

RESEARCH

Open Access



Floral extracts as fermentation modulators: improvement of cigar tobacco quality via microbial and chemical reshaping

Wanrong Hu^{1,2}, Qianying Zhang^{1,2}, Wen Cai^{1,2}, Yun Jia^{1,2}, Quanwei Zhou^{1,2}, Zhongrong Jiang^{1,2}, Li Liu³ and Dongliang Li^{1,2*}

Abstract

Background The quality of cigar tobacco leaves relies heavily on fermentation optimization. Media fermentation enhances this process through exogenous functional extracts, offering targeted improvement in leaf quality. While various plant extracts have been explored, floral extracts remain underexamined despite their rich aromatic profiles and potential to regulate microbial activity. This study investigated the effects of four floral extracts (*Prunus mume*, *Jasminum grandiflorum*, *Osmanthus fragrans*, and *Eriobotrya japonica* flower) as fermentation media on the chemical, microbial, and sensory properties of cigar tobacco leaves.

Results Chemical characterization revealed that the four floral extracts possessed distinct phenolic profiles, characterized by varying compositions of flavonoids, phenolic acids, and glycosides. As fermentation media, all four floral extracts significantly reduced total nitrogen (by up to 6.63%) and alkaloid content (by up to 9.97%), while increasing sugar levels, with *Osmanthus fragrans* extract showing the most pronounced effect (62.11% higher reducing sugar content). The extracts also promoted the accumulation of key aroma compounds, including neophytadiene and megastigmatrienones, with the *Prunus mume* group achieving 32.47% increase in total aroma content. Microbial analysis showed selective enrichment of aroma-synthesis-linked taxa, including *Pseudomonas*, *Enterobacter*, *Aspergillus*, and *Walleimia*, which strongly correlated with multiple aroma components. Sensory evaluation confirmed targeted improvements: *Prunus mume* extract enhanced aroma smoothness and richness, *Jasminum grandiflorum* extract notably improved sweetness and fluentness, *Osmanthus fragrans* extract effectively increased mellowness and cleanliness, while *Eriobotrya japonica* flower extract performed best in aftertaste.

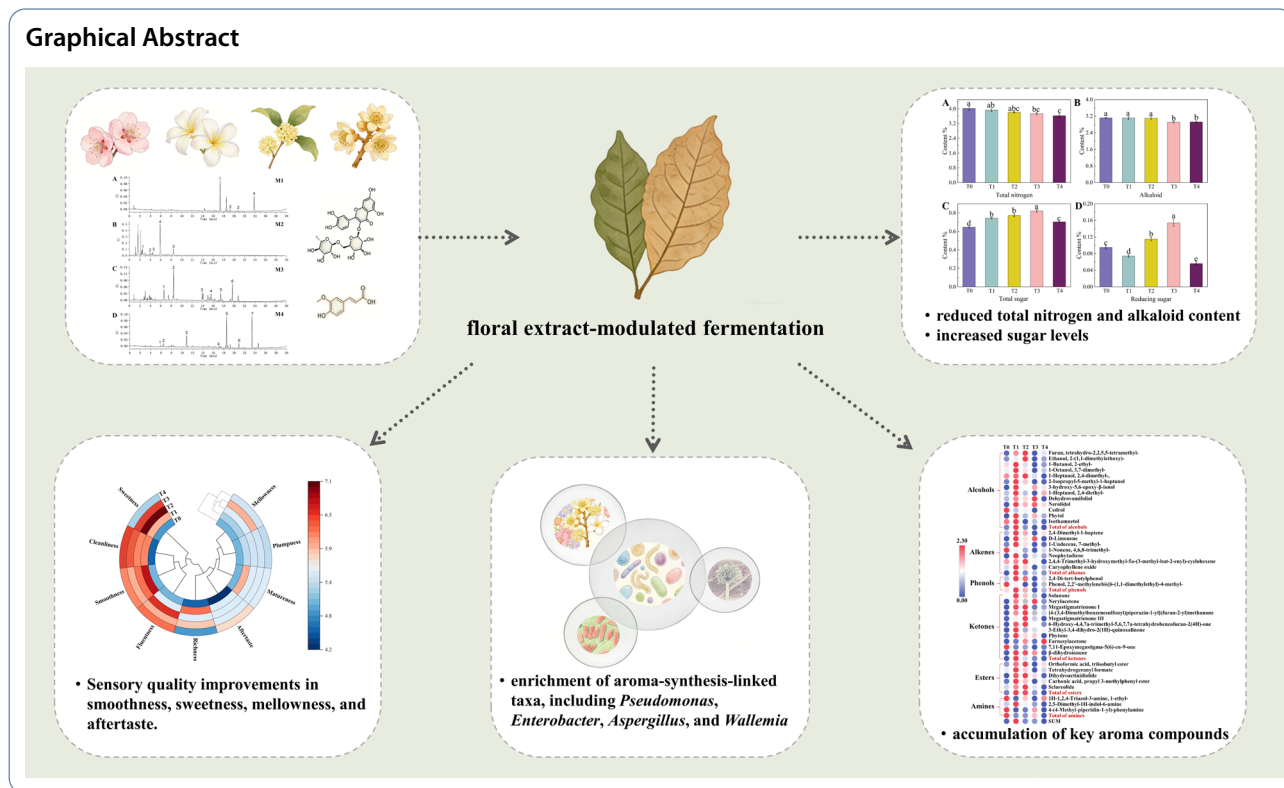
Conclusions This study demonstrates that floral extracts can act as reliable precision fermentation media by shaping microbial communities and driving desirable chemical transformations. The results provide a mechanistic basis for using floral resources to improve cigar tobacco quality and introduce a targeted, function-driven framework for developing next-generation fermentation additives.

Keywords Cigar tobacco leaves, Floral extracts, Fermentation, Microbial community, Aroma compounds, Sensory quality

*Correspondence:
Dongliang Li
sczyldl@163.com

Full list of author information is available at the end of the article

Graphical Abstract



Introduction

The pursuit of superior flavor profiles is a central endeavor in the production of premium cigars. High-quality cigars are characterized by a balanced chemical composition. Moderate levels of alkaloids provide “strength” and satisfaction, but excessive amounts can cause harshness [1]. Sugars contribute to sweetness and serve as aroma precursors [2]. Excessive nitrogenous compounds, such as proteins, produce burnt-feather odors and irritation during combustion [3]. Beyond chemistry, sensory quality is defined by attributes such as smoothness (lack of harshness), richness (aroma complexity), sweetness, and aftertaste. The post-harvest fermentation of cigar tobacco leaves (CTLs) serves as a pivotal stage for developing these profiles [4]. This critical process of fermentation relies on complex interactions between microbes and biochemical reactions which work in concert to reduce irritants and generate a diverse spectrum of aroma compounds [5]. Microorganisms colonizing the leaf surface secrete a wide array of hydrolytic enzymes, including amylases, proteases, and cellulases. These enzymes break down macromolecules such as starch, proteins, and cellulose into simpler compounds like sugars, amino acids, and organic acids [6]. Such degradation products serve as both direct flavor contributors and precursors for further transformations. For instance, sugars and amino acids participate in Maillard reactions during subsequent processing, generating pyrazines,

furans, and other compounds responsible for nutty and caramel-like aromas [7]. Concurrently, certain bacteria possess the ability to degrade nicotine and other alkaloids, thereby reducing irritation and bitterness [8].

According to extensive survey results, conventional fermentation management primarily focuses on controlling physical parameters like temperature and humidity [9, 10]. While necessary, this approach offers limited direct control over the microbial composition and metabolic pathways that ultimately define tobacco quality. A major consequence is the protracted fermentation time, as exemplified by the requirement for at least two years of fermentation for CTLs in renowned producing regions like Cuba and the Dominican Republic. Furthermore, for leaves from sub-optimal growing or air-curing conditions, conventional fermentation often fails to sufficiently remediate inherent quality defects, leaving the final product short of desired standards [11]. This variability is compounded by the inherent variability of tobacco as an agricultural product, whose quality is highly susceptible to climatic factors, leading to significant batch-to-batch inconsistencies.

To overcome these limitations in efficiency, efficacy, and consistency, a more proactive strategy has gained traction: the use of natural plant-based additives. These additives function by introducing their own characteristic flavor compounds and, more importantly, by modulating the microbial ecosystem and steering metabolic

pathways towards the desired production of flavor precursors [12]. This active intervention can potentially accelerate the fermentation process and achieve superior quality outcomes.

Beyond the well-documented spices, coffee and cocoa, the vast repertoire of aromatic botanicals remains underexplored for this purpose. Among them, edible flowers represent a unique reservoir of volatile and non-volatile compounds renowned for their captivating fragrances and historical use in flavoring foods and perfumery [13, 14]. Extracts of flowers of *Prunus mume*, *Jasminum grandiflorum*, *Osmanthus fragrans*, and *Eriobotrya japonica* are rich in terpenes, phenolics, and esters, which are known to contribute to floral, fruity, and sweet notes [15, 16]. We hypothesize that these intrinsic flavorants can not only directly impart desirable aromatic nuances to tobacco but also interact with the endogenous microbial ecosystem during fermentation. Such interactions may selectively inhibit or stimulate specific microbial taxa, thereby indirectly reshaping the metabolic pathways responsible for the degradation of macromolecules (e.g., alkaloids, proteins) and the synthesis of key aroma components.

However, a systematic investigation into the application of these floral extracts in CTL fermentation is currently lacking. It remains unclear how their distinct chemical compositions differentially influence the chemical and microbial landscapes of tobacco, and ultimately, its sensory attributes. The primary objective of this work was to systematically deconvolute the relationship between the specific chemistry of these extracts, the resultant shifts in the CTL's chemical and microbial landscape, and the ultimate sensory quality. For the first time, we integrated a multi-dimensional analysis that couples (i) detailed chemical characterization of the extracts, (ii) comprehensive analysis of the CTL's chemical evolution, (iii) bacterial and fungal community structures, (iv) correlation analysis to bridge the chemical and microbial domains, and (v) sensory quality. By establishing these links, we aimed to elucidate the specific microbial taxa and biochemical pathways that are enriched or suppressed by each additive, thereby uncovering the “why” behind their quality-enhancing effects. The findings presented herein not only provide a robust theoretical foundation for the rational development of novel, function-oriented fermentation media but also introduce a systematic, mechanism-driven research framework that can guide future innovations in cigar tobacco fermentation.

Materials and methods

Source and preparation of floral extracts

Fresh botanical materials of *Prunus mume* (Siebold) Siebold & Zucc. (Jiangsu, China), *Jasminum grandiflorum* L. (Sichuan, China), *Osmanthus fragrans* (Thunb.)

Lour. (Hubei, China), and *Eriobotrya japonica* (Thunb.) Lindl. (Zhejiang, China) were commercially sourced from a local supermarket in Chengdu, China. To preserve thermolabile compounds, the materials were lyophilized using a SCIENTZ-10N freeze dryer (Scientz, China) at -50°C for 24 h, and subsequently milled into a fine powder (<60 mesh) under an AF-06S grinder (Auari, China) at 25,000 r/min for 30 s.

The extraction protocol was designed to efficiently isolate target active compounds. Briefly, 50 g of each powdered material was suspended in 500 mL of ethanol ($\geq 99.5\%$, Kelong, China), ultrasonicated for 1 h at 35°C in a thermostatic water bath, and allowed to sediment overnight. The supernatant was sequentially filtered through three layers of qualitative filter paper (maximum pore size: $6\text{--}8\text{ }\mu\text{m}$). The filtrate was concentrated under vacuum using a rotary evaporator (RE-2000A, Yarong, China) to remove the ethanol solvent. The resulting aqueous residue was defined as the respective floral extract and stored at 4°C prior to fermentation trials. These extracts were labeled M1 (*P. mume*), M2 (*J. grandiflorum*), M3 (*O. fragrans*), and M4 (*E. japonica* flower).

