

Research Article

# Effect of Ultra-High-Pressure Treatment on the Chemical Composition and Aroma of Flue-Cured Tobacco Leaves for Heated Tobacco Products

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**Abstract:** The influences of different Ultra-High-Pressure (UHP) treatments on the chemical composition and the low-temperature pyrolysis products of Flue-Cured Tobacco Leaves (FCTL) that applied in heated tobacco products were investigated in this study. Results indicated that UHP treatment reduced total alkaloids content significantly ( $p < 0.05$ ), especially 500-600 MPa treatment group ( $2.34 \pm 0.00\%$ ,  $2.34 \pm 0.01\%$ ). Increase in sugars and potassium content were observed after UHP treatment, among which the 300 MPa group was accounted the highest for  $26.51 \pm 0.09\%$  and  $2.37 \pm 0.02\%$ . Moreover, the richness of low-temperature pyrolysis products from the FCTL were also enhanced after UHP treatment and the relative contents of aldehydes and ketones (e.g., 2-butanone and 5-hydroxymethylfurfural) were increased. The quantity of acidic and flavour compounds also raised, and those changes might contribute to a richer sensory of the FCTL. The present study served as a foundational exploration into enhancing the aroma and quality of heated tobacco products and might provide some insights for the development of novel tobacco materials.

**Keywords:** Ultra-High-Pressure Treatment, Flue-Cured Tobacco Leaves, Heated Tobacco Products, Chemical Composition, Volatile Flavour Components

## Introduction

With the continuous development of economy and society, the number of smokers is also increasing sharply around the world (Shah *et al.*, 2023). Since most consumers do not properly dispose of the cigarette butts and just simply thrown it away after smoking, this has induced a serious impact on the environment (Patel *et al.*, 2013; Wilson *et al.*, 2014). What's more, it has also been firmly established that environmental tobacco smoke constitutes a pronounced wellspring of particulate matter (PM) pollution in the submicron range, with indoor PM concentrations up to 10 times higher than idle eco-diesel engine emissions (Invernizzi *et al.*, 2004). And smoking traditional burning cigarettes and exposure to secondhand smoke pose risks to human health (Statista Research Department, 2022). In recent years, more and more people are aware of the harm of traditional burning cigarettes. Therefore, new type tobacco products have poured in and developed rapidly.

As a new type of tobacco product, heated tobacco products mainly use an external heat source to heat (usually below 350°C) tobacco materials to produce

aerosols (containing nicotine) for consumer (Ratajczak *et al.*, 2020). Its lower heating temperature reduces many harmful components that produced by high-temperature cracking of traditional combustion cigarettes, which makes it become one of the popular new type tobacco products. Meanwhile, heated tobacco products are considered to produce less harmful and potentially harmful compounds than that by the traditional burning cigarettes (Simonavicius *et al.*, 2019). However, the low heating temperature of heated tobacco products also have some defects such as lack of aroma components, insufficient taste, miscellaneous gas, strong irritation, etc., which has limited its development.

In view of the above problems in heated tobacco products, many researchers mainly focused on the exploration of heating mode, the addition and flavouring of cigarette cores, the improvement of baking technology and the improvement of modulation technology, etc. For example, Ning *et al.* (2023) used enzymatic methods to intervene in the fermentation process of tobacco and found that amylase treatment could regulate the chemical composition of tobacco and improve the quality of tobacco as well as the sensory quality of cigarette

products. However, there are few studies on the application of UHP treatment to the tobacco industry. At present, a research report suggested that the relative content of alcohols and esters, which were the key aroma substances, showed no remarkable difference between the stem silk produced from microwave-treated tobacco silk and the conventional ones (Wang *et al.*, 2017). There are also reports regarding the application of UHP technology to tobacco leaves. UHP treatment can lead to the closure of stomata on tobacco leaves and compress the intercellular spaces of the tobacco leaves, thereby narrowing the internal channels and causing changes in the sensory quality of the tobacco leaves (Tan *et al.*, 2017). Additionally, UHP technology can also activate the activity of certain enzymes within the tobacco, thereby facilitating the rapid occurrence of chemical reactions and further resulting in the release of latent aroma substances within the tobacco, enhancing the aroma components and sensory quality of the tobacco leaves. Therefore, UHP may affect the compounds of heated tobacco products as well as the sensory of FCTL (Jun *et al.*, 2007).

In recent years, UHP processing technology has gradually become a commercially viable technology and has been widely used in food homogenization, enzyme sterilization (Bao *et al.*, 2023), tissue modification, sensory quality improvement (Wang *et al.*, 2022; Balasubramaniam, 2021) etc. To prove this, the effect of UHP pretreatment on different chemical components of FCTL and the differences in volatile aroma components were investigated systematically. Continuous flow analysis was used to determine the content of conventional chemical composition in FCTL. The phenolic compounds in FCTL were quantified using high performance liquid chromatography, and electronic nose technology was also applied to detect the differences in volatile aroma components of FCTL. Furthermore, pyrolysis-gas chromatography mass spectrometry was used to analyze the differences of pyrolysis products under different pretreatment conditions of FCTL. Our study might provide some basal knowledge for the application of UHP technology in the tobacco industry.

## Materials and Methods

### *Chemicals and Materials*

Methanol (HPLC grade) was purchased from Merck (USA). Folin-Phenol, petroleum ether, sodium tetraborate decahydrate, potassium dihydrogen phosphate and borax (Analytical Reagent, AR) were purchased from Sinopharm Chemical Reagent Co., Ltd (China). The HPLC grade of formic acid, chlorogenic acid, new chlorogenic acid, cryptochlorogenic acid, rutin, scopoletin and methylthioninium chloride were purchased from Shanghai Maclin Biochemical Technology Co., Ltd (China). The raw materials of

FCTL were provided by Zhejiang Tobacco Industry Co., Ltd (China).

### *Preparation of FCTL Treated by UHP*

For the UHP treatment, about 110 g FCTL were vacuum sealed into plastic bags. Then, the samples were placed in the pressure chamber of the UHP machine (HP600 UHP machine, Baotou Kefa High Voltage Technology Co., Ltd., China), and the parameters of the UHP treatment conditions were set at 200 MPa, 300 MPa, 400 MPa, 500 MPa and 600 MPa, holding for 10 min (Tan *et al.*, 2017). The prepared sample was milled through a 60-mesh sieve for analysis.

### *Determination of Chemical Composition and Petroleum Ether Extract Content of FCTL*

The continuous flow analyser (Alliance Futura continuous flow analyser, AMS Alliance, France) was used to determine the conventional chemical components in tobacco. Among them, the determination of reducing sugar and water-soluble sugars was referred to the industry standard YC/T 159-2002. The determination of total nitrogen was referred to the industry standard YC/T 161-2002. The determination of chlorine was referred to the industry standard YC/T 162-2011. The determination of potassium was referred to the industry standard YC/T 217-2007. The determination of total plant alkaloids was referred to the industry standard YC/T 468-2021. And the determination of petroleum ether extract content was referred to the industry standard YC/T 176-2003.

### *Determination of Total Volatile Bases*

Total volatile bases in FCTL were determined using water vapor distillation according to (Feitosa *et al.*, 2023). 2.00 g of FCTL powder were added into 75 mL buffer (with 6.70 g potassium dihydrogen phosphate and 10.49 g borax) in a round-bottom. The volatile bases in FCTL were distilled with water vapor and flowed into a collection bottle containing 15 mL hydrochloric acid (0.1 mol/L). The remaining hydrochloric acid was titrated with 0.1 mol/L NaOH solution. An equal volume of buffer is used as a blank control. The volatile bases content in flue-cured tobacco was calculated by  $\text{NH}_3$ . The formula for calculating the volatile bases content in flue-cured tobacco is as follows:

$$\text{Volatile bases content (\%)} = \frac{M_{\text{NaOH}} \times (V_1 - V_2) \times 0.017 \times 1000}{m \times (1 - W)}$$

where  $M_{\text{NaOH}}$  is the concentration of the NaOH standard titration solution (mol/L),  $V_1$  is the volume of NaOH standard titration solution consumed in the blank control,  $V_2$  is the volume of NaOH standard titration solution consumed by the sample experiment,  $m$  is the sample mass (g), and  $W$  is the moisture content of the sample.

