in the control (CK) to 75 following treatment at 120 kV for 4 min (T4). The relative proportion of esters decreased from 81.38% in CK to 29.42% in T4, whereas aldehydes and ketones increased from 6.11% and 2.99% to 30.81% and 12.54%, respectively. This redistribution was accompanied by marked increases in 2-butyloct-2-enal, 2-undecanone, nonanal, (Z)-11-pentadecenal, and (E,Z)-2,13-octadecadienol. Multivariate analyses revealed treatment-dependent flavor differentiation, with T4 exhibiting the most distinct volatile profile. These findings demonstrate that DBD-CAP reconfigured the volatile profile of *C. chanhua* from ester dominance toward greater relative contributions from aldehydes and ketones, highlighting its potential for modulating the flavor characteristics of *C. chanhua* fruiting bodies.

Keywords: *Cordyceps chanhua*, dielectric barrier discharge, cold atmospheric plasma, volatile compounds, chemometrics

INTRODUCTION

Cordyceps chanhua, also known as *Cordyceps cicadae* and commonly known as Jinchanhua in Chinese, is an entomopathogenic fungus belong to the genus *Cordyceps* in the family Clavicipitaceae (Ji et al., 2025; Li et al., 2021). The fruiting bodies of *C. chanhua* have been used as both a traditional medicine and a tonic food for more than 1,600 in China. In 2020, The artificially cultivated *C. chanhua* fruiting bodies were approved by the National Health Commission of China as a novel food ingredient, facilitating their development into functional foods and related products (Chen, Tian, Chen, Han, & Zhao, 2026; Fei et al., 2026; PRC, 2021). Previous studies have identified diverse nutritional and bioactive constituents in *C. chanhua*, including polysaccharides, nucleosides, amino acids, fatty acids, sterols, and other secondary metabolites (Dong et al., 2024; He et al., 2019) that collectively determine nutritional value, technological functionality, and sensory quality (Chiu et al., 2016; Zeng et al., 2021). Although these components underpin its nutritional and functional value, the commercial acceptance of *C. chanhua*-based products depends not only on their bioactive properties but also on their sensory quality. In particular, the characteristic aroma and taste of the fruiting bodies are important determinants of consumer preference and their suitability for incorporation into foods, beverages and seasoning products.

The flavor of edible fungi is a decisive quality attribute and is produced through the combined contribution of volatile aroma compounds and water-soluble taste-active substances. Aldehydes, ketones, alcohols, esters, hydrocarbons, and terpenoids commonly contribute to mushroom-like, fruity, floral, grassy, fatty, and roasted odor notes, whereas free amino acids, 5'-nucleotides, soluble sugars/polyols and organic acids determine the principal non-volatile taste characteristics (Huang, Tsai, Lee, & Mau, 2006; Mau, Lin, & Chen, 2001; Sun et al., 2020). Glutamic and aspartic acids generally contribute umami, while several other amino acids impart sweet or bitter sensations. In addition, 5'-inosine monophosphate and 5'-guanosine monophosphate can interact synergistically with umami amino acids, substantially enhancing overall taste intensity, which can be quantitatively expressed as the equivalent umami concentration (EUC) (Mau, 2005; Yang, Lv, Liu, Bi, & Zhang, 2022). Sugars and polyols contribute sweetness and mouthfeel, whereas succinic, citric, malic, and other organic acids influence sourness, freshness, and taste balance. These compounds may also act as precursors or intermediates in oxidation, degradation, esterification and Maillard-related reactions. Consequently, processing-induced changes in either volatile or non-volatile constituents may alter the overall flavor profile, even when the major nutritional components of the raw material are largely retained.

Cold atmospheric plasma (CAP) has attracted considerable interest as an emerging non-thermal technology for food processing (Gupta & Routray, 2025; Kaur, Kumar, Singh, Kaur, & Panesar, 2024; Ucar, Ceylan, Durmus, Tomar, & Cetinkaya, 2021; Wang, Zhu, & Yi, 2026). It's generated by supplying electrical energy to a gas under atmospheric or near-atmospheric pressure, producing a partially ionized environment containing electrons, ions, excited atoms and molecules, ultraviolet photons, and reactive oxygen and nitrogen species at near-ambient bulk temperature (Harikrishna, Anil, Shams, & Dash, 2023; Ucar et al., 2021; L. H. Wang et al., 2026). Among the available plasma-generation configurations, dielectric barrier discharge (DBD) is particularly suitable for food applications because it can produce relatively uniform plasma over a large treatment area at atmospheric pressure (Figueroa-Pinochet et al., 2022; Laroque, Seó, Valencia, Laurindo, & Carciofi, 2022). Compared with conventional thermal processing, DBD-CAP can operate at relatively low temperatures, requires little or no water or chemical additives and has been investigated for microbial decontamination, enzyme inactivation, shelf-life extension, drying pretreatment and modification of food structure and functionality (Bourke, Ziuzina, Boehm, Cullen, & Keener, 2018; Misra, Pankaj, Segat, & Ishikawa, 2016; Pankaj, Wan, & Keener, 2018). These features make DBD-CAP potentially suitable for heat-sensitive food materials, in which conventional heating may cause the loss of volatile compounds or deterioration of nutritional and bioactive constituents.

Nevertheless, DBD-CAP should not be regarded as chemically inert toward food matrices. Reactive oxygen and nitrogen species generated during treatment, including hydroxyl radicals, superoxide anions, ozone, hydrogen peroxide, nitric oxide and related species, can interact with lipids, proteins, carbohydrates, pigments and low-molecular-weight flavor compounds (Baskaran & Radhakrishnan, 2025; Sruthi et al., 2022; Vedaiei & Dara, 2025; L. H. Wang et al., 2026). Depending on the applied voltage, exposure time, discharge gas, humidity, reactor configuration and food composition, these reactions may promote the cleavage, oxidation, isomerization, or formation of volatile compounds and may also modify amino acids, sugars and organic acids (Bayati, Lund, Tiwari, & Poojary, 2024; Ucar et al., 2021). DBD-CAP has been reported to modulate terpene- and

sesquiterpene-derived aromas in fruit juice, alter the flavor fingerprints of brown rice, and change both the microbial community and volatile profile of ewe milk (Campelo et al., 2020; Du et al., 2025; Liu et al., 2021; Qian et al., 2026; Wang et al., 2022). Such effects are strongly dependent on treatment intensity (Amorim et al., 2026; Liu et al., 2025). Moderate exposure may facilitate the release or formation of desirable aroma compounds, whereas excessive exposure can accelerate lipid and protein oxidation, degrade aroma-active constituents, or generate undesirable odor notes (Silveira et al., 2019; Wang et al., 2024). Free amino acid concentrations may likewise increase because of protein fragmentation or decrease through oxidation and subsequent reactions. A comprehensive assessment of CAP-treated foods should therefore extend beyond microbial or physicochemical indicators and include systematic characterization of both aroma- and taste-related compounds.