Chemical profiling of extracts

The chemical composition of the four floral extracts was characterized using ultra-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC-MS/MS). Chromatographic separation was performed using a Waters Acquity UPLC BEH C18 column ($2.1\times 150\text{ mm}$, $1.7\text{ }\mu\text{m}$, $130\text{ }\text{\AA}$) as the stationary phase. The mobile phase consisted of water (mobile phase A) and acetonitrile (HPLC grade, $\geq 99.9\%$, Merck, Germany) (mobile phase B), with the following gradient elution program: 0–2 min, 5% B; 2–16 min, 5–28% B; 16–20 min, 28–60% B; 20–26 min, 60–100% B; 26–28 min, 100% B; and 28–30 min, 100–5% B. The detection wavelength range was set at 200–600 nm, with a flow rate of 0.2 mL/min, column temperature of 25°C , and injection volume of 2 μL . Mass spectrometric analysis was conducted on a UPLC-Triple-TOF 5600+ system. The parameters were as follows: ionization mode, positive and negative electrospray ionization (ESI); mass range, m/z 100–1500; nebulizer gas (GS1), 55 psi; nebulizer gas (GS2), 55 psi; curtain gas (CUR), 35 psi; ion source temperature, 600°C (positive) and 550°C (negative). For MS1 scanning, the declustering potential (DP) was 100 V and the collision energy (CE) was 10 V. For MS/MS analysis, data were acquired in TOF MS-Product Ion-IDA mode with collision-induced dissociation (CID) energy of $40\pm 20\text{ eV}$. Prior to analysis, mass axis calibration was performed using a CDS pump to ensure mass accuracy within 2 ppm.

Compounds were identified by comparing their retention times, mass-to-charge ratios (m/z), and MS/MS

fragmentation patterns with those available in public and commercial databases, including Reaxys (Elsevier) and relevant literature. Quantitative analysis of the main phenolic compounds was performed using an external standard method. Reference standards including rutin, isoquercitrin, and ferulic acid, p-coumaric acid (purity $\geq 98\%$, Shyuan, China) were adopted.

Fermentation trial design

To evaluate the impact of the floral extracts on tobacco fermentation, a controlled experiment was set up using a uniform batch of CTLs (variety Chuxue No. 14). Each floral extract (initial concentration: 100 mg/mL) was diluted tenfold with deionized water to a final concentration of 10 mg/mL for the fermentation treatments.

For each treatment, 50 mL of a diluted extract (10 mg/mL) was evenly sprayed onto 1 kg of CTLs using a spray bottle. During spraying, the leaves were continuously turned to ensure uniform coverage. After spraying, the treated leaves were air-dried at room temperature for 30 min to allow the extract to fully absorb before fermentation. The inoculated leaves were placed in polyethylene bags and incubated in an environmental chamber (KBF720, Binder, Germany) maintained at 35 °C and 75% relative humidity under dark conditions for 20 days. The bags were randomly repositioned within the chamber every 2 days to minimize positional effects. This setup generated four treatment groups: T1 (M1 extract), T2 (M2 extract), T3 (M3 extract), and T4 (M4 extract). A control group (T0) was established by treating CTLs with an equivalent volume of water instead of the extract solution. All treatments, including the control, were performed in triplicate ($n=3$), resulting in a total of 15 independent experimental units (5 groups $\times$ 3 replicates).

At the end of the fermentation period, the leaves from each replicate were thoroughly mixed, and subsamples were collected for subsequent analyses. The samples were immediately frozen in liquid nitrogen and stored at -80 °C until further use.

Assessment of fermented tobacco quality

The following post-fermentation analyses were conducted to determine changes in key quality parameters: routine chemical analysis, aroma profiling, and microbial community structure.

Routine chemical analysis

The chemical composition of CTLs, including total nitrogen, alkaloid, total sugar, and reducing sugar was determined using Continuous Flow Analysis according to the publicly available Chinese industry standards YC/T 161–2022, YC/T 468–2021, and YC/T 159–2019 [17]. Total nitrogen is determined after acid digestion with concentrated sulfuric acid and a catalyst, where the nitrogen is

converted to ammonia, which then reacts with sodium salicylate and sodium hypochlorite in alkaline conditions to form a blue indophenol dye measured at 660 nm (YC/T 161–2022). Alkaloids are extracted with water or 5% acetic acid, and the extract reacts with p-aminobenzenesulfonic acid and cyanogen chloride (generated online from potassium thiocyanate and dichloroisocyanuric acid), with the reaction product measured at 460 nm (YC/T 468–2021). Total sugar and reducing sugar are extracted with 5% acetic acid, and the sugars react with p-hydroxybenzoic acid hydrazide in alkaline medium at 85 °C to form a yellow chromophore measured at 410 nm (YC/T 159–2019). These standardized methods are extensively validated and routinely employed in tobacco research.

Aroma profiling

The spectrum of aroma compounds was investigated by gas chromatography-mass spectrometry (GC–MS). Sample preparation involved extraction with acetonitrile followed by a clean-up step using a modified QuEChERS approach. Specifically, leaf powder samples (2 g) were transferred to 50 mL centrifuge tubes, to which 10 mL deionized water was added and agitated for 10 min. Thereafter, 10 mL acetonitrile and 50 μ L of the internal standard working solution (phenethyl acetate, 10.03 mg/mL, purity $\geq 98\%$, Sigma-Aldrich, USA) were introduced into the mixture. The samples were then subjected to shaking at 2000 rpm for a duration of 2 h. Following this, an Extraction Kit (5982–5650, Agilent, USA) was incorporated into each tube, with immediate vortex mixing to prevent aggregation. Subsequently, 1 mL of the resulting supernatant was collected and combined with 150 mg anhydrous magnesium sulfate (Macklin, China, purity $\geq 99.5\%$). The final supernatant was subjected to GC–MS analysis (7890B–5977, Agilent, USA) using an Agilent DB-5MS capillary column (60 m $\times$ 0.25 mm). The chromatographic parameters were established as follows: injector temperature, 290 °C; oven temperature gradient, initial hold at 60 °C, ramped to 250 °C at 2 °C/min, then to 290 °C at 5 °C/min, with a final hold of 20 min at 290 °C; transfer line temperature, 290 °C. Compound identification was performed using electron ionization at 70 eV, scanning over the mass range of 26–400 amu. The obtained mass spectra were compared with those in the NIST 17 mass spectral library (National Institute of Standards and Technology, Gaithersburg, USA), with a similarity threshold of $\geq 80\%$.

Microbial community structure

Microbial genomic DNA was extracted from 15 independent fermented tobacco samples (5 treatment groups $\times$ 3 biological replicates) using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, USA) according to the manufacturer's

protocol. High-throughput sequencing of the 16S rRNA gene (V3-V4 region) for bacteria and the ITS1-ITS2 region for fungi was performed using the Illumina MiSeq system (Illumina MiSeq, USA). Following sequencing, the paired-end reads were assembled using PEAR (v0.9.8). The resulting sequences were processed and clustered into operational taxonomic units (OTUs) at a 97% similarity threshold using Usearch (v11.0.667). Taxonomic assignment was performed using the RDP classifier against the SILVA database for 16S sequences and the UNITE database for ITS sequences, with a confidence threshold of 70%. Alpha diversity (Chao and Shannon indices) was computed with Mothur software (version 3.8.31). To identify bacterial and fungal taxa with significantly different abundances between treatment groups, linear discriminant analysis effect size (LEfSe, version 1.1.0) was performed. The analysis first used the non-parametric Kruskal–Wallis sum-rank test ($\alpha=0.05$) to detect features with significant differential abundance across groups. For features that passed this test, a subsequent pairwise Wilcoxon rank-sum test ($\alpha=0.05$) was applied to assess biological consistency. Finally, linear discriminant analysis (LDA) was used to estimate the effect size of each differentially abundant feature, with an LDA score threshold of >3.0 considered significant.

Data analysis

Associations between predominant microbial taxa and key chemical constituents were evaluated by computing Pearson's correlation coefficients, with significance thresholds set at $|r|>0.8$ and a p -value <0.05 . For group comparisons, a one-way ANOVA was performed, supplemented by Duncan's post hoc test for multiple comparisons, with all inferences drawn at the 95% confidence level. Statistical analyses and data visualization were performed using IBM SPSS Statistics 27 (IBM, USA), ORIGIN 2024 (OriginLab, USA), and Microsoft Excel 2016 (Microsoft, USA).

Sensory evaluation

A panel of seven trained sensory evaluators, each with extensive experience in cigar quality assessment, participated in the descriptive analysis. Prior to evaluation, the panelists underwent a calibration session using standard cigar samples to ensure consistent scoring criteria. Tobacco leaf samples were first rolled into cigar sticks (90 mm in length, 47 mm in circumference) and then conditioned in an aging cabinet (Bulldog VC528, China) at 20 °C and 60% relative humidity for 10 days before assessment. The evaluation was conducted in a controlled sensory environment with the following conditions: quiet, well-ventilated, odor-free, and free from disturbances. A local circulation smoking method was adopted, with sensory evaluation primarily conducted

when the cigar burned to between one-third and two-thirds of its length. Samples were presented in a randomized order to the panelists. To cleanse the palate between samples, evaluators were provided with water and green tea, and were instructed to take at least a 5-min break between evaluations. A 9-point structured scale was used to score nine sensory attributes: mellowness, richness, maturity, plumpness, fluentness, smoothness, sweetness, cleanliness, and aftertaste.

Results

Chemical composition of the four floral extracts

To elucidate the material basis of the fermentation media, a systematic qualitative and quantitative analysis of the four extracts from *P. mume* (M1), *J. grandiflorum* (M2), *O. fragrans* (M3), and *E. japonica* flower (M4) was conducted using UPLC-MS/MS (Fig. 1). The identified compounds with their quantitative data are summarized in Tables S1–S4. The results demonstrated that all four floral extracts were rich in various bioactive compounds. However, their specific compositional profiles and relative abundances differed significantly. As shown in Table S1, four compounds were identified in the *P. mume* extract (M1), predominantly flavonoids. Rutin was the most abundant compound (0.71 mg/100 g DW), followed by isoquercitrin and hyperoside. Additionally, the flavonol aglycone kaempferol was also detected. Five compounds were identified in the *J. grandiflorum* extract (M2) (Table S2), exhibiting greater chemical diversity. In addition to 5-dihydrocaffeoylquinic acid and its methoxy analogue, a chlorogenic acid derivative and two quercetin-derived glycosides were detected. The analysis of the *O. fragrans* extract (M3) (Table S3) revealed six flavonoid compounds, all existing in glycosidic forms. These were identified as various glycosides of flavonoid aglycones such as quercetin and luteolin. The *E. japonica* flower extract (M4) displayed the most complex chemical composition (Table S4), with seven identified compounds encompassing hydroxycinnamic acids, flavonoids, and an aldehyde. Notably, the contents of ferulic acid (13.25 mg/100 g DW) and coumaric acid (12.89 mg/100 g DW) were exceptionally high. High levels of quercetin and isorhamnetin were also detected. Furthermore, 2-hydroxycinnamic acid and 4-methoxybenzaldehyde were identified. In summary, while all four floral extracts are characterized by phenolic compounds, their specific chemical compositions are distinct. M1 is dominated by classic flavonoids; M2 features phenolic acids and flavonoid glycosides; M3 is rich in flavonoid glycosides; and M4 is marked by high levels of phenolic acids and flavonoids.