### *Determination of Phenolic Compounds Content*

The content of phenolic compounds was determined using high performance liquid chromatography (the Alliance HPLC system Waters E2695 and fluorescence detector 2489, Waters, USA), with reference to the industry standard YC/T 202-2006.

### *Electronic Nose Detection*

The Heracles NEO 100 ultra-fast gas chromatography electronic nose system (Alpha MOS, France) was used to detect volatile aroma components in present study. Dual FID detectors and two columns (MXT-5 and MXT-1701(10m, 180 $\mu$ m)) were used for analysis. About 0.5 g sample was placed into sealed headspace vial and incubated at 70°C for 20 min before electronic nose detection. Syringe needle was used to absorb 5,000  $\mu$ L volatile gases from the headspace at a constant rate (Xu *et al.*, 2024).

### *Pyrolysis-Gas Phase Mass Spectrometry Analysis*

Pyrolysis instrument (Pyroprobe 5250 pyrolysis instrument, CDS, USA) and 7890B/5977B Gas Chromatography/Mass Spectrometry (Agilent, USA) were used to analyse the pyrolysis-gas phase mass spectrometry of the FCTL treated with UHP. The method of Yildiz and Ceylan (2019) was referred to and made appropriate modifications. Briefly, 2 mg samples were accurately weighed in a special quartz tube, then the quartz tube was placed in a pyrolysis instrument for lysis in an oxygen-free atmosphere. Lysis chromatograms of samples at different temperatures were qualitatively analysed using NIST MS Search 2.3 and quantified using peak area normalization. The detail analysis parameters were as followed. Segmented pyrolysis heating procedure: the initial temperature was 50°C, held for 10 s, and heated to 250°C at a temperature of 20°C/s and maintained for 20 s; Valve box temperature: 250 °C, pyrolysis atmosphere was high-purity helium. GC/MS analysis conditions: column: DB-5 MS capillary column (60 m $\times$ 0.25 mm $\times$ 1 $\mu$ m); Inlet temperature: 280°C; Shunt ratio: 50:1; carrier flow rate: 1.0 mL/min; Heating program: 50 °C (10 min) 2 °C/min 150 °C (5 min) 5°C/min 280°C (20 min); Transmission line temperature: 280°C; Ion source temperature: 230°C; Ionization mode: EI; Ionization energy: 70 eV; Quadrupole temperature: 150°C; Scanning mode: full scan; Mass scanning range: 35~650 amu.

### *Untargeted Metabolomics Analysis*

Accurately weigh 50  $\pm$  5 mg of the sample into a 2 mL centrifuge tube, containing 400  $\mu$ L of extraction solution (methanol: water = 4:1, v/v) and four internal standards (0.02 mg/mL L-2-chloroalanine, etc.). Add a grinding bead with a diameter of 6 mm, grind at -10°C and 50 Hz for 6 minutes, and then extract by ultrasound at 5 °C (40 kHz) for 30 minutes. Subsequently, the

sample was left to stand at -20 °C for 30 minutes, and then centrifuged for 15 minutes (13000 g, 4 °C) after removal. Take the supernatant and transfer it to an injection bottle with an inner tube for analysis; take an additional 20  $\mu$ L of supernatant from each sample and mix it as a quality control (QC) sample.

### *Statistical Analysis*

The principal component analysis and main graphs of the raw data were analysed and plotted using GraphPad Prism 9.0. The significance analysis was done by SPSS 20.0 software (IBM, Chicago, USA) with a significant difference level of  $p < 0.05$ . All experimental test was repeated at least three times.

## **Results and Discussion**

### *Chemical Compositions of Tobacco*

As shown in Table 1, after UHP treatment, the content of water-soluble sugar and reducing sugar in flue-cured tobacco showed an increasing trend. The UHP treatment groups showed a trend of increasing the content of both sugars first and then decreasing with the increase of pressure. Normally, the chemical component of high-quality tobacco leaves generally contain about 18-26% water-soluble sugar, 16-24% reducing sugar, and the ratio of reducing sugar/water-soluble sugar should be about 0.9 (Wang *et al.*, 2016b). Combined with the data of reducing sugar/water-soluble sugar in Table 2, after UHP treatment, the two-sugar ratio in flue-cured tobacco is still in the category of high-quality tobacco leaves (Sun *et al.*, 2012). The chemical compositions in tobacco are the basis of its sensory quality, and the appropriate proportion of chemical compositions is of great significance to its quality and sensory style characteristics (Li *et al.*, 2016). The sugar in tobacco is thought to regulate the pungent flavor and acids in the mainstream smoke. Changes in flue-cured tobacco sugars after UHP treatment might make the prepared heated tobacco products taste more mellow when smoked.

The content of total alkaloids decreased significantly ( $p < 0.05$ ) after UHP treatment. As an important quality indicator in flue-cured tobacco, total alkaloids had an important role in enhancing the cigarette taste of momentum (Sun *et al.*, 2011). The UHP treatment of 200 MPa and 400 MPa in all treatment groups showed little effect on the total alkaloids content of flue-cured tobacco ( $p \geq 0.05$ ). As shown in Table 2, the reducing sugar/total alkaloids value of each group increased significantly after UHP treatment ( $p < 0.05$ ), and the ratio of reducing sugar/total alkaloids in the 500 MPa treatment group was the highest, but all the groups were still in the range of high-quality tobacco leaves (total alkaloids about 2.5%, reducing sugar/total alkaloids 8-12, total nitrogen/total alkaloids  $< 1$ ). The coordinated reducing sugar/total alkaloids ratio could balance the flue gas, making the smoke pure and comfortable during inhalation.

**Table 1:** Content of chemical components of flue-cured tobacco under different treatment conditions (%)

| Sample  | Water soluble sugars     | Total alkaloids        | Reducing sugar           | Chloride               | Potassium              | Total nitrogen          |
|---------|--------------------------|------------------------|--------------------------|------------------------|------------------------|-------------------------|
| RAW     | 24.88±0.01 <sup>d</sup>  | 2.46±0.00 <sup>a</sup> | 23.09±0.00 <sup>c</sup>  | 0.40±0.01 <sup>c</sup> | 1.35±0.01 <sup>f</sup> | 1.87±0.00 <sup>c</sup>  |
| UHP-200 | 25.68±0.17 <sup>c</sup>  | 2.38±0.01 <sup>b</sup> | 22.91±0.05 <sup>c</sup>  | 0.41±0.00 <sup>c</sup> | 1.53±0.00 <sup>c</sup> | 1.91±0.02 <sup>bc</sup> |
| UHP-300 | 26.51±0.09 <sup>a</sup>  | 2.35±0.01 <sup>c</sup> | 23.82±0.10 <sup>ab</sup> | 0.41±0.00 <sup>c</sup> | 2.37±0.02 <sup>a</sup> | 1.86±0.01 <sup>c</sup>  |
| UHP-400 | 26.23±0.14 <sup>b</sup>  | 2.40±0.03 <sup>b</sup> | 24.14±0.25 <sup>a</sup>  | 0.42±0.01 <sup>b</sup> | 1.45±0.00 <sup>e</sup> | 1.95±0.03 <sup>b</sup>  |
| UHP-500 | 26.29±0.01 <sup>ab</sup> | 2.34±0.00 <sup>c</sup> | 23.93±0.17 <sup>ab</sup> | 0.44±0.00 <sup>a</sup> | 1.56±0.00 <sup>b</sup> | 2.06±0.03 <sup>a</sup>  |
| UHP-600 | 26.18±0.05 <sup>b</sup>  | 2.34±0.01 <sup>c</sup> | 23.77±0.07 <sup>b</sup>  | 0.43±0.00 <sup>a</sup> | 1.50±0.00 <sup>d</sup> | 1.85±0.01 <sup>c</sup>  |

Note: Different lowercase letters in each treatment group data indicate significant differences between treatment groups ( $p < 0.05$ )