Most studies concerning CAP treatment of edible fungi have focused on microbial control, postharvest preservation, browning, moisture loss, and textural changes. Information regarding the responses of fungal flavor constituents remains limited, and to the best of our knowledge, the changes in volatile compounds of *C. chanhua* fruiting bodies following DBD-CAP treatment have not been systematically investigated. This knowledge gap restricts the rational selection of plasma-processing parameters and makes it difficult to determine whether CAP treatment preserves, improves, or diminishes the sensory quality of this food material. Accordingly, the present study investigated the effects of different DBD-CAP voltage-time combinations on the volatile components of *C. chanhua* fruiting bodies. Changes volatile profiles were systematically determined and evaluated. The results are expected to clarify the dose-dependent effects of DBD-CAP on the volatile profiles of *C. chanhua* and lay the scientific groundwork for optimizing non-thermal processing conditions for high-quality edible and medicinal fungal products.

MATERIALS AND METHODS

Samples and Reagents

Artificial cultivated *C. chanhua* fruiting bodies were obtained from Anhui Cordyceps Source Biotechnology Co., Ltd. (Anhui, China) and dried at 50 °C for 36 h. The dried samples were pulverized into powder and passed through a 100-mesh sieve and homogenized to minimize subsampling variability. The resulting powder was stored under 4 °C fridge for analysis.

Acetonitrile and methanol (HPLC grade, purity > 99.7%) were obtained from Xilong Scientific Co., Ltd. (Shantou, China). Triethylamine (analytical grade, purity > 99.0%) was supplied by Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Sodium acetate trihydrate, hydrochloric acid (analytical grade, 36-38%, w/w), acetic acid, and potassium dihydrogen phosphate (analytical grade, purity > 99.5%) were supplied by Tianjin Bodi Chemical Co., Ltd. (Tianjin, China). Amino acid standards (purity ≥ 98.0%) were purchased from Sykam GmbH (Germany).

Nucleotide standards (purity ≥ 98.0%), soluble sugar standards (purity ≥ 99.0%) and organic acid standards (purity ≥ 99.5%) were obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Ultrapure water were used throughout the experiments.

Dielectric Barrier Discharge Cold Atmospheric Plasma Treatment

The fruiting bodies of *C. chanhua* were ground into powder, and 2.0 g of each sample was weighed exactly and spread evenly in a Petri dish. The samples were then subjected to dielectric barrier discharge cold atmospheric plasma (DBD-CAP) treatment at two voltages (80 and 120 kV) for two exposure durations (2 and 4 min) to evaluate the effects of different treatment conditions on their flavor profiles. After treatment, the samples were stored under refrigeration until further analysis. The untreated sample served as the control (CK), whereas samples treated at 80 kV for 2 min, 120 kV for 2 min, 80 kV for 4 min, and 120 kV for 4 min were designated as T1, T2, T3 and T4, respectively.

Headspace Solid-phase Microextraction

A 1.0 g portion of powdered *C. chanhua* fruiting bodies was precisely weighed into a 20 mL headspace vial, which was immediately capped. The vial was equilibrated in a thermostatic water bath at 60 °C for 20 min to facilitate the release of volatile compounds. Subsequently, a 65 µm polydimethylsiloxane/ Divinybenzene (PDMS/DVB) solid-phase microextraction (SPME) fiber was inserted through the vial septum and exposed to the headspace above the sample at 60 °C for 30 min. After extraction, the fiber was retracted into the needle and immediately inserted into the GC inlet for thermal desorption and analysis.

GC-MS Analysis

Volatile compounds were analyzed using an Agilent 8890 gas chromatograph coupled to a 5977B mass selective detector. Chromatographic separation was performed on an HP-5MS UI ultra-inert capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) in splitless mode. The inlet temperature was maintained at 250 °C. High-purity helium (99.999%) was used as the carrier gas at a flow rate of 1.0 mL/min.

The oven temperature was initially maintained at 50 °C for 2 min, increased to 110 °C at 3 °C/min, and then increased to 150 °C at 2 °C/min and held for 2 min. The temperature was subsequently increased to 210 °C at 10 °C/min and maintained for 5 min. The total run time was 55 min.

Mass spectrometric detection was conducted with an electron ionization (EI) source operated at 70 eV. The ion source, quadrupole, and transfer-line temperatures were maintained at 230, 150, and 230 °C, respectively. Data were acquired in full-scan mode over a mass range of m/z 50-550.

Data Processing and Statistical Analysis

All experiments were conducted in triplicate, with results reported as the mean ± standard deviation. Microsoft Excel 2021 (Microsoft Corp., Redmond, WA, USA) was used to organize and align the retention times, characteristic ions, and peak-area data of the volatile compounds. Compounds were initially screened based on their characteristic ions, and their mass spectra were compared with those in the NIST 20 mass spectral library. Only compounds with a spectral matching score ≥ 800 were considered tentatively identified.

One-way analysis of variance (ANOVA) was performed using IBM SPSS Statistics 27 (IBM Corp., Armonk, NY, USA), with differences considered statistically significant at $P < 0.05$. Hierarchical cluster analysis (HCA) was performed using the Chiot online platform (<https://chiplot.online/>), and the results were visualized as clustered heatmaps. Principal component analysis (PCA) and orthogonal partial least-squares discriminant analysis (OPLS-DA) were conducted using SIMCA-P 14.1 (Umetrics, Umeå, Sweden). Key differential flavor compounds were screened according to the variable importance in projection (VIP) scores obtained from the OPLS-DA models, with $VIP > 1$ used as the primary selection criterion.

RESULTS AND DISCUSSION

Effects of DBD-CAP Treatment on the Volatile Profile of *C. chanhua* Fruiting Bodies

Volatile compounds were analyzed by HS-SPME-GC-MS and identified by matching their mass spectra against the NIST 20 library. In total, 82 volatile compounds were provisionally assigned to 22 esters, 15 ketones, 13 alkanes, 12 aldehydes, 7 alcohols, 6 alkenes, 3 ethers and 4 other compounds (Table 1). As shown in Fig. 1A, 42, 55, 66, 71 and 75 volatile compounds were detected in CK, T1, T2, T3 and T4, respectively. At the same exposure time, increasing the discharge voltage increased the number of detected compounds. Similarly, a longer exposure produced a broader volatile profile at a given voltage. Esters represented the largest number of compounds in every group, increasing from 15 in CK to 21 in T4. The corresponding number of ketones increased from 8 to 14, while 6 alcohols and 6 alkenes were detected in T4. Thus, T4 exhibited the greatest chemical diversity. Nevertheless, the increase in detected compounds does not necessarily indicate the formation of new volatiles, as plasma-induced matrix disruption, enhanced release, or previously low-abundance compounds exceeding the detection limit may also contribute.