Conventional chemical compositions in CTLs

To evaluate the effects of different floral extracts on the conventional chemical composition of CTLs, the

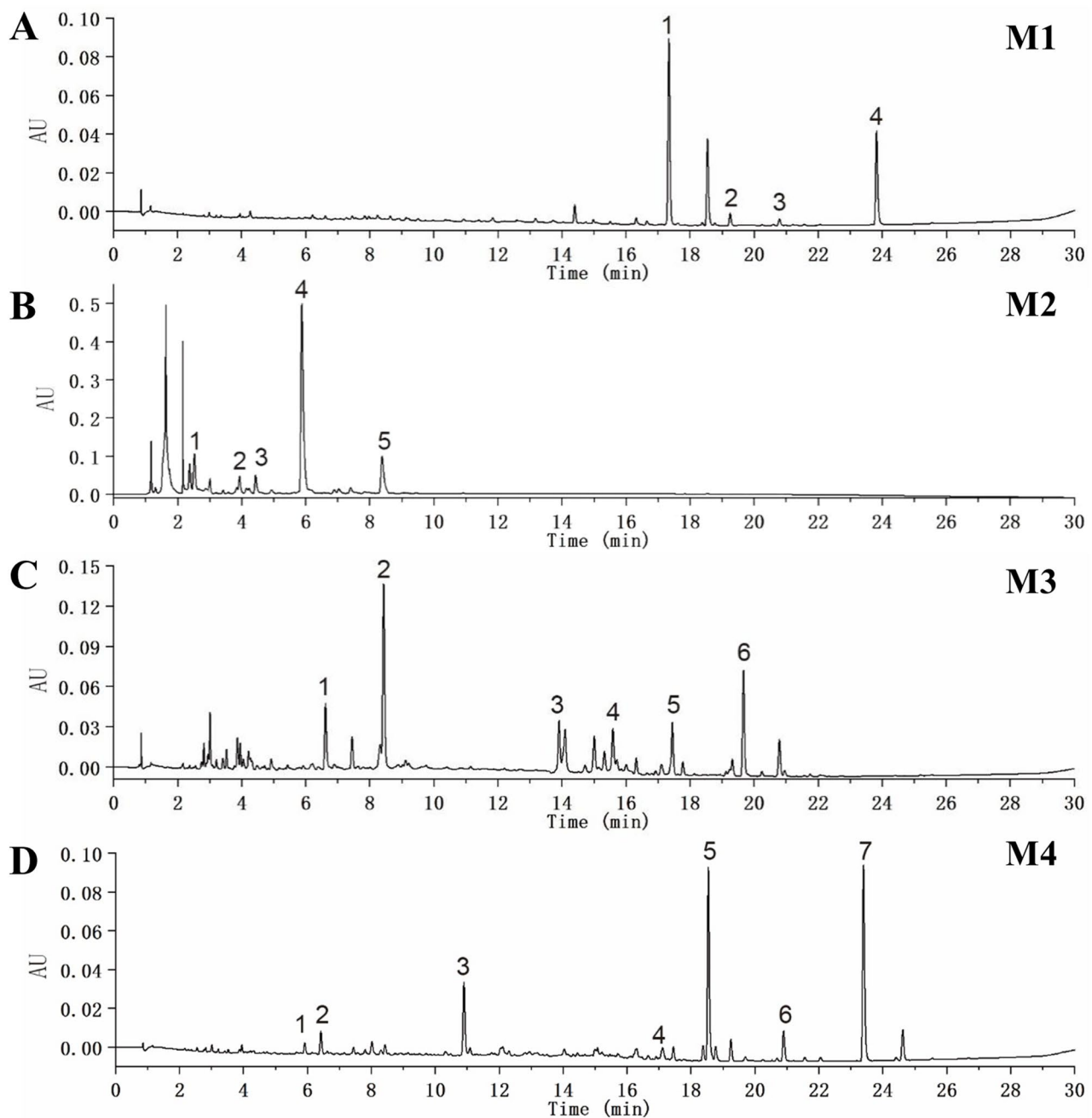


Fig. 1 Representative UPLC-MS/MS chromatograms of the four plant extracts used as fermentation media: **A***Prunus mume*, **B***lasminum grandiflorum*, **C***Osmanthus fragrans*, and **D***Eriobotrya japonica* flower

contents of total nitrogen, alkaloids, total sugars, and reducing sugars were determined across five treatment groups (T0-T4). The results are presented in Fig. 2.

As illustrated in Fig. 2A, total nitrogen content varied among treatments. Compared to the control group T0, total nitrogen was lower in T3 and T4, representing reductions of 6.63% and 6.29%, respectively. No significant differences were observed between T0, T1, and T2. A clear decreasing trend was observed for alkaloid

content (Fig. 2B). The alkaloid content in T3 and T4 was significantly reduced compared to T0. The lowest alkaloid content was found in T4 (3.62%), representing a significant reduction of 9.97% compared to T0, highlighting the pronounced effect of *Eriobotrya* extract on alkaloid degradation. The changes in sugar contents were more complex (Fig. 2C, D). The total sugar content in all plant extract-treated groups was significantly higher than that in the control group T0. Notably, the T3 group reached

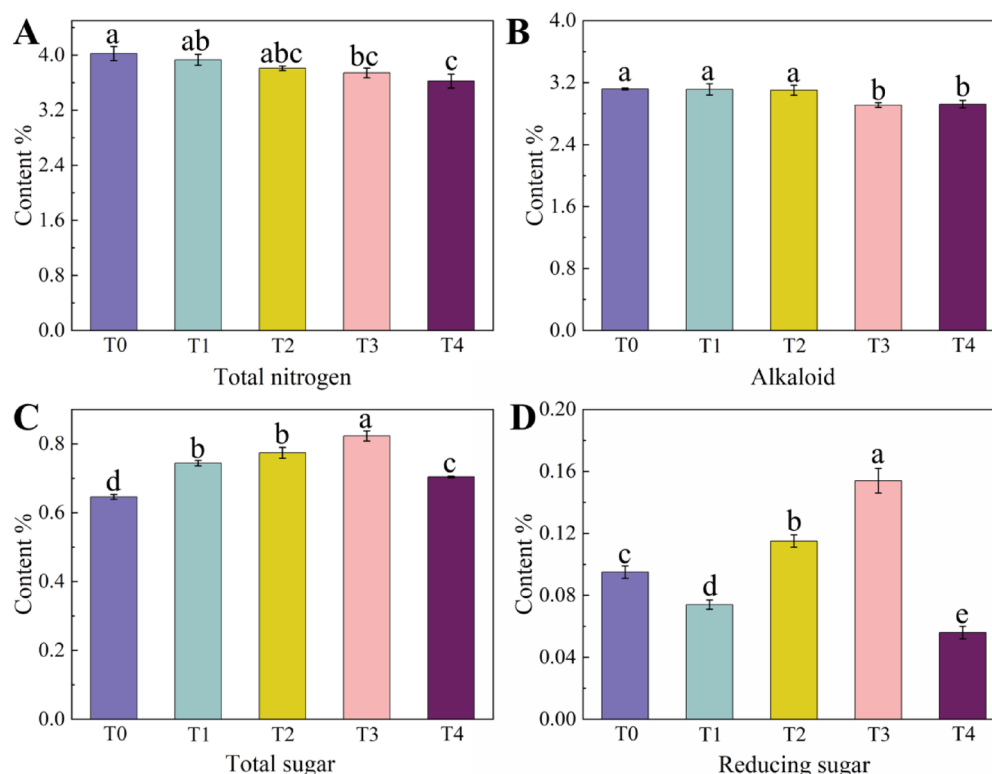


Fig. 2 Comparative analysis of total nitrogen **A**, alkaloid **B**, total sugar **C** and reducing sugar **D** contents among differently treated tobacco leaves. Data were analyzed by one-way ANOVA followed by Duncan's post hoc test. Different letters on the bar graphs indicate significant differences in the mean of each treatment at p -value < 0.05

the highest total sugar content of 0.82%, which was significantly greater than all other groups. For reducing sugar, the contents in T2 and T3 were significantly higher than those in T0. T3 showed the most remarkable increase, being 62.11% higher than T0. These results demonstrate that *O. fragrans* extract was most effective in significantly enhancing sugar accumulation, particularly for reducing sugar. In summary, all four floral extracts modulated the conventional chemical composition of CTLs, but to varying degrees. T3 and T4 were most effective in reducing total nitrogen and alkaloid content, while T3 showed the strongest enhancement of both total sugar and reducing sugar.

Aroma compounds in CTLs

GC-MS analysis was performed to investigate the effects of different floral extracts on the aroma profiles of fermented CTLs. A total of six major categories of aroma compounds were identified, including alcohols, alkenes, phenols, ketones, esters, and amines. The relative abundances of these compounds across treatment groups are presented in the heatmap (Fig. 3).

All four floral extracts modulated the aroma profiles of CTLs, but to varying degrees and with distinct patterns. The *P. mume* extract (T1) demonstrated the most

outstanding performance, with the total content of aroma components significantly increasing by 32.47% compared to the control group (T0). Besides, the T1 sample showed universal improvement across multiple aroma categories. Specifically, nerolidol increased by 40.64%, phytol by 62.10%, and megastigmatrienone I by 52.00% in T1. In the *J. grandiflorum* extract treatment (T2), moderate increases were observed for specific compounds. D-limonene increased by 50.91%, while dihydroactinidiolide and sclareolide increased by 52.05% and 28.77%, respectively. The *O. fragrans* extract treatment (T3) showed a marked increase in 3-hydroxy-5,6-epoxy- β -ionol (53.28%), a key intermediate in the carotenoid degradation pathway. Other aroma compounds in T3 showed moderate increases. The *E. japonica* flower extract treatment (T4) exhibited the smallest changes in total aroma content. However, increased levels of dehydrovomifoliol and phytol were observed compared to T0.

In summary, each floral extract imparted a distinct aroma signature to the fermented tobacco leaves. T1 showed the most comprehensive enhancement across multiple aroma categories, T2 specifically enhanced fruity-sweet notes, T3 promoted carotenoid-derived compounds, and T4 had a more moderate effect on overall aroma.

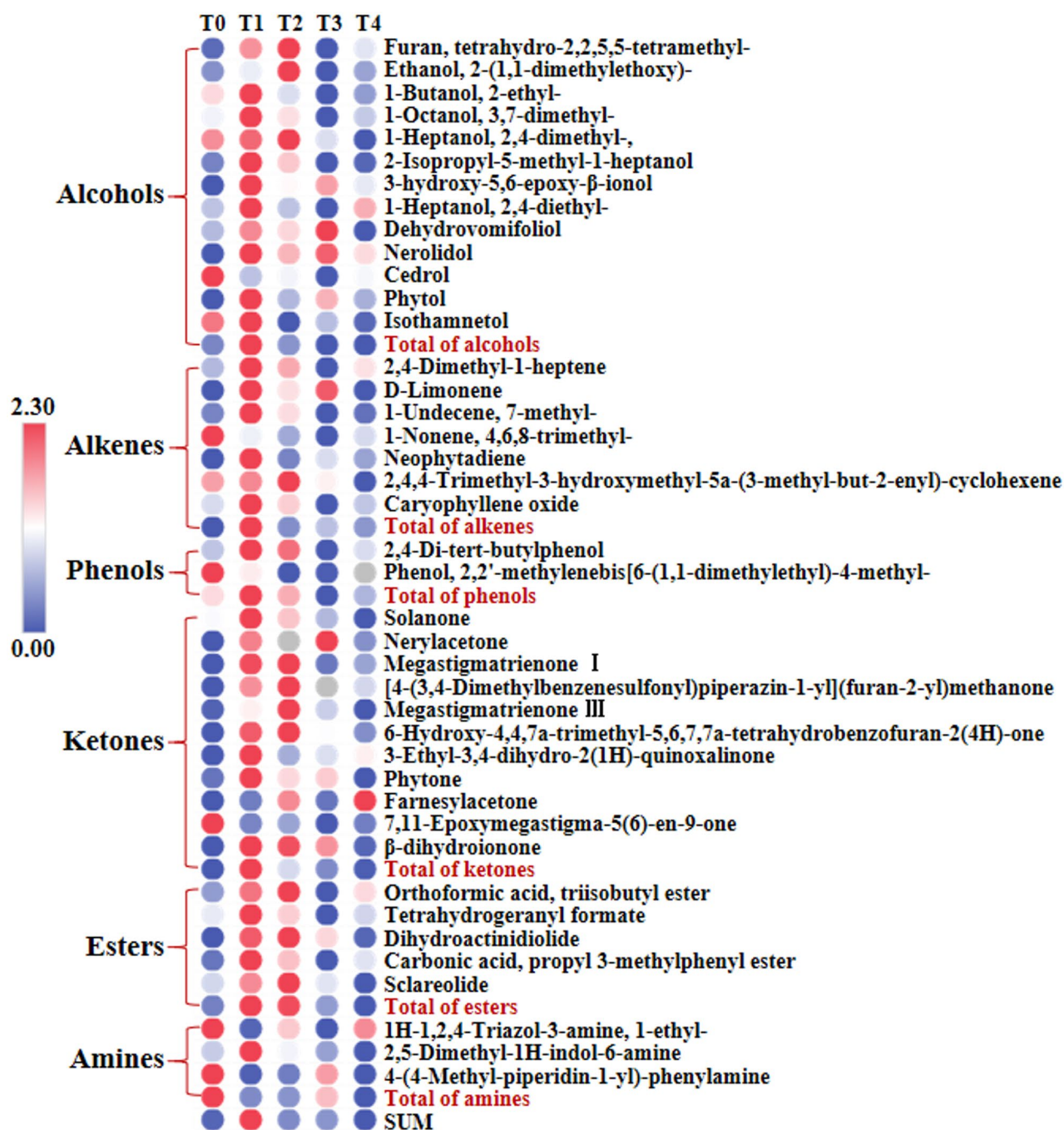


Fig. 3 Heatmap cluster analysis of key aroma compounds in CTLs fermented with different floral extracts

Microbial community analysis of CTLs

Analysis of microbial community diversity

Alpha diversity indices were used to measure the microbial community diversity within a single sample, reflecting both species richness and evenness. As shown in Fig. 4A, C, different treatments significantly impacted the alpha diversity of the bacterial communities on tobacco leaves. Notably, tobacco leaves treated with *O. fragrans* extract (T3) exhibited the highest Shannon and Chao

indices among all treatments, along with relatively high Shannon evenness.