**Table 2:** Ratio of chemical composition of flue-cured tobacco under different treatment conditions

| Sample  | Reducing sugar/water soluble sugars | Reducing sugar/total nitrogen | Reducing sugar/total alkaloids | Total nitrogen/total alkaloids | Potassium/chloride      |
|---------|-------------------------------------|-------------------------------|--------------------------------|--------------------------------|-------------------------|
| RAW     | 0.93±0.00 <sup>a</sup>              | 12.37±0.13 <sup>b</sup>       | 9.40±0.01 <sup>c</sup>         | 0.76±0.01 <sup>c</sup>         | 3.34±0.08 <sup>d</sup>  |
| UHP-200 | 0.89±0.01 <sup>d</sup>              | 12.02±0.12 <sup>bc</sup>      | 9.61±0.05 <sup>d</sup>         | 0.80±0.01 <sup>b</sup>         | 3.71±0.01 <sup>b</sup>  |
| UHP-300 | 0.90±0.00 <sup>cd</sup>             | 12.84±0.04 <sup>a</sup>       | 10.13±0.00 <sup>bc</sup>       | 0.79±0.00 <sup>bc</sup>        | 5.84±0.05 <sup>a</sup>  |
| UHP-400 | 0.92±0.00 <sup>ab</sup>             | 12.35±0.33 <sup>b</sup>       | 10.06±0.02 <sup>c</sup>        | 0.81±0.02 <sup>b</sup>         | 3.45±0.03 <sup>cd</sup> |
| UHP-500 | 0.91±0.01 <sup>bc</sup>             | 11.65±0.26 <sup>c</sup>       | 10.24±0.05 <sup>a</sup>        | 0.88±0.01 <sup>a</sup>         | 3.53±0.03 <sup>c</sup>  |
| UHP-600 | 0.91±0.00 <sup>c</sup>              | 12.87±0.05 <sup>a</sup>       | 10.15±0.01 <sup>b</sup>        | 0.79±0.00 <sup>bc</sup>        | 3.47±0.03 <sup>c</sup>  |

Chlorine and potassium in flue-cured tobacco had different changes after UHP treatment. The chlorine content did not change after treatment, while the potassium content increased significantly ( $p < 0.05$ ) and was highest in the 300 MPa treatment group. After UHP treatment, the total nitrogen content of the 400 MPa and 500 MPa treatment groups increased significantly ( $p < 0.05$ ), which might lead to a more intense taste of tobacco leaves (Chen *et al.*, 2019).

**Table 3:** Petroleum ether extract and total volatile bases content of flue-cured tobacco under different treatment conditions (%)

| Sample  | Petroleum ether extract content | Total volatile bases     |
|---------|---------------------------------|--------------------------|
| RAW     | 4.61±0.20 <sup>a</sup>          | 0.27±0.00 <sup>ab</sup>  |
| UHP-200 | 4.53±0.16 <sup>a</sup>          | 0.25±0.00 <sup>c</sup>   |
| UHP-300 | 4.47±0.01 <sup>a</sup>          | 0.25±0.00 <sup>c</sup>   |
| UHP-400 | 4.69±0.08 <sup>a</sup>          | 0.26±0.01 <sup>abc</sup> |
| UHP-500 | 4.84±0.15 <sup>a</sup>          | 0.27±0.01 <sup>a</sup>   |
| UHP-600 | 4.68±0.29 <sup>a</sup>          | 0.26±0.00 <sup>bc</sup>  |

### Petroleum Ether Extracts and Total Volatile Bases in Tobacco

As shown in Table 3, the content of the petroleum ether extract of flue-cured tobacco ranged from 0.3% to 0.5% after UHP treatment, and this had no significant ( $p \geq 0.05$ ) effect on the petroleum ether extract of roasted tobacco. Petroleum ether is a weak polarity and volatile organic solvent, which can be used as a solvent to extract many volatile and non-volatile lipids in tobacco. The petroleum ether extracts in flue-cured tobacco mainly include volatile oils, greases, resins, essential oils, pigments, waxes, lipids, organic acids, etc. (Yan *et al.*, 2007; Mo *et al.*, 2022) The petroleum ether extracts in flue-cured tobacco contain important precursors of flue-cured tobacco aroma components (including cecilia diterpene compounds and carotenoid polyterpene compounds that play an important role in aroma and taste), so they are often considered as an important indicator for evaluating tobacco aroma quality (Zhu *et*

*al.*, 2011). In summary, UHP treatment had little effect on the content of aroma components that can be extracted by petroleum ether in flue-cured tobacco.

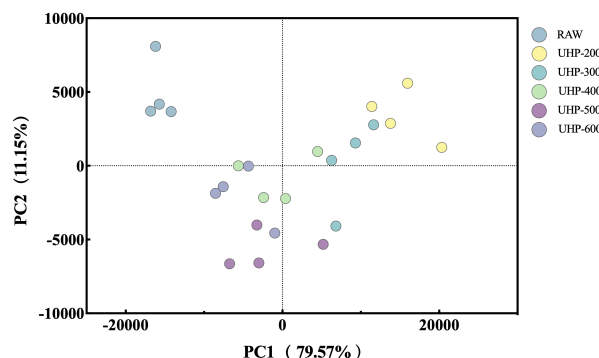
As shown in Table 3, the UHP treatment of flue-cured tobacco had a certain effect of reducing the total volatile bases. Among them, the content of total volatile bases in the 200 MPa and 300 MPa treatment groups decreased significantly ( $p < 0.05$ ). Total volatile bases in flue-cured tobacco refer to volatile amine nitrogenous compounds in tobacco, mainly including ammonia, amines, amides, and free nicotine (Niu *et al.*, 2013). As an alkaline component in flue-cured tobacco, total volatile bases can effectively neutralize acidic components in flue gas. Too high content of total volatile bases will increase the taste experience of spicy stimulation, resulting in increased smoke irritation. Low volatile bases content will lack the physiological strength of tobacco, and the richness of the smoke and the satisfaction it brings to the sense of taste will be reduced (Liu *et al.*, 2014). The reduction of total volatile bases content from flue-cured tobacco by UHP treatment might lead to a decrease in physiological intensity during the smoking process, thus resulted in a relatively flat taste.

### Phenolic Compounds Content

As shown in Figure 1, the application of high-performance liquid chromatography facilitated the identification of five prominent phenolic compounds in flue-cured tobacco, notably neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, scopoletin, and rutin. As shown in Table 4, a discernible decline in the content of principal phenolic compounds within flue-cured tobacco was observed after UHP treatment ( $p < 0.05$ ). Interestingly, there was no statistically significant difference in the content of major phenolic compounds between the UHP treatment groups. These results collectively suggested that the content of phenolic compounds was relatively stable in the UHP range of

200 MPa-600 MPa. Extensive scientific inquiry has underscored the pivotal role played by polyphenols, regarded as vital secondary metabolites within the tobacco plant, in the genesis of fragrant attributes (Shen *et al.*, 2021). Subsequent research on the effect of UHP on phenolic compounds in flue-cured tobacco can further expand the pressure range or adjust the time of UHP treatment. Previous study has shown that in living tobacco leaf tissues, almost all phenolic compounds were in the form of glycosides and esters. However, the coexistence of glycosides and hydrolytic enzymes in distinct cells of the same organ within the tobacco plant posed a challenge in terms of accessibility of both. We assumed that UHP treatment compressed the microstructure of the flue-cured tobacco leaf tissues while increasing the possibility of cell destruction. This disruption could lead to the release of hydrolytic

enzymes, consequently diminishing the content of polyphenolic substances within the tobacco leaves.