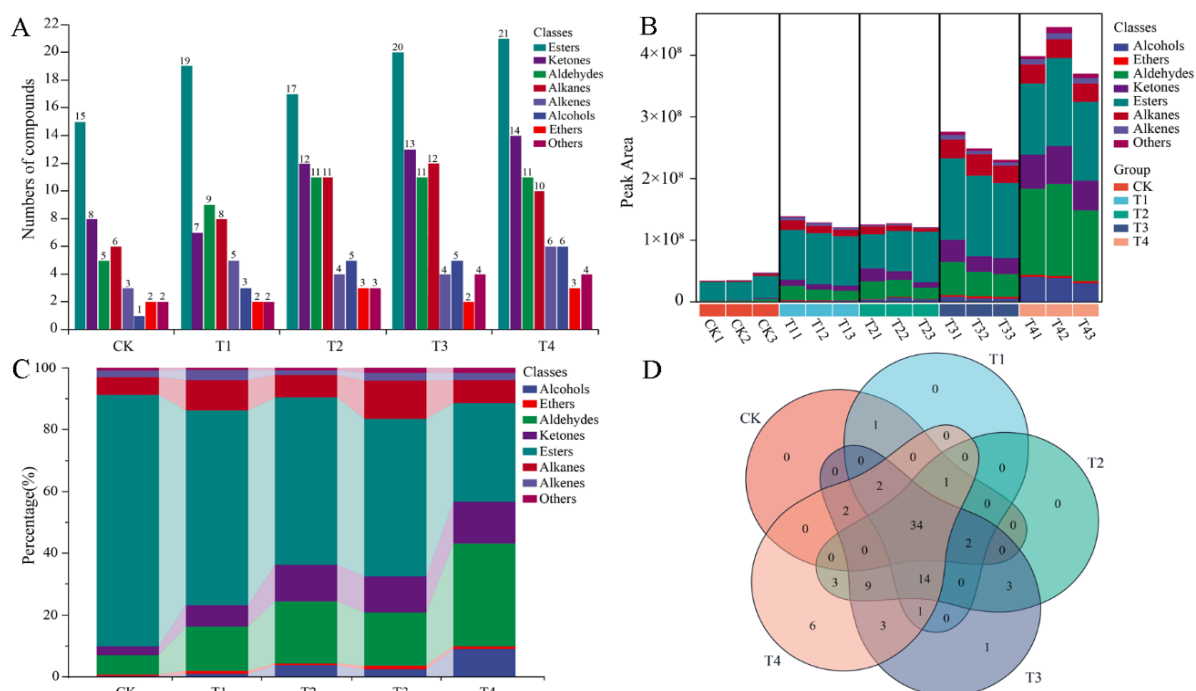


Figure 1: Analysis of volatile compounds in fruiting bodies of *C. chanhua* under different treatments

The summed GC-MS peak area showed a similar treatment-related pattern (Fig. 1B). CK produced the lowest total chromatographic response, whereas substantially higher signals were recorded for T1 and T2. Extending the treatment time further increased the response in T3 and T4, with T4 showing the highest value. At 80 kV, the total peak area after 4 min (T3) was clearly higher than that after 2 min (T1); the same pattern was more pronounced between T2 and T4 at 120 kV. Therefore, the statement that exposure for 2 and 4 min at 80 kV caused little change is not supported by Fig. 1B.

DBD-CAP also markedly reshaped the relative distribution of the volatile classes (Fig. 1C). The CK profile was strongly dominated by esters, which accounted for 81.38% of the total peak area, whereas aldehydes and ketones represented only 6.11% and 2.99%, respectively. As the treatment conditions intensified, the relative contribution of esters generally decreased, while those of aldehydes, ketones and alcohols increased. In T4, esters accounted for 29.42%, whereas aldehydes and ketones increased to 30.81% and 12.54%, respectively. The volatile profile therefore changed from a highly ester-dominated pattern toward a more diverse composition involving aldehydes, ketones, alcohols and esters.

Changes in individual volatile compounds further supported this compositional change. In the CK group, the chromatographic feature eluting at 50.73 min, tentatively assigned to methyl isohexadecanoate and methyl petroselinate accounted for 47.62% and 21.33% of the total peak area, respectively, together representing 68.95% of the volatile profile. Their relative contributions decreased markedly to 11.47% and 7.03%, respectively, in T4. By contrast, several aldehydes, ketones and alcohols exhibited pronounced increases. The relative peak area of 2-butyloct-2-enal increased from 3.24% in CK to 15.50% in T4, whereas that of 2-undecanone increased from 0.57% to 6.78%. Nonanal was not detected in CK but accounted for 3.97% of the total peak area in T4. Likewise, Z-11-pentadecenal and (E,Z)-2,13-octadecadienol were absent from CK but reached 6.48% and 7.30% in T4, respectively. These changes were major contributors to the increased relative proportions of aldehydes, ketones, and alcohols following high-intensity DBD-CAP treatment. Because several aldehydes and ketones possess relatively low odor thresholds, their accumulation may alter the fatty, green, and mushroom-like aroma characteristics of the fruiting bodies. Nevertheless, their actual contributions to the overall aroma profile should be further verified using odor activity values and sensory evaluation.

The distribution of shared and treatment-specific features provided further evidence of volatile-profile differentiation (Fig. 1D). Thirty-four compounds occurred in all five groups and may constitute a relatively stable core volatile profile. A further 14 compounds were detected in every DBD-CAP-treated group but not in CK, suggesting a close association with plasma exposure. Nine additional compounds were shared by T2, T3, and T4 but were absent from CK and T1, indicating that increasing the voltage or extending the exposure time further broadened the detectable volatile spectrum. No compounds were unique to CK, T1, or T2. T3 contained one unique compound, 4-methyltetradecane, whereas six features were unique to T4: 3-methyl-3-decen-2-one, 1-nonadecene, 2-phenyl-2,4-octadienol, cyclohexadecane, widdrol and phthalic acid, cyclohexyl isohexyl ester.

Collectively, increasing the DBD-CAP voltage and exposure time enhanced the total chromatographic response and broadened the detectable volatile profile, accompanied by a transition from ester dominance toward greater contributions from aldehydes, ketones and alcohols.