Analysis of the fungal communities (Fig. 4B, D) revealed a different pattern of change compared to bacteria. All treatment groups with floral extracts (T1-T4) showed markedly higher fungal Shannon index and Shannon evenness than the control group T0. The *O. fragrans* extract treatment (T3) again showed the most pronounced effect, with its fungal community reaching

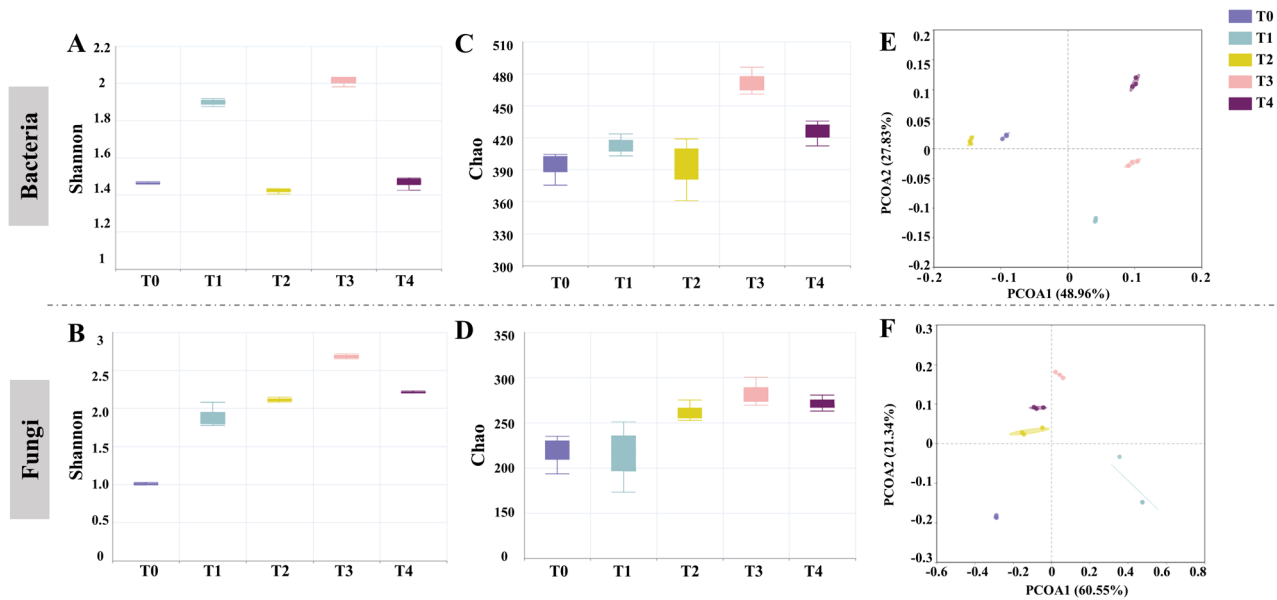


Fig. 4 Diversity analysis of microbial communities in CTLs subjected to different floral extract treatments: **A** Shannon index of bacterial communities, **B** Shannon index of fungal communities, **C** Chao index of bacterial communities, **D** Chao index of fungal communities, **E** Principal coordinate analysis (PCoA) of bacterial communities, **F** PCoA of fungal communities

the peak Shannon index and species richness. This indicates that the T3 treatment resulted in the highest fungal diversity and the lowest dominance, representing the most balanced community structure. Treatments with *P. mume* (T1) and *E. japonica* flower (T4) extracts also significantly increased fungal diversity, whereas the *J. grandiflorum* treatment (T2) had a slightly less pronounced effect compared to T1, T3, and T4.

To further visualize the overall structural differences in microbial communities among treatments, principal coordinate analysis (PCoA) was performed based on Bray–Curtis distance matrix. For bacterial communities (Fig. 4E), samples from different treatments were clearly separated along the first two principal coordinates, with the T3 group distinctly clustering apart from the others. Similarly, fungal community PCoA (Fig. 4F) also revealed clear separation among treatments, further confirming that floral extract amendments significantly reshaped the microbial community composition. Notably, samples within the same treatment group clustered closely, indicating good experimental reproducibility.

Bacterial OTU analysis (Fig. 5A) revealed a total of 950 OTUs across all samples. The control group T0 contained 49 unique OTUs, while the floral extract treatment groups exhibited significantly higher numbers of unique OTUs: T1 had 72, T2 had 42, T3 had 97, and T4 had 68. Notably, T3 showed the highest number of unique OTUs, indicating its most pronounced effect on shaping the bacterial community structure. Additionally, 285 OTUs were shared among all treatment groups, suggesting a stable core microbiome across different treatments.

Fungal OTU analysis (Fig. 5B) showed a similar trend. A total of 556 fungal OTUs were identified across all samples. The control group T0 contained only 7 unique OTUs, while the flower extract groups had significantly more unique OTUs: T1 had 10, T2 had 30, T3 had 56, and T4 had 54. Among these, T3 and T4 exhibited the highest numbers of unique OTUs, further supporting the strong influence of *O. fragrans*, and *E. japonica* flower extracts on fungal community structure. A total of 142 fungal OTUs were shared among all groups. The number of OTUs shared between T0 and flower extract groups was relatively low; for example, T0&T1 shared only 2 OTUs, and T0&T3 shared 10 OTUs. In summary, flower extract treatments significantly increased the number of unique bacterial and fungal OTUs in CTLs, with the most pronounced effects observed in *O. fragrans* (T3) and *E. japonica* flower (T4) extracts.

Community structure analysis

At the phylum level (Fig. 6A), the bacterial communities across all samples were dominated by Bacillota (38.80–62.04%), Cyanobacteriota (21.93–45.86%), and Actinomycetota (6.46–16.92%). Compared to the control group (T0), flower extract treatments significantly altered the relative abundance patterns of these major phyla. The abundance of Actinomycetota was significantly higher in the T3 (16.52%) and T1 (13.41%) groups than in T0 (10.77%). Notably, Pseudomonadota exhibited exceptional enrichment in the T1 group (19.27%), significantly surpassing all other treatment groups, indicating a specific promotive effect of *P. mume* extract on this phylum.

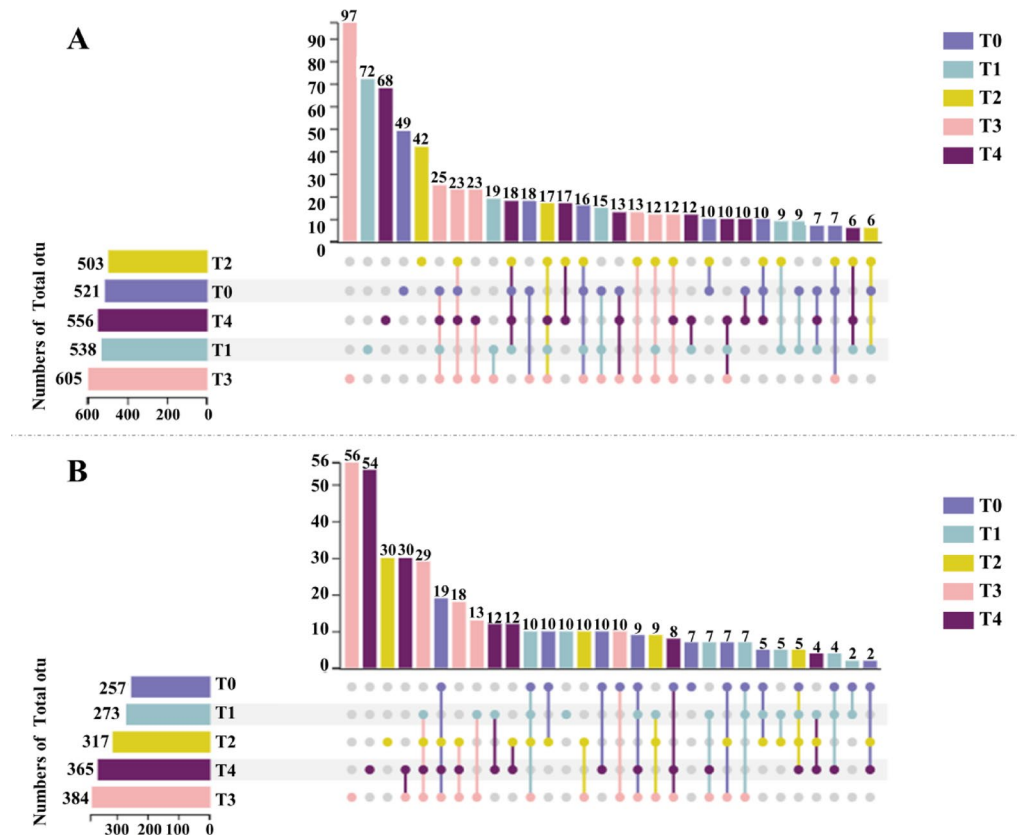


Fig. 5 Upset of bacterial **A** and fungal **B** communities of CTLs

At the genus level (Fig. 6B), the core bacterial composition consisted of *Staphylococcus* (36.13–58.64%) and *Corynebacterium* (5.89–15.23%). *Aerococcus* became one of the dominant genera in the T3 (12.65%), while its abundance was negligible ($\leq 2.30\%$) in T0 and other treatments. *Pseudomonas* was specifically enriched in the T1 group (15.61%), in contrast to its low abundance ($< 0.60\%$) in T0, T3, and T4. Furthermore, *Leuconostoc* was identified as a signature genus for the T4 treatment (1.44%), being virtually absent in other groups.

The fungal community at the phylum level was highly conserved (Fig. 6C), with Ascomycota overwhelmingly dominant (42.71–94.33%). Nevertheless, flower extract treatments induced significant internal restructuring. Compared to the T0 group, the proportion of unclassified fungal sequences increased significantly in T1 and T3 treatments (up to 52.94%). Concurrently, the abundance of Basidiomycota showed an increasing trend in treated groups, suggesting that the treatments may have activated or promoted previously under-characterized fungal lineages.

At the genus level (Fig. 6D), the fungal community in the control group (T0) was absolutely dominated by *Alternaria* (82.19–82.69%). Flower extract treatments fundamentally disrupted this pattern. The T1 treatment (*P. mume* extract) caused a drastic reduction in

Alternaria abundance to 8.49%, accompanied by an explosive increase in *Aspergillus* (up to 47.00%). Treatments T3 (*O. fragrans* extract) and T4 (*E. japonica* flower extract) significantly elevated the relative abundances of genera with potential metabolic activity, such as *Fusarium*, *Plectosphaerella*, and *Acremonium*, which were present at minimal levels ($< 0.70\%$) in T0.

LEfSe analysis and functional prediction

To further statistically identify key microbial genera that exhibit significant inter-group differences in CTLs following treatment with different flower extracts, LEfSe analysis was employed. Using an LDA score threshold of > 3.0 , signature bacterial and fungal genera specifically enriched in the control group (T0) and each treatment group were identified (Fig. 7A, B). These genus-level taxonomic units serve as key biomarkers distinguishing the microbial community structures among groups. T1 was significantly enriched with *Pseudomonas* and *Enterobacter*. The signature genera for T3 were *Aerococcus*, *Corynebacterium* and *Sphingomonas*. T4 specifically exhibited enrichment of *Staphylococcus* and *Leuconostoc*.