**Fig. 1:** Analysis chart of electronic nose detection PCA of flue-cured tobacco under different UHP treatment

**Table 4:** Concentration of main phenolic substances in flue-cured tobacco under different treatment conditions (mg\*g<sup>-1</sup>)

| Sample  | Neochlorogenic acid    | Chlorogenic acid         | Cryptochlorogenic acid | Scopoletin             | Rutin                   |
|---------|------------------------|--------------------------|------------------------|------------------------|-------------------------|
| RAW     | 1.36±0.03 <sup>a</sup> | 13.71±0.12 <sup>a</sup>  | 3.03±0.08 <sup>a</sup> | 0.91±0.01 <sup>a</sup> | 10.10±0.40 <sup>a</sup> |
| UHP-200 | 1.23±0.02 <sup>b</sup> | 12.82±0.07 <sup>c</sup>  | 2.70±0.05 <sup>b</sup> | 0.86±0.00 <sup>b</sup> | 9.10±0.21 <sup>b</sup>  |
| UHP-300 | 1.24±0.02 <sup>b</sup> | 13.12±0.12 <sup>b</sup>  | 2.75±0.08 <sup>b</sup> | 0.86±0.00 <sup>b</sup> | 9.29±0.38 <sup>b</sup>  |
| UHP-400 | 1.24±0.03 <sup>b</sup> | 13.00±0.10 <sup>bc</sup> | 2.73±0.05 <sup>b</sup> | 0.87±0.01 <sup>b</sup> | 9.27±0.32 <sup>b</sup>  |
| UHP-500 | 1.24±0.02 <sup>b</sup> | 12.89±0.11 <sup>c</sup>  | 2.72±0.05 <sup>b</sup> | 0.85±0.03 <sup>b</sup> | 9.51±0.43 <sup>ab</sup> |
| UHP-600 | 1.24±0.03 <sup>b</sup> | 12.87±0.13 <sup>c</sup>  | 2.74±0.08 <sup>b</sup> | 0.86±0.00 <sup>b</sup> | 9.10±0.32 <sup>b</sup>  |

#### Analysis of Electronic Nose Test

To elucidate the variances in volatile aromatic constituents within flue-cured tobacco specimens subjected to diverse UHP treatment regimens, an electronic olfactory apparatus was applied to holistically assess the collective profile of volatile elements that present in the samples (Wang *et al.*, 2016a). Utilizing Principal Component Analysis (PCA), an in-depth exploration was conducted on the electronic olfaction response data derived from distinct treatment conditions, whereby the signal intensities associated with flavor-related constituents were leveraged to accentuate the differentials amidst the samples (Yang *et al.*, 2021).

The Heracles Neo ultra-fast gas-phase electronic nose was harnessed to capture the chromatographic data pertaining to sample exposed to varying UHP treatment parameters. Subsequently, a PCA model was constructed as depicted in Figure 1. Remarkably, the cumulative contribution of variance attributed to the first principal component (PC1) and the second principal component (PC2) reached 90.72%. It was discerned that the samples subjected to UHP treatments occupied distinct and delineated regions within the PCA analysis plot in comparison to untreated group. This spatial segregation signified pronounced differences in their volatile constituents. Specifically, along PC1, the elevation of treatment pressure (ranging from 200 MPa to 600 MPa) led to an alignment of volatile aromatic constituents in the treatment groups that approached closer to those within the untreated group. Conversely, the PC2 dimension unveiled an accentuation of the scent

divergence between the treatment groups' flue-cured tobacco samples and their untreated group as the treatment pressure escalated.

The cumulative findings underscored the discernible impact of UHP treatments on the intricate composition of volatile aromatic constituents within flue-cured tobacco samples.

#### Tobacco Pyrolysis-Gas Phase Mass Spectrometry Results

Thermal cracking was combined with GC-MS to simulate and identify the volatile flavour components that might be produced during heating process of heated tobacco products. A total of 51 compounds were identified in the pyrolysis products of tobacco samples when subjected to thermal decomposition at 350°C. These compounds include 3 olefinic substances, 17 ketones, 4 aldehydes, 3 alcohols, 6 esters, 7 acids, 2 furans, 2 pyridines, 1 alkaloid, and 6 other compounds. In general, different UHP treatment conditions had little effect on the composition of cracking products, but they did have some influence on the quantity and concentration of certain types of compounds. In general, the five UHP treatment conditions had a small effect on the compositional species of the cleavage products, but had an effect on the quantity and concentration of compounds such as ketones and esters. As shown in Table 5, UHP-200 and UHP-400 treatments increased the number of pyrolysis products of tobacco under the same conditions, especially the number of ketones and acids.

Among all the UHP treatment conditions, the 400 MPa treatment group had the highest number of ketone cracking products (15 ketones), as well as the highest overall quantity of cracking products. Aldehydes and

ketones are believed to be mainly the products of sugar decomposition in tobacco and the products of Maillard reaction between amino substances in tobacco and reducing sugars (Wang *et al.*, 2021).