Table 1: Volatile compounds composition of fruiting bodies of *C. chanhua* under different treatments

No.	RT	Compounds	Formula	Score	Mw	CAS	CK	T1	T2	T3	T4
1	4.25	Hexanal	C ₆ H ₁₂ O	86	100.1	66-25-1	—	—	—	0.26±0.07 ^a	0.49±0.25 ^a
2	7.78	Methyl caproate	C ₇ H ₁₄ O ₂	91	130.1	106-70-7	0.30±0.05 ^a	0.60±0.41 ^a	0.38±0.13 ^b	2.94±1.34 ^a	2.45±0.65 ^a
3	9.81	6-Heptenoic acid	C ₇ H ₁₂ O ₂	80	128.1	1119-60-4	—	—	—	0.25±0.12 ^b	0.49±0.33 ^a
4	11.78	Methyl heptanoate	C ₈ H ₁₆ O ₂	83	144.1	106-73-0	0.29±0.09 ^a	—	—	0.32±0.08 ^a	0.37±0.13 ^a
5	12.37	3-Octen-2-one	C ₈ H ₁₄ O	91	126.1	1669-44-9	0.45±0.14 ^a	—	—	0.50±0.05 ^a	0.28±0.08 ^b
6	14.43	Tetramethyl-pyrazine	C ₈ H ₁₀ N ₂	87	136.1	1124-11-4	0.59±0.18 ^a	0.15±0.02 ^c	0.29±0.22 ^{bc}	0.46±0.05 ^{ab}	0.26±0.06 ^{bc}
7	14.92	Undecane	C ₁₁ H ₂₄	97	156.2	1120-21-4	0.27±0.07 ^a	0.36±0.06 ^a	0.78±0.27 ^b	0.82±0.30 ^b	0.32±0.12 ^a
8	15.26	Nonanal	C ₉ H ₁₈ O	99	142.1	124-19-6	—	1.07±0.23 ^{cd}	1.84±0.78 ^{bc}	2.44±0.81 ^b	3.99±1.10 ^a
9	16.27	Methyl caprylate	C ₉ H ₁₈ O ₂	96	158.1	111-11-5	—	0.30±0.04 ^a	0.31±0.18 ^a	0.42±0.11 ^a	0.38±0.19 ^a
10	18.02	4-Hydroxy- α -Thujone	C ₁₀ H ₁₈ O ₂	83	168.1	158612-68-1	—	—	0.22±0.14 ^a	0.22±0.08 ^a	0.16±0.03 ^a
11	18.20	3-Methylundecane	C ₁₂ H ₂₆	90	170.2	1002-43-3	—	—	0.11±0.10 ^a	0.19±0.08 ^a	—
12	19.50	Dodecane	C ₁₂ H ₂₆	98	170.2	112-40-3	1.33±0.16 ^b	1.87±0.20 ^{ab}	1.78±1.09 ^{ab}	2.92±0.93 ^a	1.40±0.44 ^b
13	20.64	3-Nonenoic acid, methyl ester	C ₁₀ H ₁₈ O ₂	97	170.1	13481-87-3	—	0.21±0.24 ^{ab}	1.02±0.62 ^{ab}	0.76±0.32 ^{ab}	1.29±1.07 ^a
14	20.75	Methyl nonylate	C ₁₀ H ₂₀ O ₂	96	172.1	1731-84-6	2.17±0.48 ^a	2.27±0.25 ^a	2.47±1.15 ^a	2.07±0.59 ^a	3.28±2.12 ^a
15	21.19	3-Methyl-3-decen-2-one	C ₁₁ H ₂₀ O	88	168.2	54411-03-9	—	—	—	—	0.13±0.02 ^a
16	22.49	Octahydronaphthalen-4a-ol	C ₁₁ H ₁₈ O	88	154.1	55693-34-0	—	—	0.18±0.06 ^a	0.20±0.05 ^a	0.20±0.03 ^a
17	22.84	6-Pentadecanone	C ₁₁ H ₂₂ O	80	170.2	927-49-1	—	—	—	0.19±0.03 ^a	0.15±0.01 ^b
18	22.98	2-Butylhept-2-enalzhin	C ₁₁ H ₂₀ O	82	168.2	73757-26-3	0.24±0.18 ^a	0.42±0.13 ^{bc}	0.38±0.08 ^{bc}	0.47±0.03 ^b	0.71±0.07 ^a
19	23.29	Carvenone oxide	C ₁₀ H ₁₆ O ₂	85	168.1	1000186-21-3	0.27±0.21 ^b	0.53±0.13 ^a	0.49±0.10 ^{ab}	0.60±0.01 ^a	0.73±0.07 ^a
20	23.71	α -Tridecene	C ₁₃ H ₂₆	92	182.2	2437-56-1	—	0.31±0.06 ^a	—	0.42±0.07 ^a	0.31±0.04 ^a
21	23.82	2-Undecanone	C ₁₁ H ₂₀ O	98	170.2	112-12-9	0.57±0.29 ^a	3.23±0.48 ^{bc}	8.11±4.44 ^a	5.85±0.49 ^{ab}	6.81±1.18 ^a
22	24.05	Tridecene	C ₁₃ H ₂₆	97	184.2	629-50-5	1.48±0.66 ^b	2.92±0.53 ^{ab}	2.16±0.94 ^{ab}	3.38±0.97 ^a	1.66±0.50 ^b
23	24.48	3-Butylcycloheptanone	C ₁₁ H ₂₀ O	86	168.2	1000164-85-6	—	—	0.53±0.17 ^b	0.29±0.05 ^c	1.01±0.08 ^a
24	25.07	1-Nonadecene	C ₁₉ H ₃₈	87	266.3	18435-45-5	—	—	—	—	0.11±0.01 ^a
25	25.32	Methyl caprate	C ₁₁ H ₂₂ O ₂	91	186.2	110-42-9	0.28±0.07 ^b	0.55±0.14 ^a	0.16±0.06 ^b	0.28±0.06 ^a	0.19±0.09 ^a
26	25.96	Carbonic acid, decyl dodecyl ester	C ₂₃ H ₄₆ O ₂	84	370.3	1000383-16-1	—	0.25±0.02 ^a	0.16±0.06 ^b	0.27±0.08 ^a	0.15±0.04 ^a
27	26.15	Cyclodecanone	C ₁₀ H ₁₈ O	81	154.1	1502-06-3	0.18±0.03 ^b	0.10±0.02 ^b	0.37±0.21 ^a	0.41±0.02 ^a	0.56±0.10 ^a
28	26.65	2,4-Dimethyl-dodecane	C ₁₄ H ₃₀	88	198.2	6117-99-3	—	—	0.08±0.07 ^b	0.21±0.06 ^a	—
29	27.09	γ -Nonalactone	C ₉ H ₁₆ O ₂	94	156.1	104-61-0	0.31±0.13 ^a	0.40±0.08 ^a	0.23±0.24 ^a	0.32±0.04 ^a	0.29±0.03 ^a
30	27.20	4-Methyltetradecane	C ₁₄ H ₃₀	88	212.3	25117-24-2	—	—	—	0.23±0.08 ^a	—
31	27.45	3-Methyltridecene	C ₁₄ H ₃₀	93	198.2	6418-41-3	0.32±0.18 ^b	0.65±0.06 ^a	0.48±0.18 ^b	0.87±0.28 ^a	0.40±0.11 ^b
32	27.65	2-Butyloct-2-enal	C ₁₂ H ₂₂ O	98	182.2	13019-16-4	3.25±1.72 ^{2a}	5.62±0.60 ^{ab}	11.24±3.05 ^{ab}	6.59±0.47 ^a	15.58±1.48 ^a
33	28.24	(Z)-5-Tetradecene	C ₁₄ H ₂₈	83	196.2	41446-62-2	—	0.32±0.06 ^a	0.28±0.12 ^a	0.33±0.07 ^a	0.33±0.05 ^a
34	28.37	2-Butyl-2,7-octadien-1-ol	C ₁₂ H ₂₄ O	90	182.2	1000131-72-0	—	—	0.20±0.09 ^b	0.21±0.03 ^b	0.46±0.03 ^a
35	28.56	1-Tetradecanol	C ₁₄ H ₃₀ O	91	214.2	112-72-1	—	0.21±0.05 ^{ab}	0.13±0.07 ^b	0.27±0.06 ^a	0.14±0.04 ^a
36	28.96	Tetradecane	C ₁₄ H ₃₀	98	198.2	629-59-4	1.14±0.60 ^b	2.10±0.46 ^a	1.17±0.40 ^b	1.74±0.39 ^{ab}	0.92±0.23 ^b