LEfSe analysis of the fungal community also revealed clear differential signature genera at the genus level (Fig. 7B). In the control group (T0), *Alternaria* was significantly identified as the most discriminating

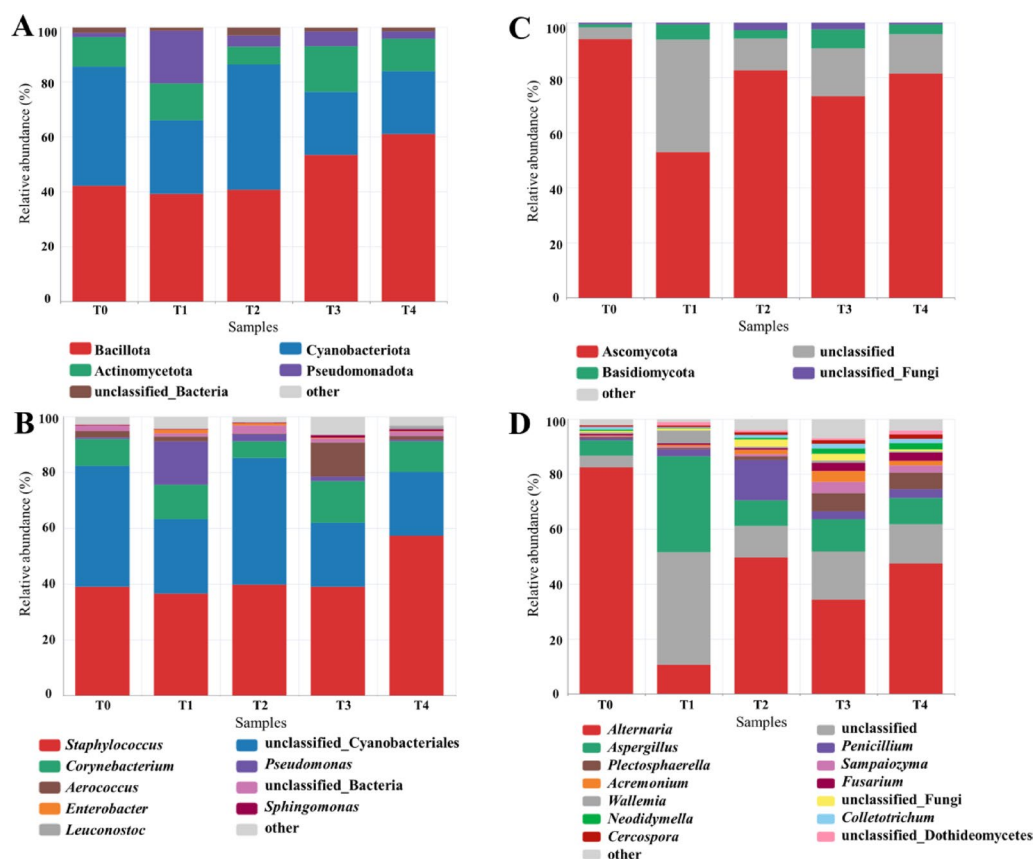


Fig. 6 Microbial community composition of cigar tobacco leaves. The relative abundance of bacterial taxon at **A** phylum and **B** genus level, respectively. The relative abundance of fungal taxon at **C** phylum and **D** genus level, respectively

signature genus, consistent with its absolute dominance in untreated leaves. In T1, *Aspergillus* and *Wallemia* were significantly enriched. The signature fungal genera for T3 included *Plectosphaerella*, *Fusarium*, *Acremonium*, *Colletotrichum*, and *Sampaiozyma*. T4 was associated with the enrichment of *Neodidymella* and *Cercospora*. These signature genera not only serve as robust discriminators between groups but are also likely directly linked to key functions during tobacco fermentation.

PICRUSt2 was employed to predict the KEGG metabolic pathways of the bacterial community during fermentation. As shown in Fig. 7C, metabolic pathways dominated across all treatment groups, with carbohydrate metabolism, amino acid metabolism, and energy metabolism exhibiting the highest relative abundances. This pattern was consistent across the control (T0) and all floral extract-treated samples (T1-T4). Despite this overall similarity, treatment-specific differences were observed in several pathway categories. The T1 group showed higher abundances than other treatments in multiple core metabolic pathways, particularly carbohydrate metabolism, amino acid metabolism, membrane transport, and energy metabolism. T2 also exhibited elevated abundances in certain pathways, but to a lesser

extent than T1. In pathways related to xenobiotics biodegradation and metabolism, both T1 and T2 showed enrichment compared to T0, T3, and T4. Additionally, the biosynthesis of other secondary metabolites pathway was notably enriched in T1, while T2, T3, and T4 showed levels comparable to or slightly above T0. Other pathway categories, such as lipid metabolism, nucleotide metabolism, and glycan biosynthesis and metabolism, showed relatively minor variation among treatments, with T1 generally exhibiting the highest abundances followed by T2.

Association of microbial functional groups with chemical compounds

To investigate the interactions between microbial community structure and chemical composition in CTLs fermented with different plant extracts, we performed correlation analysis to assess the associations between bacterial and fungal communities and key chemical components as well as volatile aroma compounds.

As shown in Fig. 8A, several bacterial genera showed significant correlations with chemical compounds ($P < 0.05$). Notably, both *Pseudomonas* and *Enterobacter* were significantly positively correlated with a suite of

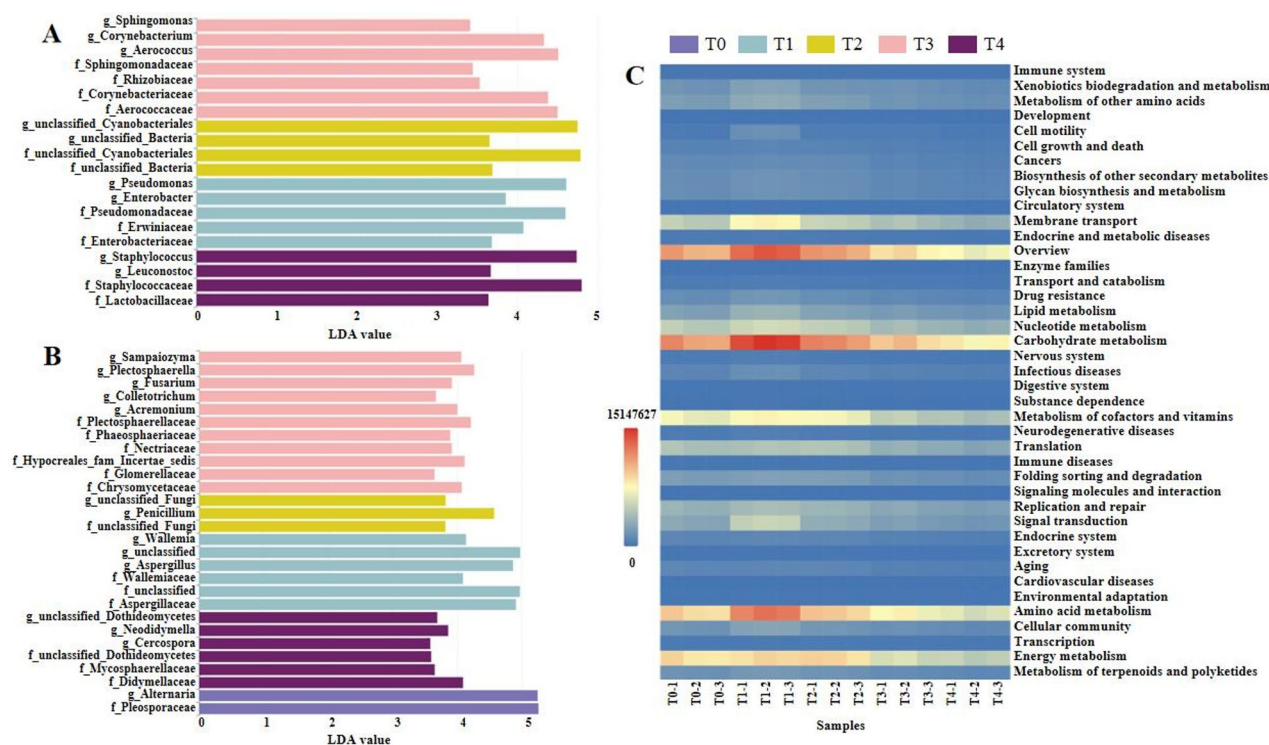


Fig. 7 Analysis of LEfSe in bacterial **A** and fungal **B** communities at different cigar tobacco leaves. Functional annotation of KEGG metabolic pathway in bacterial communities **C**

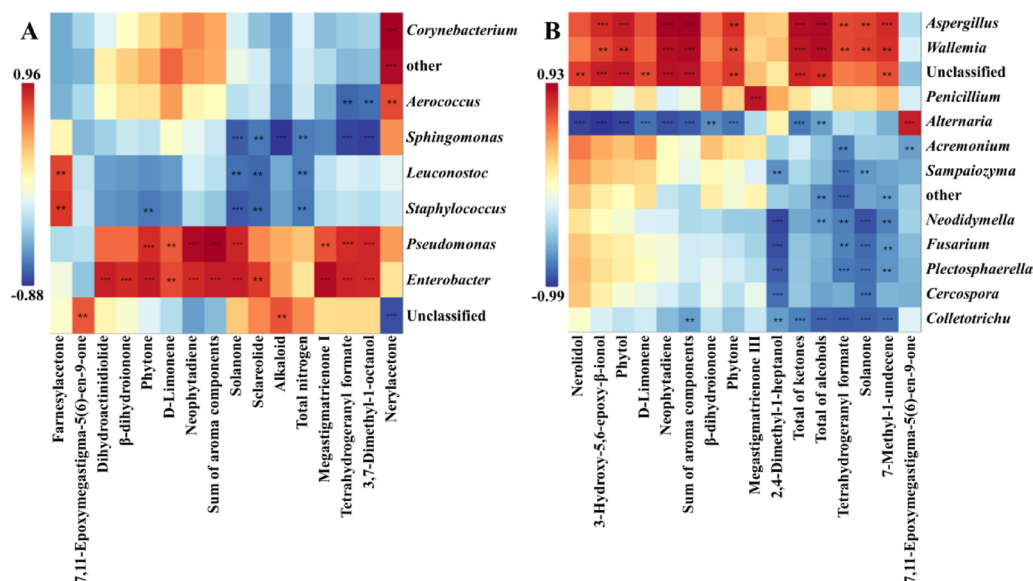


Fig. 8 Correlation analysis of chemical components and microbial community in CTLs; **A** bacteria, **B** fungi. Only results with P value < 0.05 and correlation coefficient absolute value ($|r| > 0.8$) are shown in the figure (* $0.01 \leq p$ -value < 0.05 ; ** $0.001 \leq p$ -value < 0.01 , *** p -value < 0.001)

key aroma compounds, including phytone, d-limonene, neophytadiene, solanone, megastigmatrinenone I, Tetrahydrogeranyl formate, 3,7-dimethyl-1-octanol, and the total of aroma components. *Enterobacter* exhibited additional significant positive correlations with dihydroactinidiolide, β -dihydroionone, and sclareolide. In contrast,

Sphingomonas, *Staphylococcus* and *Leuconostoc* showed significant negative correlations with total nitrogen, sclareolide, and solanone. Among fungal communities, *Aspergillus* and *Wallmania* were significantly positively correlated with several aroma components (Fig. 8B).