**Table 5:** The thermal cracking products under different conditions of flue-cured tobacco under UHP

| Number    | Compound                                                                                        | CAS        | Formula                                        | Relative peak area/% |           |            |           |            |            |
|-----------|-------------------------------------------------------------------------------------------------|------------|------------------------------------------------|----------------------|-----------|------------|-----------|------------|------------|
|           |                                                                                                 |            |                                                | RAW                  | UHP-200   | UHP-300    | UHP-400   | UHP-500    | UHP-600    |
| Alkenes   |                                                                                                 |            |                                                | 2                    | 2         | 2          | 3         | 2          | 2          |
| ALKE1     | 2-Octene, (E)-                                                                                  | 13389-42-9 | C <sub>8</sub> H <sub>16</sub>                 | -                    | -         | -          | 0.19±0.01 | -          | -          |
| ALKE2     | Neophytadiene                                                                                   | 504-96-1   | C <sub>20</sub> H <sub>38</sub>                | 2.56±0.13            | 2.18±0.02 | 2.07±0.06  | 2.58±0.03 | 2.05±0.02  | 2.06±0.12  |
| ALKE3     | Cyclodecacyclotetradecene, 14,15-didehydro-1,4,5,8,9,10,11,12,13,16,17,18,19,20-tetradecahydro- | 14113-61-2 | C <sub>22</sub> H <sub>32</sub>                | 0.33±0.01            | 0.32±0.01 | 0.39±0.01  | 0.43±0.03 | 0.29±0.01  | 0.39±0     |
| Ketones   |                                                                                                 |            |                                                | 14                   | 14        | 14         | 15        | 14         | 13         |
| KET1      | 2,3-Butanedione                                                                                 | 431-03-8   | C <sub>4</sub> H <sub>6</sub> O <sub>2</sub>   | 0.69±0.18            | 0.51±0.01 | 0.75±0.28  | 0.75±0.11 | 0.62±0.12  | 0.69±0.16  |
| KET2      | 2-Propanone, 1-hydroxy-                                                                         | 116-09-6   | C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>   | 1.93±0.07            | 1.8±0.01  | 2.26±0.01  | 2.06±0.01 | 2.05±0.04  | 1.93±0.04  |
| KET3      | 2-Propanone, 1-(acetyloxy)-                                                                     | 592-20-1   | C <sub>5</sub> H <sub>8</sub> O <sub>3</sub>   | 0.44±0               | 0.38±0.01 | 0.44±0     | 0.39±0.01 | 0.38±0     | 0.44±0.01  |
| KET4      | 4-Cyclopentene-1,3-dione                                                                        | 930-60-9   | C <sub>5</sub> H <sub>4</sub> O <sub>2</sub>   | 1.07±0.02            | 1.04±0.01 | 1.23±0.02  | 1.13±0    | 1.05±0.01  | 1.12±0.02  |
| KET5      | 2(5H)-Furanone                                                                                  | 497-23-4   | C <sub>4</sub> H <sub>4</sub> O <sub>2</sub>   | 0.38±0               | 0.37±0    | 0.45±0     | 0.4±0     | 0.41±0     | 0.41±0     |
| KET6      | 1,2-Cyclopentanedione                                                                           | 3008-40-0  | C <sub>5</sub> H <sub>6</sub> O <sub>2</sub>   | 0.79±0.01            | 0.8±0     | 0.98±0.01  | 0.95±0.02 | 0.95±0.07  | 0.88±0.01  |
| KET7      | 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one                                                    | 10230-62-3 | C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>   | 0.98±0.05            | 1.02±0    | 1.11±0.02  | 1.06±0.04 | 0.98±0.01  | 1.04±0     |
| KET8      | Furyl hydroxymethyl ketone                                                                      | 17678-19-2 | C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>   | 0.32±0               | 0.32±0    | 0.41±0.01  | 0.33±0.01 | 0.35±0     | 0.36±0.01  |
| KET9      | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-                                             | 28564-83-2 | C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>   | 7.12±0.31            | 7.04±0.01 | 7.99±0.26  | 7.69±0.03 | 6.94±0.02  | 7.37±0.11  |
| KET10     | 6-Ethyl-5,6-dihydro-2H-pyran-2-one                                                              | 19895-35-3 | C <sub>7</sub> H <sub>10</sub> O <sub>2</sub>  | 0.49±0.02            | 0.48±0.01 | 0.44±0     | 0.49±0.01 | 0.43±0.02  | 0.48±0.01  |
| KET11     | 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-                                                         | 1073-96-7  | C <sub>6</sub> H <sub>6</sub> O <sub>4</sub>   | 0.43±0.02            | 0.46±0    | 0.5±0.01   | 0.49±0    | 0.47±0.01  | 0.45±0.03  |
| KET12     | 5-(Hydroxymethyl) dihydrofuran-2(3H)-one                                                        | 10374-51-3 | C <sub>5</sub> H <sub>8</sub> O <sub>3</sub>   | -                    | -         | -          | 0.23±0.02 | -          | -          |
| KET13     | 6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)-                                           | 54868-48-3 | C <sub>13</sub> H <sub>22</sub> O              | 0.26±0               | 0.25±0    | 0.27±0     | 0.29±0    | 0.22±0.01  | 0.27±0     |
| KET14     | Megastigmatrienone                                                                              | 38818-55-2 | C <sub>13</sub> H <sub>18</sub> O              | 0.21±0               | 0.19±0.01 | -          | 0.21±0.01 | 0.19±0.01  | -          |
| KET15     | 2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-                                    | 34318-21-3 | C <sub>13</sub> H <sub>20</sub> O <sub>2</sub> | -                    | -         | -          | 0.2±0     | -          | -          |
| KET16     | 3(2H)-Furanone, 4-hydroxy-5-methyl-                                                             | 19322-27-1 | C <sub>5</sub> H <sub>6</sub> O <sub>3</sub>   | -                    | -         | 0.22±0.02  | -         | -          | -          |
| KET17     | 1,2-Cyclopentanedione, 3-methyl-                                                                | 765-70-8   | C <sub>6</sub> H <sub>8</sub> O <sub>2</sub>   | 0.27±0.02            | 0.24±0.01 | 0.3±0      | -         | 0.27±0     | 0.25±0     |
| Aldehydes |                                                                                                 |            |                                                | 3                    | 3         | 4          | 3         | 3          | 3          |
| ALDE1     | Furfural                                                                                        | 98-01-1    | C <sub>5</sub> H <sub>4</sub> O <sub>2</sub>   | 3.3±0.04             | 3.19±0.02 | 4.01±0.05  | 3.17±0.02 | 3.33±0.08  | 3.63±0.01  |
| ALDE2     | 2-Furancarboxaldehyde, 5-methyl-                                                                | 620-02-0   | C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>   | 1.91±0.04            | 1.78±0.01 | 2.06±0.06  | 1.79±0.02 | 1.77±0.02  | 2.02±0.01  |
| ALDE3     | 5-Hydroxymethylfurfural                                                                         | 67-47-0    | C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>   | 8.58±0.27            | 9.58±0.35 | 12.45±0.62 | 9.58±0.01 | 10.18±0.27 | 10.98±0.36 |
| ALDE4     | Pentanal                                                                                        | 110-62-3   | C <sub>5</sub> H <sub>10</sub> O               | -                    | -         | 0.82±0.02  | -         | -          | -          |
| Alcohols  |                                                                                                 |            |                                                | 3                    | 3         | 3          | 3         | 3          | 3          |
| ALCO1     | 2-Furanmethanol                                                                                 | 98-00-0    | C <sub>5</sub> H <sub>6</sub> O <sub>2</sub>   | 2.03±0.02            | 2.28±0.01 | 2.8±0      | 2.48±0    | 2.4±0.04   | 2.11±0.05  |
| ALCO2     | Furaneol                                                                                        | 3658-77-3  | C <sub>6</sub> H <sub>8</sub> O <sub>3</sub>   | 1.14±0               | 0.67±0.01 | 1.29±0.01  | 1.22±0    | 1.17±0.09  | 1.21±0.05  |
| ALCO3     | Thunbergol                                                                                      | 25269-17-4 | C <sub>20</sub> H <sub>34</sub> O              | 0.23±0               | 0.21±0.02 | 0.28±0.04  | 0.69±0.01 | 0.22±0     | 0.24±0     |
| Esters    |                                                                                                 |            |                                                | 2                    | 3         | 3          | 5         | 5          | 4          |
| EST1      | Propanoic acid, 2-oxo-, methyl ester                                                            | 600-22-6   | C <sub>4</sub> H <sub>6</sub> O <sub>3</sub>   | 0.51±0.01            | 0.46±0.01 | 0.6±0.01   | 0.51±0    | 0.52±0.02  | 0.54±0     |
| EST2      | 1,2-Propanediol, 1-acetate                                                                      | 627-69-0   | C <sub>5</sub> H <sub>10</sub> O <sub>3</sub>  | -                    | 0.32±0.01 | 0.35±0.02  | 0.32±0.01 | 0.29±0.01  | 0.3±0.04   |
| EST3      | Butyrolactone                                                                                   | 96-48-0    | C <sub>4</sub> H <sub>6</sub> O <sub>2</sub>   | -                    | -         | -          | 0.2±0     | 0.19±0.02  | -          |
| EST4      | 2-Hydroxy-gamma-butyrolactone                                                                   | 19444-84-9 | C <sub>4</sub> H <sub>6</sub> O <sub>3</sub>   | 0.26±0.03            | -         | 0.27±0.04  | 0.23±0.06 | 0.27±0.03  | 0.25±0.06  |
| EST5      | Scopoletin                                                                                      | 92-61-5    | C <sub>10</sub> H <sub>8</sub> O <sub>4</sub>  | -                    | 0.32±0.06 | -          | 0.42±0.06 | -          | 0.23±0.01  |
| EST6      | Methyl nitrite                                                                                  | 624-91-9   | CH <sub>3</sub> NO <sub>2</sub>                | -                    | -         | -          | -         | 0.25±0.01  | -          |
| Acids     |                                                                                                 |            |                                                | 4                    | 7         | 3          | 6         | 4          | 4          |
| ACID1     | 2-Propenoic acid                                                                                | 79-10-7    | C <sub>3</sub> H <sub>4</sub> O <sub>2</sub>   | -                    | 0.19±0.02 | -          | 0.23±0.06 | -          | -          |
| ACID2     | Acetic acid, (acetyloxy)-                                                                       | 13831-30-6 | C <sub>4</sub> H <sub>6</sub> O <sub>4</sub>   | -                    | 0.24±0.01 | -          | 0.27±0    | 0.27±0.01  | 0.27±0.02  |
| ACID3     | 2-Furancarboxylic acid                                                                          | 88-14-2    | C <sub>5</sub> H <sub>4</sub> O <sub>3</sub>   | 0.48±0.03            | 0.57±0.04 | 0.47±0.03  | 0.57±0.02 | 0.55±0.06  | 0.62±0.03  |
| ACID4     | n-Hexadecanoic acid                                                                             | 57-10-3    | C <sub>16</sub> H <sub>32</sub> O <sub>2</sub> | 0.85±0               | 1.25±0.1  | 1.04±0.15  | 1.42±0.08 | 1.11±0.21  | 1.12±0.03  |
| ACID5     | 10E,12Z-Octadecadienoic acid                                                                    | 2420-56-6  | C <sub>18</sub> H <sub>32</sub> O <sub>2</sub> | -                    | 0.24±0.03 | -          | 0.26±0.02 | -          | -          |
| ACID6     | 9,12,15-Octadecatrienoic acid, (Z, Z, Z)-                                                       | 463-40-1   | C <sub>18</sub> H <sub>30</sub> O <sub>2</sub> | 0.25±0.02            | 0.5±0.08  | 0.32±0.08  | 0.57±0.05 | 0.44±0.11  | 0.37±0.03  |
| ACID7     | Acetic acid                                                                                     | 64-19-7    | C <sub>2</sub> H <sub>4</sub> O <sub>2</sub>   | 5.99±1.69            | 5.93±1.02 | -          | -         | -          | -          |