37	29.30	Hexahydroseuodionone	C ₁₃ H ₂₆ O	92	198.2	1604-34-8	—	—	0.33±0.14 ^a	0.29±0.03 ^b	0.47±0.09 ^a
38	29.80	2-Hexyl-1-decanol	C ₁₆ H ₃₄ O	87	242.3	2425-77-6	0.16±0.08 ^b	0.27±0.01 ^a	0.08±0.01 ^c	0.17±0.04 ^a	—
39	30.04	2,10-Dimethyl-9-undecenal	C ₁₃ H ₂₆ O	90	196.2	1000131-85-9	—	0.28±0.05 ^a	0.35±0.13 ^a	0.40±0.07 ^a	0.30±0.04 ^a
40	30.84	Methyl 9-oxononanoate	C ₁₀ H ₁₈ O ₃	88	186.1	1931-63-1	—	—	0.34±0.05 ^b	0.29±0.02 ^b	0.56±0.10 ^a
41	31.34	1-Tetradecanol, methyl ether	C ₁₅ H ₃₂ O	90	228.2	1000406-29-1	0.21±0.05 ^b	0.39±0.13 ^a	0.20±0.06 ^b	0.43±0.13 ^a	0.19±0.06 ^b
42	31.74	Geranyl acetone	C ₁₃ H ₂₆ O	90	194.2	689-67-8	0.19±0.06 ^c	0.42±0.07 ^a	0.33±0.09 ^{ab}	0.40±0.05 ^a	0.22±0.06 ^{bc}
43	32.51	2-Methyl-cyclododecanone	C ₁₃ H ₂₆ O	81	196.2	16837-94-8	0.17±0.07 ^b	0.26±0.00 ^b	0.07±0.03 ^c	0.19±0.02 ^{ab}	—
44	32.72	1-Butyl-2-pentyl-cyclopentane	C ₁₁ H ₂₂ O ₂	83	196.1	5073-65-4	—	0.32±0.03 ^a	0.63±0.11 ^b	0.43±0.03 ^b	1.27±0.08 ^a
45	32.93	Cyclotridecanone	C ₁₀ H ₁₈ O ₂	93	168.1	54814-64-1	—	—	0.67±0.12 ^b	0.38±0.04 ^a	1.34±0.09 ^a
46	33.21	8-Selinene	C ₁₅ H ₂₄	96	204.2	17066-67-0	0.38±0.20 ^{bc}	0.80±0.16 ^a	0.29±0.05 ^c	0.56±0.02 ^b	0.36±0.05 ^{bc}
47	33.73	1-Heptadecene	C ₁₇ H ₃₄	94	238.3	6765-39-5	1.20±0.86 ^{ab}	1.51±0.63 ^a	0.63±0.21 ^{ab}	1.02±0.07 ^{ab}	0.52±0.15 ^b
48	33.91	Phytane	C ₁₈ H ₃₈	87	282.3	638-36-8	—	0.42±0.09 ^a	0.15±0.04 ^b	0.33±0.07 ^a	0.19±0.05 ^b
49	34.14	Pentadecane	C ₁₅ H ₃₂	89	212.3	629-62-9	0.56±0.14 ^a	0.49±0.15 ^a	0.21±0.05 ^b	0.40±0.08 ^{ab}	0.20±0.06 ^b
50	34.47	Dibutylcresol	C ₁₅ H ₃₂ O	99	518.1	497-39-2	—	—	0.12±0.03 ^b	0.23±0.02 ^a	0.13±0.03 ^b
51	35.51	Methyl laurate	C ₁₃ H ₂₆ O ₂	81	214.2	111-82-0	0.49±0.09 ^b	0.67±0.09 ^a	0.24±0.05 ^c	0.47±0.02 ^b	0.17±0.06 ^b
52	35.67	8-Hexylpentadecane	C ₂₁ H ₄₄	80	296.3	13475-75-7	0.70±0.07 ^b	0.96±0.23 ^a	0.20±0.07 ^c	0.61±0.04 ^b	0.29±0.11 ^a
53	36.26	2,6,10-Trimethyltetradecane	C ₁₇ H ₃₄	83	240.3	14905-56-7	—	—	0.10±0.03 ^b	0.20±0.05 ^a	—
54	36.88	Tetradecyl ether	C ₂₄ H ₅₀ S ₂	80	402.3	27458-90-8	0.39±0.07 ^{bc}	0.53±0.14 ^{ab}	0.22±0.06 ^c	0.64±0.14 ^a	0.23±0.07 ^a
55	37.57	1-Cyclododecylethanone	C ₁₄ H ₂₈ O	82	210.2	28925-00-0	0.52±0.49 ^b	1.49±0.43 ^a	0.49±0.27 ^b	0.81±0.15 ^{ab}	0.69±0.42 ^b
56	37.74	2-Pentylidenonol	C ₁₃ H ₂₆ O	81	228.2	1653-34-5	—	0.48±0.08 ^a	0.87±0.28 ^b	0.75±0.02 ^{bc}	1.69±0.26 ^a
57	37.97	2,6,10-Trimethyl-9-undecenal	C ₁₄ H ₂₈ O	82	210.2	141-13-9	0.37±0.18 ^b	0.93±0.10 ^a	0.39±0.10 ^b	0.72±0.15 ^a	0.35±0.11 ^b
58	38.23	1,11-Tridecadiene	C ₁₃ H ₂₆	80	180.2	1000130-76-4	0.46±0.13 ^a	0.48±0.08 ^a	0.20±0.07 ^b	—	0.53±0.07 ^a
59	38.59	(Z)-9-Tetradecenal	C ₁₄ H ₂₈ O	81	210.2	53939-27-8	—	—	0.08±0.02 ^b	—	0.13±0.01 ^a
60	39.39	Hexadecane	C ₁₆ H ₃₄	94	226.3	544-76-3	—	—	0.29±0.04 ^b	0.24±0.02 ^b	0.47±0.06 ^a
61	39.77	1-(Cyclododec-1-en-1-yl)ethanone	C ₁₄ H ₂₈ O	85	208.2	65938-08-1	0.44±0.17 ^{bc}	1.00±0.13 ^a	0.32±0.10 ^c	0.63±0.12 ^b	0.36±0.10 ^c
62	41.04	2-Phenyl-2,4-octadienol	C ₁₄ H ₁₈ O	94	202.1	1000131-83-7	—	—	—	—	0.13±0.05 ^a
63	42.09	Cyclohexadecane	C ₁₆ H ₃₄	89	224.3	295-65-8	—	—	—	—	0.33±0.10 ^a
64	43.00	(E,Z)-2,13-octadecadienol	C ₁₈ H ₃₆ O	89	266.3	1000131-10-0	—	0.46±0.19 ^c	3.17±1.43 ^b	1.34±0.19 ^c	7.33±1.38 ^a
65	43.27	Cyclopentadecanone	C ₁₅ H ₃₀ O	82	224.2	502-72-7	0.36±0.22 ^{ab}	0.54±0.08 ^a	0.22±0.09 ^b	0.42±0.02 ^{ab}	0.25±0.07 ^b
66	43.47	Z-11-Pentadecenal	C ₁₅ H ₃₀ O	91	592.2	556-68-3	—	0.38±0.17 ^c	2.87±1.29 ^b	1.18±0.16 ^c	6.51±1.26 ^a
67	44.98	Widdrol	C ₁₅ H ₂₆ O	82	222.2	6892-80-4	—	—	—	—	0.18±0.05 ^a
68	45.83	Methyl myristate	C ₁₅ H ₃₀ O ₂	96	242.2	124-10-7	0.40±0.10 ^a	0.29±0.01 ^a	0.23±0.14 ^a	0.26±0.03 ^a	0.42±0.13 ^a
69	45.93	7-Pentadecene-7-carbaldehyde	C ₁₆ H ₃₀ O	80	238.2	1000432-46-3	1.76±0.22 ^b	4.68±1.56 ^a	1.15±0.37 ^b	1.93±0.51 ^b	1.17±0.38 ^b
70	47.02	Muscone	C ₁₆ H ₃₄ O	82	238.2	541-91-3	—	—	0.09±0.05 ^b	—	0.28±0.05 ^a
71	47.91	2-Phenoxyethyl phenyl ether	C ₁₆ H ₁₈ O ₂	89	214.1	104-66-5	—	—	0.11±0.07 ^b	—	0.31±0.06 ^a
72	48.43	Methyl pentadecanoate	C ₁₆ H ₃₂ O ₂	94	256.2	7132-64-1	0.32±0.11 ^a	0.38±0.02 ^a	—	—	—
73	49.21	Phthalic acid, cyclohexyl isohexyl ester	C ₁₆ H ₂₄ O ₄	92	304.2	1000315-34-0	—	—	—	—	0.13±0.03 ^a
74	49.82	Methyl palmitoleate	C ₁₇ H<								