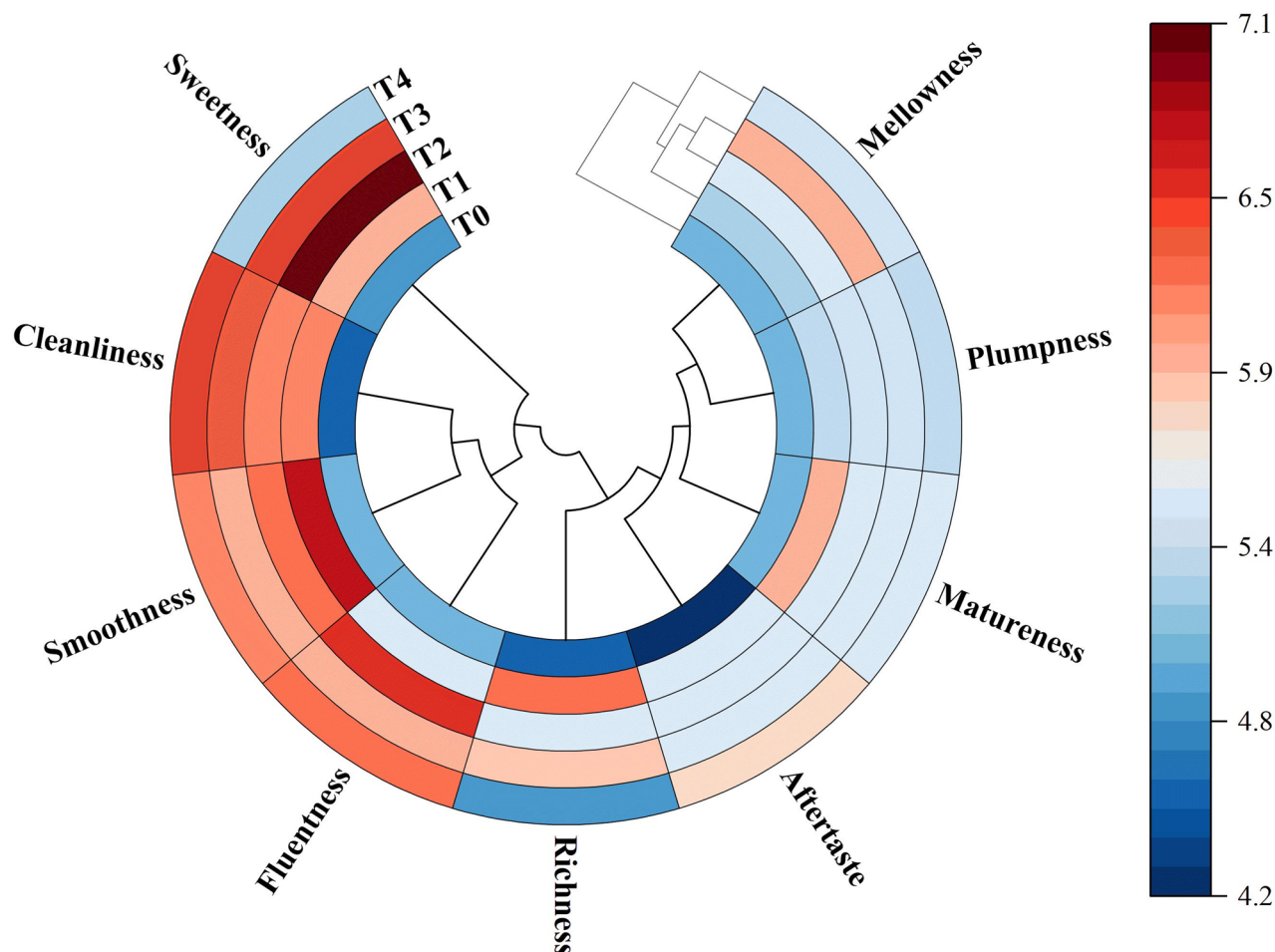


Fig. 9 Effects of different media on sensory quality of CTLs

Conversely, *Alternaria* was significantly negatively correlated with phytol and nerolidol.

Sensory evaluation of CTLs

To systematically evaluate the effects of fermentation with different plant extracts on the sensory quality of CTLs, a professional sensory assessment was conducted. A 9-point scoring system was used, covering nine dimensions: mellowness, richness, matureness, plumpness, fluentness, smoothness, sweetness, cleanliness, and aftertaste (Fig. 9).

As shown in Fig. 9, all plant extract-treated groups (T1–T4) outperformed the control group (T0) in multiple sensory attributes. Specifically, the T0 group scored relatively low in several indicators, particularly in aftertaste (4.2) and cleanliness (4.5), indicating that untreated fermentation resulted in noticeable irritation and residual harshness.

In contrast, fermentation with plant extracts significantly improved the sensory profile of the tobacco leaves. The T1 (*P. mume*) group excelled in smoothness (6.8), along with notable improvements in richness and

sweetness. The T2 (*J. grandiflorum*) group achieved the highest score in sweetness (7.1) and performed best in fluentness (6.6), suggesting a pronounced enhancement in sweet sensation and smoke smoothness. The T3 (*O. fragrans*) group scored highest in mellowness (6.0) and cleanliness (6.4), indicating its effectiveness in reducing off-flavors and improving overall harmony. The T4 (*E. japonica* flower) group showed the best aftertaste (5.8), along with clear improvements in fluentness and smoothness, highlighting its potential in enhancing smoke persistence and comfort.

In summary, all four plant extracts improved the sensory quality of CTLs to varying degrees. *P. mume* extract significantly enhanced aroma smoothness. *J. grandiflorum* extract notably improved sweetness and fluentness. *O. fragrans* extract excelled in mellowness and cleanliness. *E. japonica* flower extract showed strong potential in aftertaste and fluentness.

Discussion

Floral extracts as functional fermentation media

The UPLC-MS/MS analysis revealed that the four floral extracts possessed distinct chemical profiles, characterized by varying compositions of flavonoids, phenolic acids, and glycosides (Sect. "Chemical composition of the four floral extracts"). These differences provide a chemical basis for their potentially distinct roles in CTL fermentation. Flavonoids, which dominated M1 (*P. mume*) and were also abundant in M4 (*E. japonica*), are well-documented for their antioxidant activities [18]. During fermentation, flavonoids can serve as precursors for volatile aroma compounds through microbial or enzymatic transformations, contributing to the development of tobacco flavor [18]. Phenolic acids, particularly abundant in M4 (ferulic acid and coumaric acid), are key products of the plant phenylpropanoid pathway. Abundant phenolic acids and flavonoids in four extracts endows them with significant potential for inhibiting harmful microorganisms, regulating the fermentation process, and serving as a flavor and taste modulator [19]. Glycosides, which were prominent in M2 (*J. grandiflorum*) and M3 (*O. fragrans*), are important aroma precursors [20]. During fermentation, microbial glycoside hydrolases can cleave the glycosidic bonds, releasing aglycones that directly contribute to aroma or serve as substrates for further transformations [21]. The enrichment of glycoside-rich extracts may therefore promote the accumulation of specific aroma compounds through hydrolysis of these bound precursors. This is consistent with the observed increase in aroma compounds in T2 and T3 (Sect. "Aroma compounds in CTLs"), particularly the enhancement of fruity-sweet notes in T2 and carotenoid-derived compounds in T3.

The distinct chemical profiles of the four extracts suggest that they may influence the fermentation process through different mechanisms. M1, rich in flavonoids, may primarily act as a redox modulator and antioxidant. M2 and M3, abundant in glycosides, may serve as reservoirs of aroma precursors. M4, characterized by high phenolic acid content, may exert stronger antimicrobial effects while directly contributing phenolic flavor notes. These differential properties provide a rational basis for the observed treatment-specific effects on microbial community structure, chemical transformations, and ultimately sensory outcomes.

The concentration used in this study (10 mg/mL, equivalent to 0.5% of dry plant material) elicited significant biological effects on microbial community structure and tobacco chemical composition. This suggests that the floral extracts may function as metabolic "triggers" rather than simple nutrient sources, activating microbial responses through signaling or substrate-induction mechanisms even at this moderate concentration. This

hypothesis is supported by the marked increase in unique OTU numbers (Fig. 5) and the clear separation of treatment groups in PCoA analysis (Fig. 4), indicating that the extracts can profoundly reshape microbial community structure through cascading effects on microbial metabolism and community assembly.

Modulation of tobacco chemical composition by floral extracts

All four floral extracts significantly reduced total nitrogen and alkaloid content in CTLs, with the most pronounced effects observed in T3 and T4. Total nitrogen serves as a crucial indicator for the overall content of proteins and nitrogenous compounds in tobacco. Elevated levels can lead to undesirable burnt-feather odors and irritating ammonia during combustion, resulting in a harsh and bitter smoke [3]. Consequently, the significant reduction in total nitrogen observed in T3 and T4 suggests a potential decrease in smoke irritation and an improvement in smoke mildness. Alkaloids are the primary contributors to tobacco's "impact," but excessive levels cause strong irritation and a pronounced bitterness, demonstrating a negative correlation with sensory evaluation scores [1]. The significant reduction achieved by two of the extracts (T3 and T4) indicates that fermentation with these additives is a viable strategy for mitigating irritation and enhancing the smoothness of CTLs. The mechanism may involve microbial degradation, as certain bacteria enriched in these treatments (e.g., *Pseudomonas*) are known to participate in alkaloid metabolism [22].

Sugars play a beneficial role in neutralizing alkaline substances in the smoke, imparting a noticeable sweetness and mellowness [2]. More importantly, sugars act as key precursors for the Maillard reaction and caramelization, facilitating the formation of numerous pleasant aroma compounds such as furans, furanones, and pyrazines, thereby improving the internal quality of tobacco products [7]. The substantial increase in sugars, especially reducing sugar in the T3, strongly implies a significant potential enhancement in its sensory quality, characterized by greater aroma complexity and heightened sweetness.

In conclusion, the four plant extracts differentially modulated the chemical foundation of the CTLs, thereby influencing their final sensory attributes. *P. mume*, *J. grandiflorum*, and *O. fragrans* (M3) extracts laid the material foundation for developing a sweet and rich aroma by drastically increasing sugar contents, whereas *E. japonica* extract (T4) exhibited the greatest potential in reducing smoke irritation and bitterness by efficiently lowering alkaloid and total nitrogen levels. These chemical changes provide a robust theoretical basis for interpreting the subsequent sensory evaluation results.

Shaping of aroma profiles in CTLs by floral extracts

The four floral extracts differentially modulated the aroma profiles of CTLs (Sect. "Aroma compounds in CTLs"), with each treatment promoting the accumulation of specific compound classes.

The substantial increases in nerolidol, phytol, and megastigmatrienone I in T1 (40.64–62.10%) suggest that M1 (*P. mume*) broadly activated multiple aroma biosynthetic pathways. Nerolidol is a sesquiterpene alcohol that imparts sweet violet and fruity-floral notes [23]. Phytol, a diterpene derived from chlorophyll degradation, contributes green and oily notes that can add depth to tobacco aroma [24]. Megastigmatrienone I, a carotenoid degradation product, is known for its sweet, fruity, and typical tobacco-like aroma and is considered a key contributor to cigar quality [25]. The *P. mume* medium potentially contributed its own aroma precursors to the tobacco leaves. Furthermore, it effectively activated or promoted the biosynthesis and accumulation of endogenous macromolecular aroma compounds like alcohols and ketones. Collectively, these processes laid the foundation for a mellow smoke with rich aroma nuances.

In T2, the specific increases in D-limonene, dihydroactinidiolide, and sclareolide (28.77–52.05%) point to targeted enhancement of fruity and sweet aroma nuances. D-limonene provides fresh citrus and lemon-like scents [26], while dihydroactinidiolide and sclareolide are known for their strong fruity-sweet characteristics [27]. The increase in these compounds ultimately specifically enhancing the fresh, fruity-sweet nuance in the tobacco.

It shows a significant 53.28% increase in 3-hydroxy-5,6-epoxy- β -ionol in the T3 sample, a compound directly related to the osmanthus scent and a key precursor to potent aroma compounds like β -damascenone. Its accumulation implies that the addition of the *O. fragrans* medium potentially activated or enhanced the carotenoid degradation pathway (the β -damascenone pathway) in tobacco [28]. It not only explains the notable enhancement of sweetness in T3 but also provides strong evidence for the targeted regulation of key tobacco aroma pathways via exogenous media.

The relatively modest changes in total aroma content in T4, coupled with increases in dehydrovomifoliol and phytol, indicate that M4 (*E. japonica*) exerted a more subtle influence on aroma metabolism. Dehydrovomifoliol is another carotenoid degradation product associated with smooth and mellow notes, while phytol contributes to the green, leafy background of tobacco aroma. This suggests that the *E. japonica* flower medium might benefit the smoothness and delicacy of the smoke without drastically altering the overall aroma structure. This moderate modulation aligns with the high phenolic acid content of M4, which may have prioritized antimicrobial effects over direct stimulation of aroma pathways.