Table 5: Continued

| Number    | Compound                                     | CAS       | Formula                                        | Relative peak area/% |            |           |            |           |           |
|-----------|----------------------------------------------|-----------|------------------------------------------------|----------------------|------------|-----------|------------|-----------|-----------|
|           |                                              |           |                                                | RAW                  | UHP-200    | UHP-300   | UHP-400    | UHP-500   | UHP-600   |
| Furans    |                                              |           |                                                | 1                    | 1          | 1         | 1          | 1         | 1         |
| FURA1     | Furan, 2-methyl-                             | 534-22-5  | C <sub>5</sub> H <sub>6</sub> O                | -                    | -          | 0.29±0    | 0.24±0     | 1.92±2.4  | 0.26±0    |
| FURA2     | Furan, 3-methyl-                             | 930-27-8  | C <sub>5</sub> H <sub>6</sub> O                | 0.25±0               | 0.23±0     | -         | -          | -         | -         |
| Pyridines |                                              |           |                                                | 1                    | 1          | 1         | 2          | 2         | 1         |
| PYRI1     | 2,3'-Dipyridyl                               | 581-50-0  | C <sub>10</sub> H <sub>8</sub> N <sub>2</sub>  | 0.27±0               | 0.29±0.01  | 0.23±0    | 0.29±0     | 0.26±0.03 | 0.3±0     |
| PYRI2     | Pyridine                                     | 110-86-1  | C <sub>5</sub> H <sub>5</sub> N                | -                    | -          | -         | 0.2±0      | 0.19±0.01 | -         |
| Alkaloids |                                              |           |                                                | 1                    | 1          | 1         | 1          | 1         | 1         |
| PYRR1     | Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)- | 54-11-5   | C <sub>10</sub> H <sub>14</sub> N <sub>2</sub> | 53.57±0.85           | 50.31±0.17 | 49.04±0.5 | 50.73±0.21 | 46.95±1   | 53.17±0.3 |
| Others    |                                              |           |                                                |                      |            | 2         | 4          | 3         | 4         |
| OTHE1     | 2,5-Furandione, 3-methyl-                    | 616-02-4  | C <sub>5</sub> H <sub>4</sub> O <sub>3</sub>   | 0.22±0               | 0.21±0     | 0.22±0.01 | 0.23±0.01  | 0.21±0    | 0.24±0    |
| OTHE2     | . beta. -D-Glucopyranose, 1,6-anhydro-       | 498-07-7  | C <sub>6</sub> H <sub>10</sub> O <sub>5</sub>  | 0.39±0.04            | 0.65±0.13  | 0.25±0.28 | 0.23±0     | 0.65±0.27 | 0.61±0.01 |
| OTHE3     | Propanenitrile, 3,3'-oxybis-                 | 1656-48-0 | C <sub>6</sub> H <sub>8</sub> N <sub>2</sub> O | -                    | 0.31±0.01  | 0.39±0    | 0.32±0     | -         | 0.34±0    |
| OTHE4     | 13-Docosenamide, (Z)-                        | 112-84-5  | C <sub>22</sub> H <sub>43</sub> NO             | -                    | -          | -         | 0.32±0.08  | 0.23±0.06 | 0.22±0.02 |
| OTHE5     | Hydrazine, 1,2-dimethyl-                     | 540-73-8  | C <sub>2</sub> H <sub>8</sub> N <sub>2</sub>   | -                    | -          | -         | -          | 5.93±0.24 | -         |
| OTHE6     | 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-  | 126-11-4  | C <sub>4</sub> H <sub>9</sub> NO <sub>5</sub>  | -                    | 0.32±0.03  | -         | -          | 0.27±0.05 | -         |
| 合计        | 51                                           |           |                                                | 33                   | 39         | 35        | 43         | 40        | 36        |

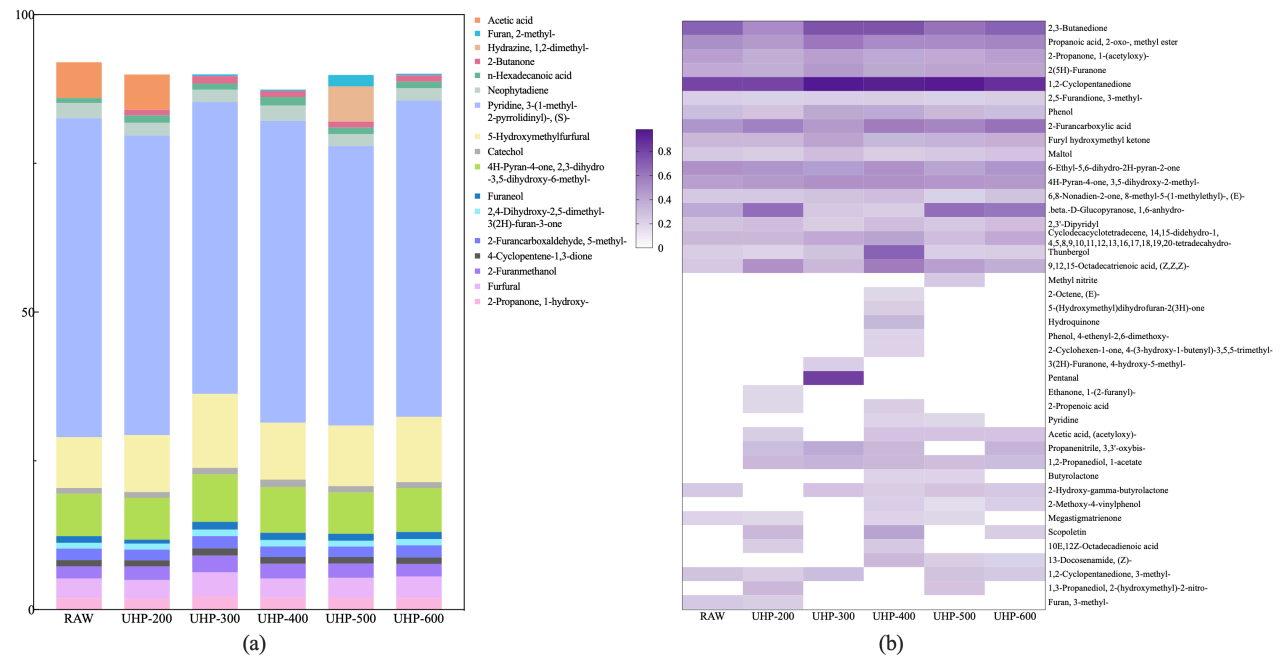


Fig. 2: Accumulation map and heat map of the relative peak area of the pyrolysate at 350°C before and after the UHP treatment of FCTL. (a) represents the compound accumulation map of the relative peak area of the pyrolysis product greater than 1%, and (b) represents the heat map of the compound with the relative peak area of the cracker less than or equal to 1%

Based on the relative abundance of each pyrolysis product, compounds with relative peak areas greater than 1% and compounds with equal to or less than 1% relative peak areas were separated. They were then plotted separately in Figure 2(a) (stacked plot of pyrolysis products with relative peak areas greater than 1%) and Figure 2(b) (heat map of pyrolysis products with relative peak areas equal to or less than 1%).