Reactive oxygen and nitrogen species generated by DBD-CAP may promote lipid oxidation, cleavage of unsaturated fatty acids, and the hydrolysis or transformation of esters, thereby facilitating the formation or release of aldehydes, ketones, and alcohols (Bayati et al., 2024; Cheng, Li, Yang, & Sun, 2026). Plasma-induced modification of the powdered matrix may also improve volatile extractability (Vedaei & Dara, 2025). These explanations remain mechanistic hypotheses and require validation using lipid-oxidation indices, precursor analysis, and targeted identification of reaction products.

Hierarchical Cluster Analysis of Volatile Compounds

The composition and relative abundance of volatile compounds in *C. chanhua* fruiting bodies varied substantially across the DBD-CAP treatments. To visualize these differences, the relative peak areas of 82 identified volatile compounds were Z-score normalized and subjected to hierarchical cluster analysis (Fig. 2). The three replicates from each treatment clustered closely, indicating satisfactory within-group reproducibility. Based on the sample dendrogram, the 15 samples were separated into three major clusters: CK and T1 formed the first cluster, T2 and T3 constituted the second and T4 formed an independent third cluster. The close association between CK and T1 suggested that treatment at 80 kV for 2 min caused only a limited alteration in the overall volatile profile. T2 and T3 also displayed similar compositional patterns, whereas the clear separation of T4 from the remaining groups indicated that treatment at 120 kV for 4 min exerted the strongest effect on volatile composition. Nevertheless, hierarchical clustering reflects overall compositional similarity and does not, by itself, establish statistically significant differences between groups.

CK and T1 were characterized by relatively high standardized peak areas of several esters and alkanes, including methyl petroselinate, pentadecane, methyl palmitoleate, methyl pentadecanoate, and methyl margarate. Conversely, 2-undecanone, 4-hydroxy- α -thujone, octahydronaphthalen-4a-ol, nonanal, and cyclodecanone showed relatively low standardized abundances in this cluster. Although CK and T1 exhibited similar overall volatile patterns, more compounds were detected in T1 than in CK (55 vs. 42). This finding suggested that treatment at 80 kV for 2 min increased the detectability of some low-abundance volatiles without markedly restructuring the predominant volatile profile.

The T2-T3 cluster was distinguished by the relative enrichment of several linear or branched alkanes and ethers, such as undecane, 3-methylundecane, 4-methyltetradecane, 2,4-dimethyldodecane, 2,6,10-trimethyltetradecane, dodecane, tridecane, 3-methyltridecane, 1-tetradecanol methyl ether, and tetradecyl ether. In contrast, 1,11-tridecadiene, 3-methyl-3-decen-2-one, 1-nonadecene, 2-phenyl-2,4-octadienol, cyclotridecanone, and widdrol exhibited relatively low standardized abundances. A greater number of volatile compounds was detected in T3 than in T2 (71 vs. 66). However, because these treatments differed in both voltage and exposure time, their direct comparison cannot isolate the effect of either individual factor.

T4 exhibited a distinct volatile signature characterized by increased relative abundances of several aldehydes, ketones, alcohols, and other compounds. High standardized peak areas were observed for (E,Z)-2,13-octadecadienol, (Z)-11-pentadecenal, hexanal, muscone, 2-phenoxyethyl phenyl ether, and methyl 9,12-heptadecadienoate. Moreover, 3-methyl-3-decen-2-one, 1-nonadecene, 2-phenyl-2,4-octadienol, cyclohexadecane, widdrol, and the chromatographic feature tentatively assigned to

phthalic acid, cyclohexyl isohexyl ester were detected exclusively in T4, thereby contributing to its separation from the other groups. By comparison, methyl petroselinate, methyl palmitoleate, methyl pentadecanoate, methyl margarate, and the feature tentatively assigned to methyl isohexadecanoate were present at relatively low levels in T4.