In conclusion, the four floral extracts shaped the aroma profiles of CTLs through distinct mechanisms. *P. mume* broadly enhanced multiple pathways, *J. grandiflorum* selectively enriched fruity-sweet compounds, *O. fragrans* specifically activated carotenoid degradation, and *E. japonica* exerted milder, background-level effects. These aroma changes provide the chemical basis for the sensory improvements observed in Sect. "Sensory evaluation of CTLs".

Microbial community restructuring by floral extracts

The addition of floral extracts significantly reshaped both bacterial and fungal communities in CTLs, with each extract inducing a distinct microbial signature.

All four floral extracts increased both the diversity and evenness of the fungal communities compared to the control (Fig. 4B, D). Besides, T3 (*O. fragrans*) also significantly enhanced bacterial diversity (Fig. 4A, C). It suggested that *O. fragrans* extract may have supplied additional metabolic niches, thereby supporting the persistence of rare OTUs and fostering a more complex and stable bacterial community structure. The higher numbers of unique OTUs in treated samples (Fig. 5) indicate that the extracts introduced or promoted previously underrepresented microbial taxa. This diversification of the microbial community may contribute to more complex metabolic interactions during fermentation, potentially enhancing the range of biochemical transformations occurring in the tobacco leaves.

The specific enrichment of *Pseudomonas* and *Enterobacter* in T1 (Fig. 7A) is particularly noteworthy. *Pseudomonas* is known as a potent decomposer of organic matter and a key contributor to flavor metabolism [29], with additional documented ability to degrade nicotine and other alkaloids [22]. It may adjust smoke strength and indirectly influence aroma balance by altering precursor availability. *Enterobacter* has been reported to enhance aroma quality and volume in fermented tobacco, potentially through the secretion of glycoside hydrolases that release bound aromatic compounds [30]. The positive correlations of these genera with multiple aroma compounds (Fig. 8A) support their involvement in aroma formation during fermentation. In T3, the enrichment of *Aerococcus*, *Corynebacterium* and *Sphingomonas* (Fig. 7A) coincided with significant reductions in total nitrogen and alkaloids and enhanced sugar accumulation (Sect. "Conventional chemical compositions in CTLs"). *Aerococcus* play important roles in the synergistic degradation of complex organic compounds and the synthesis of specific secondary metabolites [31]. *Corynebacterium* is known for its role in amino acid metabolism and has been linked to the formation of aroma compounds such as pyrazines in fermented products [32]. The positive correlation of *Corynebacterium* with nerylacetone (Fig. 8A)

further supports its contribution to aroma development. *Sphingomonas* is documented for its ability to degrade nicotine and polyphenols like chlorogenic acid [33], which could explain its inverse relationship with total nitrogen observed in Fig. 8A and its contribution to the reduced nitrogenous compound levels in T3. T4 showed enrichment of *Staphylococcus* and *Leuconostoc* (Fig. 7A). *Staphylococcus* participates in fat metabolism and carotenoid degradation [34], processes that might consume or transform precursors of certain aroma compounds like solanone, leading to the balance of aroma precursor. *Leuconostoc* is a lactic acid bacterium commonly associated with fermented foods, though its role in tobacco fermentation remains less characterized.

The most striking change in fungal communities was the dramatic reduction of *Alternaria* in all treated samples (from 82% in T0 to below 9% in T1) and its replacement by other genera (Fig. 6D). As a known pathogenic fungus associated with leaf spots and spoilage [35], the presence of *Alternaria* might be linked to the degradation of these alcohols or indicate an unfavorable microbial state for their accumulation. Its suppression by floral extracts may therefore contribute to improved tobacco quality by reducing potential off-flavors or undesirable metabolic activities. The enrichment of *Aspergillus* and *Wallemia* in T1 (Fig. 7B) correlated positively with several aroma components (Fig. 8B). *Aspergillus* secretes hydrolytic enzymes (e.g., amylases, proteases) that degrade sugars and proteins, promoting the synthesis of flavor compounds [36]. *Wallemia* has been associated with increased total aroma content in similar studies [37]. Notably, *Penicillium* was significantly enriched in T2, as revealed by both the community composition analysis (Fig. 6D) and LEfSe analysis (Fig. 7B). Its significant positive correlation with megastigmatrienone III (Fig. 8B) suggests a potential role in the synthesis of specific ketones, possibly through the decomposition of complex organic matter such as carbohydrates and proteins [38]. The enrichment of *Plectosphaerella*, *Fusarium*, and *Acremonium* in T3 suggested that these genera may contribute to the specific metabolic activities observed in this treatment, including enhanced carotenoid degradation (Sect. "Aroma compounds in CTLs"). The identification of these treatment-specific signature genera provides clear research targets for their specific mechanistic roles in the formation of tobacco quality.

The PICRUST2 analysis provided insights into the functional potential of the bacterial communities (Fig. 7C). Metabolic pathways dominated across all treatments, with carbohydrate metabolism, amino acid metabolism, and energy metabolism showing the highest abundances. This reflects the active metabolic state of microorganisms during tobacco fermentation.

The significantly higher abundances of carbohydrate metabolism, amino acid metabolism, and membrane transport pathways in T1 suggest that *P. mume* extract may act as a functional fermentation medium that enhances overall microbial metabolic activity and substrate utilization capacity. This heightened metabolic potential aligns with the comprehensive aroma enhancement observed in T1 (Sect. "Aroma compounds in CTLs"). The elevated membrane transport pathways in T1 further suggest enhanced nutrient uptake and intercellular communication, which may facilitate more efficient coordinated metabolism within the microbial community. In pathways related to xenobiotics biodegradation and metabolism, the T1 and T2 groups also showed significant enrichment, implying that *P. mume* and *J. grandiflorum* extracts may promote the microbial degradation of exogenous compounds in tobacco leaves, thereby improving sensory quality. Additionally, the biosynthesis of other secondary metabolites pathway was notably enriched in T1, suggesting that this treatment may enhance the microbial synthesis of beneficial aroma precursors.

Collectively, these functional predictions indicate that the floral extracts, particularly *P. mume*, do not merely introduce exogenous aromatic compounds into tobacco leaves. More importantly, they modulate microbial community succession and metabolic functions, creating a more active and specialized metabolic environment that drives the synthesis of aroma compounds and the degradation of undesirable constituents. The distinct functional profiles of T1 and T2 suggest that different extracts may enhance tobacco quality through partially overlapping but distinct metabolic mechanisms.

Sensory implications of chemical and microbial changes

The sensory evaluation results (Sect. "Sensory evaluation of CTLs") demonstrated that each floral extract imparted a distinct sensory signature to the fermented tobacco leaves. These improvements can be linked to the chemical and microbial changes observed in previous sections.

T1 (*P. mume*) showed the highest scores in smoothness and richness, with notable improvements in sweetness. This aligns with the comprehensive aroma enhancement observed in T1, particularly the increases in nerolidol, phytol, and megastigmatrienone I, which contribute to floral, green, and sweet tobacco-like notes [23, 24]. The enrichment of *Pseudomonas* and *Enterobacter* in T1, both positively correlated with multiple aroma compounds, suggests that microbial modulation played a key role in these sensory improvements. T2 (*J. grandiflorum*) achieved the highest sweetness score and excelled in flueness. This corresponds to the specific enhancement of fruity-sweet aroma compounds in T2, including D-limonene, dihydroactinidiolide, and sclareolide [26, 27]. The

enrichment of *Penicillium* in T2 (Fig. 7B) and its positive correlation with megastigmatrienone III (Fig. 8B) further supports the involvement of fungal metabolism in shaping this sensory profile. T3 (*O. fragrans*) scored highest in mellowness and cleanliness. These attributes may be linked to the significant reduction in total nitrogen and alkaloids in T3, which would decrease harshness and irritation [1]. The enrichment of *Aerococcus*, *Corynebacterium*, and *Sphingomonas* in T3, all associated with degradation of nitrogenous compounds [31, 32], provides a microbial basis for these chemical changes. The increase in 3-hydroxy-5,6-epoxy- β -ionol (Sect. "Aroma compounds in CTLs"), a precursor to the potent aroma compound β -damascenone [28], may also contribute to the improved mellowness. T4 (*E. japonica*) showed the best aftertaste, along with improvements in fluentness and smoothness. This correlates with the most pronounced alkaloid reduction among all treatments (9.97% in T4), which would reduce bitterness and lingering harshness. The enrichment of *Staphylococcus* and *Leuconostoc* in T4 suggests their potential involvement in these quality improvements, though their precise roles require further investigation.

In conclusion, the sensory improvements can be linked to treatment-specific chemical changes. These include reductions in nitrogenous compounds and enhancements in key aroma compounds. Such chemical shifts were mediated by distinct changes in microbial community structure. These findings demonstrate that floral extracts improve cigar tobacco quality through integrated chemical and microbial mechanisms, with each extract imparting a unique sensory signature.

Limitations and future directions

It should be noted that the present study focused exclusively on the effects of non-volatile compounds (e.g., flavonoids and phenolic acids) from floral extracts on cigar tobacco quality. Volatile components, including particularly terpenes and other aroma-active compounds known to be abundant in flowers such as *P. mume*, *J. grandiflorum*, *O. fragrans*, and *E. japonica*, were not investigated [15]. Given the rich diversity of volatile organic compounds in these floral species, they may also contribute to tobacco quality modulation, either through direct aroma deposition or by serving as substrates for microbial transformation during fermentation. Therefore, the potential roles of terpenes and other volatile organic compounds warrant further investigation using complementary analytical approaches such as GC–MS profiling of the extracts or essential oil-based fermentation trials.

Conclusion

This study provides a mechanistic demonstration that floral extracts can be precision-engineered as functional fermentation media. Beyond their inherent aromas, extracts from *P. mume*, *J. grandiflorum*, *O. fragrans*, and *E. japonica* flower actively remodeled the microbial consortia in cigar tobacco leaves. This targeted microbial steering—particularly the enrichment of *Pseudomonas*, *Enterobacter*, *Aspergillus*, and *Wallemia*—drove a cascade of desirable biochemical transformations: the effective reduction of nitrogenous compounds and alkaloids, the enhancement of sugar metabolism, and the elevated synthesis of key aroma constituents such as neophytadiene and megastigmatrienones. Consequently, each extract imparted a unique sensory signature, translating biochemical shifts into perceptible quality improvements in smoothness, sweetness, mellowness, and aftertaste.

The significance of this work lies in shifting the paradigm from using plant additives as mere flavorants to employing them as ecological and metabolic modulators. This approach offers a sustainable, natural pathway to not only enhance the quality and consistency of cigar tobacco but also to diversify its flavor profiles in a targeted manner. Future research should focus on isolating the specific bioactive molecules responsible for the observed microbial selection and elucidating their molecular mechanisms of action to fully realize the potential of precision flora-based fermentation.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40538-026-00948-6>.

Supplementary Material 1

Author contributions

WH: investigation, performing, and writing. QYZ and WC: writing review and editing. YJ and QWZ: experiments performing. ZJ and LL: data analysis. DL: conceptualization and methodology. All authors reviewed and approved the final manuscript.

Funding

This research was funded by the Science and Technology Program of China Tobacco Sichuan Industrial Co., Ltd. (No. 2023510000340551).

Data availability

The sequencing data presented in this study are available in online repositories. The names of the repositories and their corresponding accession numbers are as follows: <https://www.ncbi.nlm.nih.gov/>, PRJNA1417530 and PRJNA1417537. Additional data underlying this paper will be shared on reasonable request to the corresponding author.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

This research has been confirmed for publication in the journal.

Competing interests

The authors declare no competing interests.