Among the pyrolysis products with relative peak areas greater than 1%, the content of acetic acid, which originally present in tobacco, decreased to non-detectable

levels in the high-pressure treatment groups. This reduction might contribute to the diminished pungent flavour in the high-pressure treatment group. On the other hand, 2-butanone emerged as a newly introduced flavour compound after UHP treatment, characterized by a pleasant fruity aroma (Cheng, 2010). 5-Hydroxymethylfurfural, an important intermediate in the Maillard reaction, exhibited increased relative abundance following UHP treatment. Among the treatment groups, the 300 MPa group demonstrated the highest relative content (12.45±0.62%). The elevation in the relative



content of 5-hydroxymethylfurfural could potentially enhance the caramel sweetness and roasted aroma of tobacco during smoking (Yao *et al.*, 2021). The outcomes presented herein exhibit paralleled with findings reported by (Baxter *et al.*, 2005) on the alteration of volatile flavour components following UHP treatment of orange juice. It was hypothesized that after undergoing UHP treatment, certain enzymes within FCTL underwent activation, thereby inducing rapid reactions between certain chemicals in the leaves. Consequently, this phenomenon facilitated the liberation of latent aromatic compounds. As the most abundant component in the pyrolysis products of tobacco at 350°C, nicotine exhibited a decreased relative content after UHP treatment. However, no distinct linear relationship was observed between the treatment pressure and the reduction in nicotine content. This outcome aligns well with the current demand to reduce nicotine levels in tobacco products.

In the pyrolysis products with relative peak areas less than or equal to 1%, it could be visually observed from Figure 2(b) that the UHP treatment induced the generation of numerous compounds in tobacco pyrolysis that were not present originally. This phenomenon was particularly pronounced in 200 MPa, 400 MPa, and 500

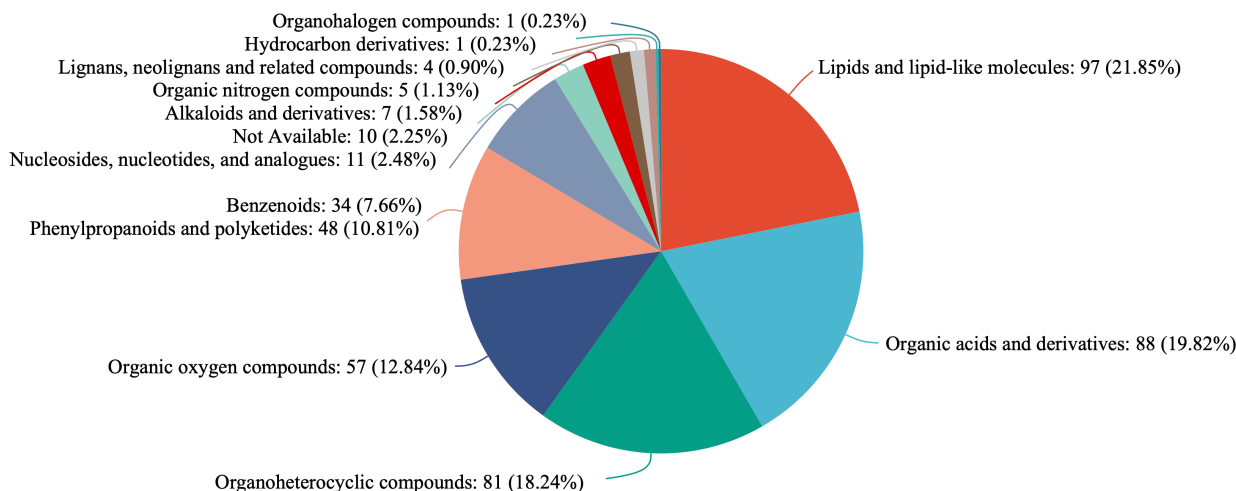
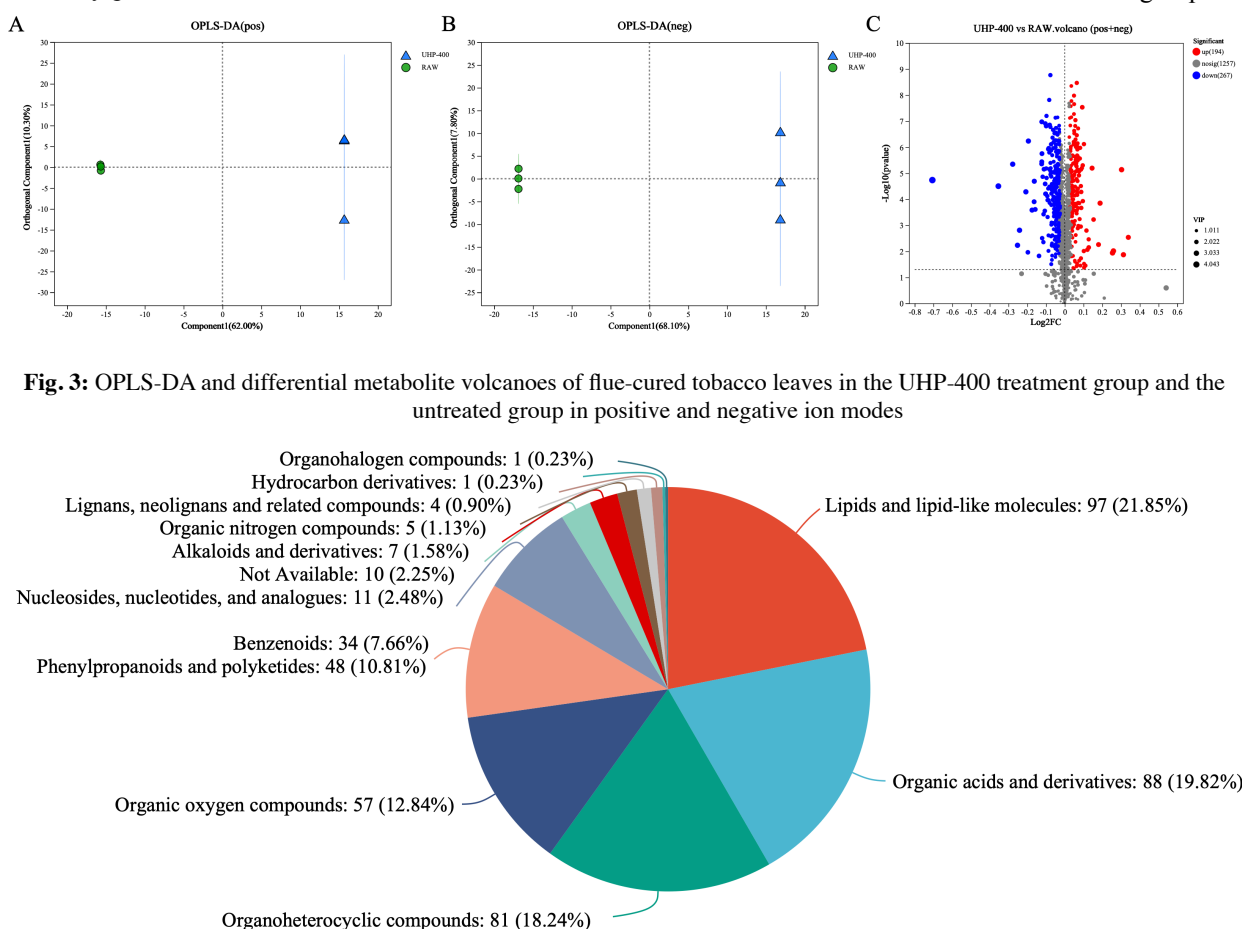
MPa treatment groups. Thus, it could be stated that UHP treatment enhanced the diversity of pyrolysis products in flue-cured tobacco for heated tobacco products, as evidenced by the increased richness of compounds.

### Untargeted Metabolomics Analysis Results

Based on the sensory evaluation results of tobacco leaves before and after Ultra-High Pressure pretreatment, UHP-400 group and untreated tobacco leaves (RAW) were selected for untargeted metabolomics analysis.

According to the OPLS-DA results in Figure 3, compared with the untreated group, the UHP-400 treated group had better separation in the first orthogonal component (positive ion mode: 62.00%, negative ion mode: 68.10%) and higher similarity in the second orthogonal component (positive ion mode: 10.30%, negative ion mode: 7.80%). The permutation test showed that the model was more reliable (positive ion mode:  $R^2 = 0.995$ ,  $Q^2 = 0.2557$ ; negative ion mode:  $R^2 = 0.9992$ ,  $Q^2 = 0.2654$ ).

In the volcano map of positive and negative ion mode merging (Figure 3C), 267 metabolites significantly decreased and 194 metabolites significantly increased in the tobacco leaves of the UHP-400 treatment group.