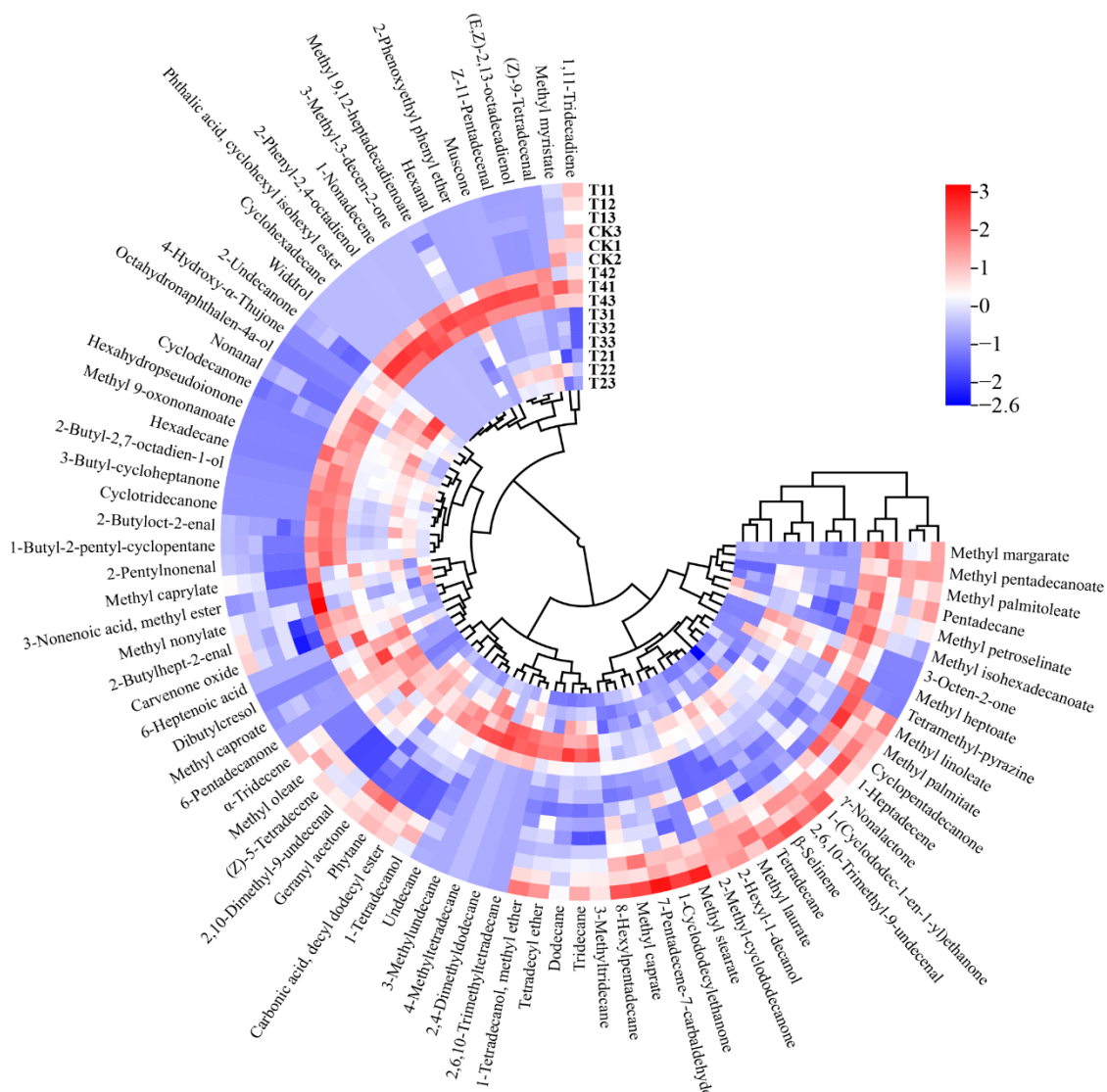


Figure 2: Cluster analysis of volatile compounds in fruiting bodies of *C. chanhua* under different treatments

When treatment duration was held constant, increasing the voltage generally increased the number of detectable volatile compounds. A similar increase was observed with prolonged exposure at a fixed voltage. Thus, increasing the DBD-CAP voltage and/or treatment duration progressively shifted the volatile profile from ester dominance toward greater relative contributions from aldehydes, ketones, and alcohols, with the most pronounced change occurring in T4. This compositional redistribution may be associated with reactive-species-mediated lipid oxidation, ester transformation, and the release of volatile precursors. However, because the heatmap was constructed from relative peak-area data, newly detected compounds may have resulted from their signals exceeding the detection threshold rather than from de novo formation. Further confirmation using

retention indices, authentic standards, procedural blanks, and lipid oxidation markers is therefore required to clarify the underlying mechanisms.

Principal Component Analysis of Volatile Compounds

Principal component analysis (PCA) was performed using the relative peak areas of 82 tentatively identified volatile compounds to evaluate the overall effects of DBD-CAP treatment on the volatile profiles of *C. chanhua* fruiting bodies.

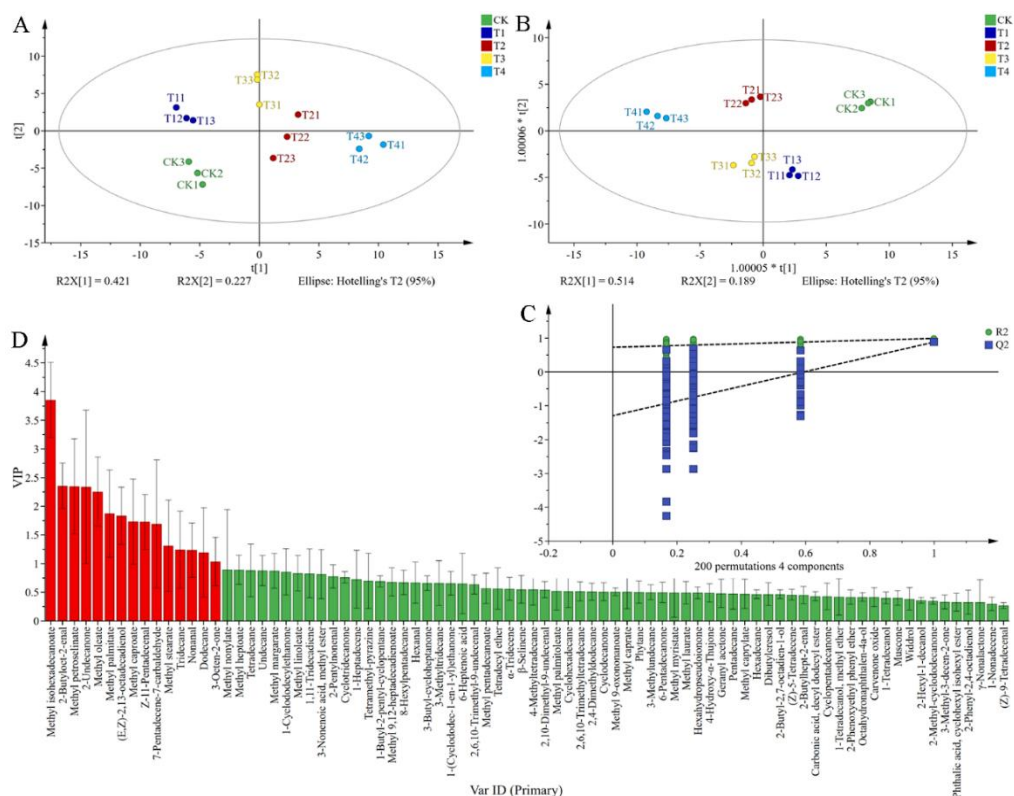


Figure 3: Chemometrics analysis of volatile compounds (A) Principal Component Analysis; (B) Orthogonal partial least squares discriminant analysis; (C) results of 200 displacement tests; (D) and variable importance values.