Author details

¹China Tobacco Technology Innovation Center for Cigar, China Tobacco Sichuan Industrial Co., Ltd., Chengdu 610101, China

²Cigar Fermentation Technology Key Laboratory of Tobacco Industry, China Tobacco Sichuan Industrial Co., Ltd., Chengdu 610101, China

³Industry Efficient Utilization to Domestic Cigar Tobacco Key Laboratory of Sichuan Province, Shifang 610094, China

Received: 2 February 2026 / Accepted: 3 April 2026

Published online: 16 April 2026

References

- Yang WW, Wang K, Luo ZW, Tang X, Kuang LP. Research progress on bitter components in e-liquid used in electronic cigarette. *Flavour Fragr Cosmet.* 2025;2:34–9. <https://doi.org/10.20099/j.issn.1000-4475.2024.0090>.
- Chen J, Li Y, He X, Jiao FC, Xu ML, Hu BB, et al. Influences of different curing methods on chemical compositions in different types of tobaccos. *Ind Crops Prod.* 2021;167:113534. <https://doi.org/10.1016/j.indcrop.2021.113534>.
- Jiang CD, Kong DC, Li YY, Sun JG, Chen ZG, Yang MF, et al. Degradation and mechanism analysis of protein macromolecules by functional bacteria in tobacco leaves. *Front Microbiol.* 2024;15:1416734. <https://doi.org/10.3389/fmicb.2024.1416734>.
- Yang T, Lin XL, Chen R, Wang RQ, Li T, Shen FL, et al. Integrated metagenomics and metabolomics analysis reveals dynamic changes of microbiota and metabolic profile during fermentation of cigar tobacco (*Nicotiana tabacum* L.) leaves. *Front Genet.* 2025;16:1662815. <https://doi.org/10.3389/fgene.2025.1662815>.
- Zhang MZ, Guo DF, Wang HQ, Wu GL, Shi YQ, Zhou JL, et al. Analyzing microbial community and volatile compound profiles in the fermentation of cigar tobacco leaves. *Appl Microbiol Biotechnol.* 2024;108(1):243. <https://doi.org/10.1007/s00253-024-13043-3>.
- Zhang L, Luo ZH, Yang MC, Li SG, Xin YH, Cai B, et al. Diversity of fermentation microbes and changes of hydrolytic enzyme activities of cigar leaf raw materials. *J Agric Sci Technol.* 2021;23(10):171–80. <https://doi.org/10.13304/j.nykjdb.2020.0534>.
- B M, J S, A Ð, a B M, Ş D. Carbohydrates—key players in tobacco aroma formation and quality determination. *Molecules.* 2020;25(7):1734. <https://doi.org/10.3390/molecules25071734>.
- Huang XZ, Wang Z, Yang HJ, Shen LW, Zhang Y, Zhang JD, et al. Microbial nicotine degradation technology: from metabolic engineering to industrial application. *J Biol.* 2025;42(5):17–23. <https://doi.org/10.3969/j.issn.2095-1736.2025.05.017>.
- Jia Y, Guo SD, Hu WR, Zhang QY, Wang Y, Zhang ZC, et al. Effects of different fermentation temperatures on microbiomes of cigar tobacco leaves. *Front Bioeng Biotechnol.* 2025;13:1550383. <https://doi.org/10.3389/fbioe.2025.1550383>.
- Zhang GH, Yang WL, Xia HC, Hong RL, Zhao H, Su YL, et al. Generic fermentation technology parameters and process control for cigar tobacco leaves. *J Yunnan Agr Univ.* 2025. [https://doi.org/10.12101/j.issn.1004-390X\(n\).202304037](https://doi.org/10.12101/j.issn.1004-390X(n).202304037).
- Zhao YZ, Wang SX, Wang J, Wang YH, Pan Y, Guo DF, et al. Current status and perspectives on the research and technological application of domestic cigar tobacco leaf fermentation. *Chin Tob Sci.* 2025;46(3):118–25. <https://doi.org/10.13496/j.issn.1007-5119.2025.03.014>.
- Hu WR, Cai W, Liu J, Li M, Chen Q, Jia Y, et al. Effects of active compounds extracted from natural spices on the quality of cigar tobacco leaves during fermentation. *Chem Biol Technol Agric.* 2025;12(1):69. <https://doi.org/10.1186/s40538-025-00788-w>.
- Giannetti V, Biancolillo A, Marini F, Mariani MB, Livi G. Characterization of the aroma profile of edible flowers using HS-SPME/GC-MS and chemometrics. *Food Res Int.* 2024;178:114001. <https://doi.org/10.1016/j.foodres.2024.114001>.
- Chetia I, Vijayakumar A, Badwaik LS. Edible flowers' flavor, safety and their utilization as functional ingredients: a review. *J Food Sci Technol.* 2025;62(1):11–23. <https://doi.org/10.1007/s13197-024-06071-4>.
- Li ZQ, Zhang JD, Yang L, Li XL, Meng Q, Li Y, et al. Intelligent chemical profiling of 73 edible flowers by liquid chromatography-high resolution mass spectrometry combined with HRMS database and their authentication based on large-scale fingerprints. *Food Chem.* 2024;446:138683. <https://doi.org/10.1016/j.foodchem.2024.138683>.
- Abbaszadeh F, Fakhri S, Varnamkhasti BS, Moradi SZ, Khirehgesh MR, Echeverria J. Phytochemicals from edible flowers prevent neurodegenerative diseases via the gut-brain axis. *Food Biosci.* 2025;63:105681. <https://doi.org/10.1016/j.fbio.2024.105681>.
- Yao L, Shan YL, Chen GY, Wang J, Yang JP, Yu J, et al. Influence of tobacco flower bud extract on microbial community and aroma quality during cigar leaves fermentation. *Front Bioeng Biotechnol.* 2025;13:1647801. <https://doi.org/10.3389/fbioe.2025.1647801>.
- Wang XW, Zhang SX, Li WQ, Hu YK, Zhang BH, Du CY, et al. Non-targeted metabolomics analysis of metabolite changes in middle leaves of tobacco 'Cuibi 1' during curing process. *J Fujian Agric For Univ.* 2025;54(6):841–52. [https://doi.org/10.13323/j.cnki.j.fafu\(nat.sci\).202501018](https://doi.org/10.13323/j.cnki.j.fafu(nat.sci).202501018).
- Wang JT, Zhu JJ, An ZC, Dang ZM, Wang XJ. Metabolic characteristics of different flue-cured tobacco varieties and grades and quality improvement effects of nicotine-degrading bacteria on tobacco leaves. *Acta Microbiol Sin.* 2025;1–10. <https://doi.org/10.13343/j.cnki.wxsb.20250537>.
- Wei JB, Song K, Zang ZP, Yang HJ, Gao YZ, Zhang JD, et al. Influence of specific tobacco endophytic *Bacillus* on tobacco leaf quality enhancement during fermentation. *Front Microbiol.* 2024;15:1468492. <https://doi.org/10.3389/fmicb.2024.1468492>.
- Wu X, Hu Y, Wang Q, Liu J, Fang S, Huang DW, et al. Study on the correlation between the dominant microflora and the main flavor substances in the fermentation process of cigar tobacco leaves. *Front Microbiol.* 2023;14:1267447. <https://doi.org/10.3389/fmicb.2023.1267447>.
- Nie WX, He ZL, Gu MJ, Zhou T, Xu J, Zhong JJ, et al. Improved bacterial cellulose production by *Acetobacter oryzoeni* MGC-N8819 in tobacco waste extract coupled with nicotine removal by *Pseudomonas* sp. JY-Q/5Δ. *Int J Biol Macromol.* 2024;293:139336. <https://doi.org/10.1016/j.jbiomac.2024.139336>.
- Feng W, Zhao YF, Zhang X, Bai RH, Huang Y. Advances in the impact of oolong tea manufacturing processes on aromatic compounds. *Food Ferment Ind.* 2025;24:428–38. <https://doi.org/10.11882/j.issn.0254-5071.2025.12.005>.
- Guo YJ, Guo ZX, Bian JM, Xie QT, Li GY, Wang K, et al. The effect of different drying methods on the aroma components of *Osmanthus fragrans* concrete and absolute were analyzed by combining GC-MS with ROAV analysis. *Food Sci.* 2024;45(14):162–71.
- Liao F, Tian YN, Li QX, Li ST, Xu LP, Bai B, et al. Synthesis of megastigmatrienone precursors and their pyrolysis characteristics. *J Light Ind.* 2025. <https://doi.org/10.12187/2025.05.010>.
- Chang J, Li Q, Wei XD, Liu DY, Xu YM, Wei JX, et al. Research progress on the biosynthesis of terpenoid flavor and latent fragrance compounds. *China Biotechnol.* 2024. <https://doi.org/10.13523/j.cb.2404044>.
- Chen X, Zhang TD, Deng GB, Yang CX, Yin JC. Analysis of the volatile components extracted from Hongda flowers and their application in tobacco. *J Yunnan Agr Univ.* 2018;33(4):663–8. [https://doi.org/10.12101/j.issn.1004-390X\(n\).201702004](https://doi.org/10.12101/j.issn.1004-390X(n).201702004).
- Cao YN, Cao JM, Lin RY, Luo CG, Cai XJ, Yan D, et al. Study on key chemical basis of high aroma trait of Zhongyan 207 and its parent 0953. *Chin Tob Sci.* 2023;44(4):71–8. <https://doi.org/10.13496/j.issn.1007-5119.2023.04.010>.
- Pan QW, Tian XY, Xu WJ, Chen JY, Zhang X. Application of biotechnology in improving the quality of low-grade tobacco leaves. *China Brew.* 2025. <https://doi.org/10.13995/j.cnki.11-1802/ts.043541>.
- Xiao GW, Wang L, Pan TT, Chen ZL, Shen XC, Shi T, et al. Analysis of the microbial communities of flue-cured tobacco and their correlation with chemical components and aroma characteristics. *Tob Sci Technol.* 2025. <https://doi.org/10.13496/j.issn.1007-5119.2025.05.009>.
- Wang HQ, Guo DF, Ding NH, Zhang MZ, Shi YQ, Wu XF, et al. Characterization of microbial communities, free amino acids and volatile flavor compounds in cigar tobacco leaves from Yunnan province. *J Light Ind.* 2024;4:97–108. <https://doi.org/10.12187/2024.04.013>.
- Shangguan LL, Liu ZX, Xu LL, Yang Q, Zhang XL, Yao L, et al. Effect of *Corynebacterium glutamicum* fermentation on the volatile flavors of the enzymatic hydrolysate of soybean protein isolate. *Foods.* 2024;13(16):2591. <https://doi.org/10.3390/FOODS13162591>.
- Ning Y, Mai J, Hu BB, Lin ZL, Chen Y, Jiang YL, et al. Study on the effect of enzymatic treatment of tobacco on HnB cigarettes and microbial succession during fermentation. *Appl Microbiol Biotechnol.* 2023;107(13):4217–32. <https://doi.org/10.1007/s00253-023-12577-2>.

34. Pei Q, Jiang X, Li ZQ, Xu H, Xie MY, Xiong T, et al. Study on quality enhancement during cigar tobacco fermentation by *Staphylococcus nepalensis*: insights into microbial community, volatile substances and sensory evaluation. *Front Microbiol.* 2025;16:1526178. <https://doi.org/10.3389/FMICB.2025.1526178>.
35. Wang HQ, Guo DF, Zhang MZ, Wu GL, Shi YQ, Zhou JL, et al. Correlation study on microbial communities and volatile flavor compounds in cigar tobacco leaves of diverse origins. *Appl Microbiol Biotechnol.* 2024;108(1):236. <https://doi.org/10.1007/S00253-024-13032-6>.
36. Mu Y, Chen Q, Parales RE, Lu ZM, Hong Q, He J, et al. Bacterial catabolism of nicotine: catabolic strains, pathways and modules. *Environ Res.* 2020;183:109258. <https://doi.org/10.1016/j.envres.2020.109258>.
37. Hu WR, Liu L, Zhang JB, Zhou QW, Jiang ZR, Jia Y, et al. Development and mechanism study of functional fermentation media for reducing alkaloid content in cigar tobacco leaves. *Chem Biol Technol Agric.* 2025;12(1):120. <https://doi.org/10.1186/s40538-025-00847-2>.
38. Sun DJ, Fan YM, Yu JY, Zhu FR, Cheng Z, Zhang T. Screening, identification of β -glucosidase-producing fungi and optimization of their liquid fermentation conditions. *China Feed.* 2025. <https://doi.org/10.15906/j.cnki.cn11-2975/s.2024060039-06>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.