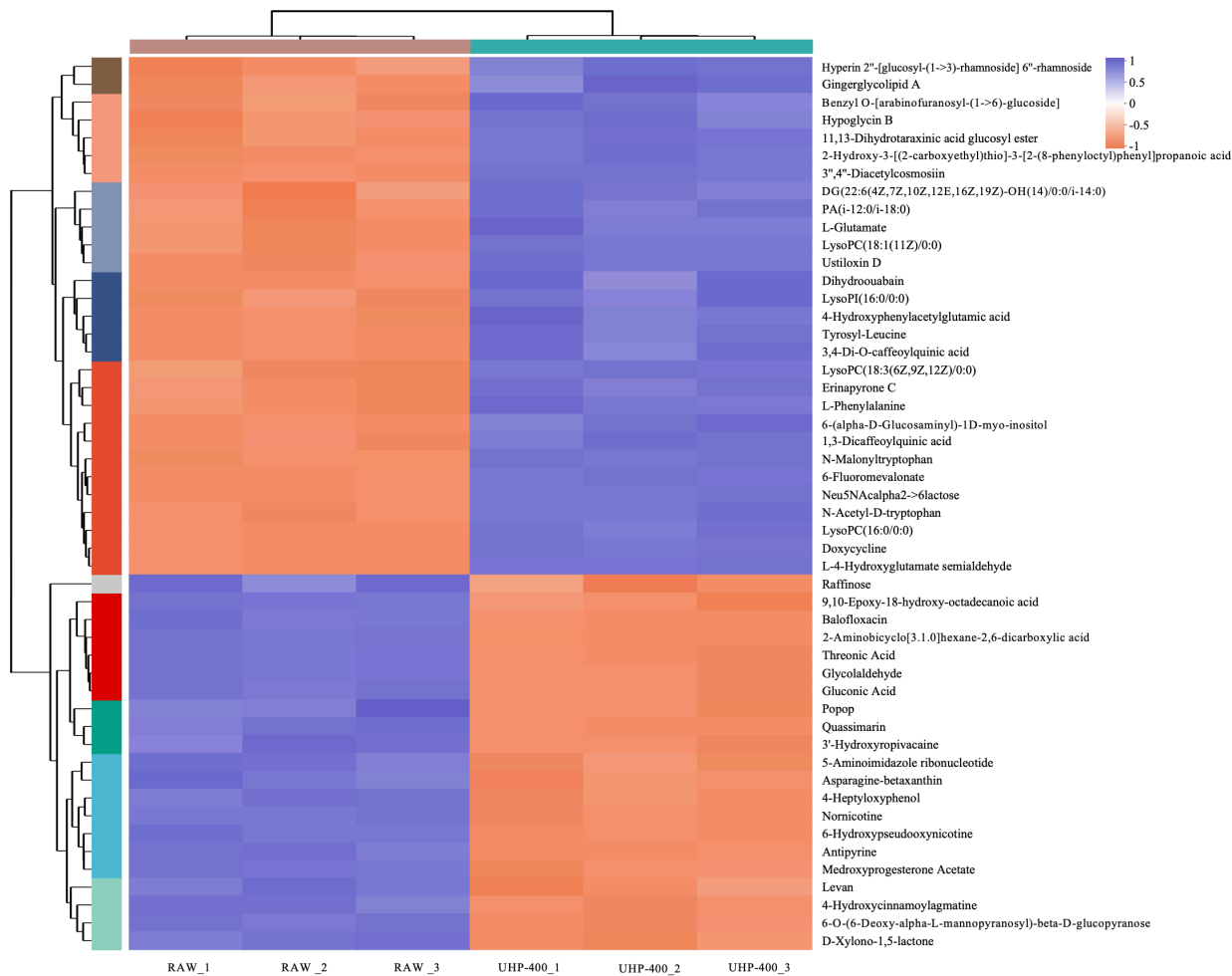




The differential metabolites (461 species) of UHP-400 treated and untreated tobacco leaves were classified according to the HMDB database. OPLS-DA and differential metabolite volcanoes of flue-cured tobacco leaves in the UHP-400 treatment group and the untreated group were analyzed in positive and negative ion modes. According to the classification results, the highest differential metabolites in the UHP-400 treated and untreated tobacco leaves were lipids and lipid molecules, organic acids and their derivatives, and organic heterocyclic compounds. This indicates that the effects of Ultra-High Pressure treatment on tobacco leaves are mainly reflected in metabolites such as lipids and lipoids, organic acids and their derivatives, and organic heterocyclic compounds.

Select the top 50 metabolites with relative content between the UHP-400 treatment group and the untreated group to draw a cluster analysis heatmap (Figure 5).

Among these metabolites, 29 metabolites showed a decrease in relative content and 21 metabolites showed an increase in relative content. Among the 29 metabolites with relatively decreased content, there are 9 organic acids and their derivatives, 8 lipids and lipoids molecules, 5 organic oxygen-containing compounds, 4 phenylpropanoid compounds and polyketide compounds, 2 organic heterocyclic compounds, and 1 other class. Based on cluster analysis, the decrease in the relative content of various amino acids and lipid molecules may be related to the promotion of glucose and lipid metabolism in tobacco leaves during the ultra-high pressure treatment process. Among the 21 metabolites with relatively increased content, there are 8 organic oxygen-containing compounds, 3 lipids and lipoids molecules, 5 organic heterocyclic compounds, 3 organic acids and their derivatives, 1 benzene ring compound, 1 phenylpropanoid compound, and polyketide compound.



**Fig. 5:** Heat map of cluster analysis of differential metabolites in flue-cured tobacco leaves treated with UHP-400 and untreated tobacco leaves

After UHP-400 treatment, the relative content of amino acids such as L-Glutamate, L-Asparagine, 5-hydroxyindoleacetyl glycine, and L-tryptophan increased, while the relative content of xanthine decreased. A study

has shown that the content of amino acids such as glutamic acid, aspartic acid, and glycine in tobacco leaves is relatively high. UHP-400 treatment further promotes the increase of these amino acid contents,

which is consistent with the results of Heinemann and Hildebrandt (2021) that different pressure environmental conditions can lead to the accumulation of amino acids and their derived secondary metabolites. Glutamic acid can cause fluctuations in xanthine content, which is closely related to the formation of the characteristic flavor of Tie Guan Yin oolong tea (Li *et al.*, 2023). At the same time, the decrease in xanthine content can alleviate the bitterness and astringency of Tie Guan Yin. These results also provide some reference for the improvement of sensory quality of roasted tobacco after the Ultra-High Pressure treatment.

## Conclusion

In this study, FCTL for heated tobacco products were treated with UHP. The changes of chemical compositions and lysis products in flue-cured tobacco before and after treatment were of particular interest. The results indicated that UHP treatment conditions exert a certain influence on the conventional chemical constituents, total volatile compounds in FCTL. There was a noteworthy increase in sugar compounds after UHP treatment, accompanied by a reduction in nicotine content, thereby achieving the objective of diminishing nicotine while enhancing its quality. Moreover, the volatile flavor constituents within the FCTL underwent transformations post UHP treatment. In the thermal pyrolysis products, products associated with the degradation of sugars, such as aldehydes and acids increased, consequently enriching the aromatic constituents of the tobacco. The results of untargeted metabolomics analysis indicate that the increase in the content of amino acids such as glutamic acid and aspartic acid, as well as the fluctuations in the content of compounds such as xanthine, may be related to the formation of characteristic flavors and the improvement of sensory quality in the heated cigarettes produced by this treatment group. This study established foundational research for enhancing and enriching the aroma of heated tobacco products, aiming to provide insights for the development of novel tobacco products.

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## Author's Contributions

**Xinbo Lu, Jie Yin, Ying Yuan and Jun Xia:** Investigation, methodology, and original draft preparation.

**Huawen Wang and Xingqian Ye:** Data curation.

**Dan Wu and Jun Wang:** Validation.

**Jinhu Tian and Jian Jiang:** Project administration, supervision, review, and editing.

## Conflict of Interest

The authors declared that there were no conflicts in present study.

## Data Availability

Data will be made available on request.

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