As shown in Fig. 3A, PC1 and PC2 explained 42.1% and 22.7% of the total variance, respectively, corresponding to a cumulative contribution of 64.8%. The full PCA model exhibited satisfactory explanatory and predictive abilities, with $R^2X(\text{cum})=0.850$ and $Q^2(\text{cum})=0.613$. The three replicates within each treatment clustered closely, and all samples were located within the 95% Hotelling's T² confidence ellipse, indicating good analytical reproducibility and the absence of apparent outliers. CK and T1 were positioned on the negative side of PC1 but were separated along PC2, whereas T2 and T4 were located on the positive side of PC1. T3 was primarily distributed in the positive region of PC2. Notably, the large separation between CK and T4 indicated that treatment at 120 kV for 4 min induced the most pronounced alteration in the overall volatile profile. These findings demonstrated that both treatment voltage and exposure duration contributed to the compositional variation in volatile compounds.

Orthogonal Partial Least Squares Discriminant Analysis of Volatile Compounds

An OPLS-DA model was subsequently constructed to improve discrimination among the treatment groups. As illustrated in Fig. 3B, the two displayed components explained 51.4% and 18.9% of the X-matrix variance, respectively, accounting for 70.3% in total. The overall model showed high goodness of fit and predictive ability, with $R^2X(\text{cum})=0.980$, $R^2Y(\text{cum})=0.981$, and $Q^2(\text{cum})=0.811$. Clear separation was achieved among the five groups, while the biological replicates within each group remained tightly clustered. CK was located on the positive side of the first score dimension, whereas T4 was positioned on the negative side, resulting in the greatest separation between these groups. T1, T2, and T3 occupied distinct regions along the two score dimensions. Compared with the unsupervised PCA, OPLS-DA provided clearer discrimination and confirmed that the different DBD-CAP treatments produced distinguishable volatile signatures.

The robustness of the OPLS-DA model was assessed using a 200-permutation test (Fig. 3C). The Q^2 values of the permuted models were generally lower than that of the original model ($Q^2=0.811$), and the Q^2 regression line exhibited a negative intercept of approximately -1.3 . These results indicate that the predictive performance of the model was unlikely to have arisen from random class assignment. Together with the high R^2Y and Q^2 values, the permutation test supported the overall fit and predictive capacity of the model. Nevertheless, because the R^2 regression intercept was relatively high and each treatment contained only three replicates, the model should be interpreted primarily as exploratory evidence. Additional assessment using CV-ANOVA and an independent validation dataset would further strengthen the robustness of the discrimination.

Based on variable importance in projection (VIP) scores, 15 volatile compounds with $VIP>1$ were identified as candidate discriminant compounds contributing to the separation among treatment groups (Fig. 3D). The chromatographic feature assigned to methyl isohexadecanoate exhibited the highest VIP score and therefore made the greatest contribution to model discrimination. Relatively high VIP scores were also observed for 2-butyloct-2-enal, methyl petroselinate, 2-undecanone, and methyl oleate. The remaining candidate compounds comprised methyl palmitate, (E,Z)-2,13-octadecadienol, methyl caproate, (Z)-11-pentadecenal, 7-pentadecene-7-carbaldehyde, methyl stearate, tridecane, nonanal, dodecane, and 3-octen-2-one. These candidate discriminants encompassed esters, aldehydes, ketones, alcohols, and alkanes, indicating that group separation resulted from coordinated changes across multiple chemical classes rather than variation in a single class. When considered together with the relative peak-area data, the model discrimination primarily reflected a decrease in the relative contribution of predominant esters and a concomitant enrichment of aldehydes, ketones, and alcohols under the more intensive DBD-CAP conditions. Nevertheless, VIP scores indicate the contribution of individual variables to model discrimination and do not independently establish statistical significance or sensory relevance. These candidate markers should therefore be further evaluated using univariate analysis, fold-change criteria, retention indices, authentic standards, and odor activity values.

CONCLUSIONS

This study demonstrated that dielectric barrier discharge cold atmospheric plasma (DBD-CAP) treatment substantially reshaped the volatile profiles of *C. chanhua* fruiting bodies in

a voltage and time dependent manner. GC-MS identified 82 volatile compounds, with the number detected rising from 42 in the untreated control (CK) to 75 under the most intensive condition (T4: 120 kV, 4 min). Increasing treatment voltage and/or time progressively changed the profile away from ester dominance (declining from 81.38 % in CK to 29.42 % in T4) toward greater relative contributions of aldehydes (from 6.11 % to 30.81 %), ketones (from 2.99 % to 12.54 %) and alcohols. Multivariate analyses (hierarchical clustering, PCA and OPLS-DA) confirmed clear treatment-dependent differentiation, with T4 exhibiting the most distinct volatile signature, characterized by elevated relative peak areas of compounds such as 2-butyloct-2-enal, 2-undecanone, nonanal, (Z)-11-pentadecenal, and (E,Z)-2,13-octadecadienol. In contrast, milder conditions (e.g., 80 kV for 2 min) produced profiles comparatively closer to the control.

The observed redistribution is consistent with reactive-species-mediated oxidation, cleavage of volatile precursors and altered compound release from the sample matrix. Overall, mild DBD-CAP treatment offered a more favorable balance between increasing volatile diversity and limiting extensive compositional change, whereas 120 kV for 4 min induced the greatest transformation. These findings demonstrated the potential of DBD-CAP as a non-thermal approach to modulate the aroma-related chemical composition of *C. chanhua* fruiting bodies. Nevertheless, because compound identification and comparison were based primarily on mass spectral matching and relative peak areas, further studies should incorporate retention indices, internal-standard-based quantification, GC-olfactometry, odor activity values and sensory evaluation to confirm compound identification and determine whether the observed compositional changes translate into perceptible aroma differences.

Author Contributions

Huixin Song: Data curation, formal analysis, investigation, writing-original draft. **Haojun Zhang:** Methodology, investigation, writing-original draft. **Yang Wang:** investigation, writing-original draft. **Tengfei Zhang:** Methodology, formal analysis. **Shijun Yu:** conceptualization, methodology, supervision, validation, funding acquisition, writing-review & editing. All authors have read and agreed to the published version of the manuscript.

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Data will be made available on request.

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