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Research article

Analysis and application evaluation of the flavour-precursor and volatile-aroma-component differences between waste tobacco stems

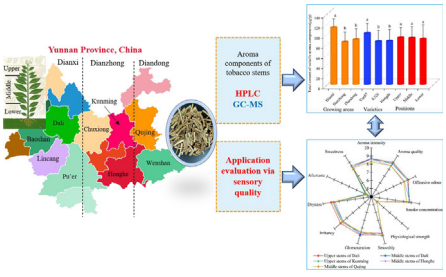
Xing Fan [a], Wenhua Zi [a,b,*], Jincheng Ao [c], Boyu Li [a], Junfeng Qiao [a], Yong Wang [a], Yonghong Nong [a]

[a] Faculty of Energy and Environment Science, Yunnan Normal University, Kunming 650500, PR China
[b] Yunnan Key Laboratory of Rural Energy Engineering, Kunming 650500, PR China
[c] College of Plant Protection, Yunnan Agricultural University, Kunming 650201, PR China

HIGHLIGHTS

- Differences in flavour precursors and aroma components in tobacco stems were systematically studied.
- Flavour precursors and total volatile substances were the highest in the Dianxi area, Yun 87 variety and upper parts.
- The aroma components and sensory quality of tobacco stems have obvious regional characteristics.
- Higher flavour components in tobacco stems provide a better contribution to improving cigarette quality.
- The use of middle and upper tobacco stems are preferred and classify to process to promote industrial applicability.

GRAPHICAL ABSTRACT



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ABSTRACT

The development and utilization of waste resources have important scientific significance and social value. As a renewable and clean resource, the flavour components of waste tobacco stems (WTS) make a significant contribution to the development of waste resources. To evaluate the industrial applicability of the different WTS as raw materials, the present study systematically examined the differences in flavour precursors and volatile aroma components in tobacco stems (TS) from typical tobacco (*Nicotiana tabacum L.*) growing areas in Yunnan Province through HPLC and GC–MS analysis. Meanwhile, the contribution to improving the quality of cigarette products is discussed accordingly. The results showed that the contents of flavour precursors, carotenoid and hexane degradation products, neophydiene and total volatile aroma substances in TS were the highest in the Dianxi area, Yun 87 variety and the upper part of Yunnan Province, while the contents of Maillard reaction products and phenylalanine degradation products were the highest in the Diandong area and middle part. The aroma components and sensory quality of TS have obvious regional characteristics in Yunnan. Accordingly, the higher flavour components improve cigarette quality. Most importantly, the middle and upper TS should be preferred to use and classified to process according to the tobacco growing areas, which is beneficial for promoting industrial applicability in cigarette preparation. This study provides a theoretical reference for the industrial value-added applicability of TS in the aspects of flavour extraction and cigarette preparation.

* Corresponding author.
E-mail addresses: kgclxwh@163.com, ziwh180010@ynnu.edu.cn (W. Zi).

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1. Introduction

As a renewable and clean resource, the value-added utilization of biomass resources has important scientific significance and social value for environmental protection, resource conservation and economic contributions in the energy field (Saha and Basak, 2019; Sherwood, 2020). Waste tobacco stems (WTS) is a kind of natural biomass material that is a byproduct of the tobacco (*Nicotiana tabacum L.*) industry after the tobacco leaves are harvested. Usually, tobacco stems (TS) can be transformed into a viable product through different preparation methods. For example, TS is used to prepare expanded cut stems via high temperature-high humidity and reconstituted tobacco sheets via paper-making (Zi et al., 2013a, 2013b). TS have been applied in cigarettes to reduce harmful components and raw material consumption (Liu et al., 2019a; Zhao et al., 2019). However, more than 60% of TS has been disposed of as rubbish worldwide (Chen et al., 2017; Yan et al., 2018; Zhang et al., 2019). This has led to an enormous waste of natural plant resources and serious environmental pollution. Therefore, the reutilization of this industrial waste and the exploitation of potential biomass material are important and indispensable.

For a long time, much research has focused on TS pretreatment and processing technological innovation to improve its utilization. Some pretreatments and innovation processes, e.g., microwave (Zi et al., 2013a, 2013b, 2019), high-pressure (Zhou, 2019), soaking (Zhou, 2019), enzymes (Xue et al., 2014), microorganisms (Chen et al., 2013), chemical additives (Chen et al., 2018) and other pretreatments, can effectively degrade macromolecular substances such as sugar, pectin, cellulose and lignin and improve conventional chemical compounds and increase the content of aroma components in TS. In particular, the technological innovation of microwaves expanding the TS has provided a new direction for the value-added utilization of agricultural waste resources. The TS of microwave expansion can be prepared into porous material, and the product has good filling and physical adsorption properties (Zi et al., 2013a, 2013b), which has been widely used as a cigarette formulation material (Zi et al., 2019), reconstituted tobacco leaf modified particles (Zeng et al., 2012; Song et al., 2021; Banožić et al., 2020) and filter adsorption material (Zi et al., 2019). This technology can effectively avoid environmental pollution and energy consumption caused by water washing or extraction pretreatment, high temperature, and high humidity expansion in the traditional treatment process of TS.

The components of TS are similar to those of tobacco leaves, which contain a large number of aroma components, and their content directly determines the utilization value for the extraction of useful chemicals and fragrance. Tobacco essential oil prepared from TS has a variety of crucial aroma components (Banožić et al., 2020). It is a necessary additive in reconstituted tobacco leaves, which can give off the original tobacco fragrance and conceal the odour and woodiness of reconstituted tobacco. Expanded cut stems have the advantages of high filling value, good combustibility, and low tar release in cigarette products and have become an essential ingredient in cigarette formulations (Zi et al., 2019; Zhao et al., 2019). However, when the expanded cut stems are combusted, the aroma is weak, the woody smell is heavier, and the irritation is more remarkable, which affects the amount and scope of use in cigarette products. These are mainly affected by the higher content of cell wall substances and the lower content of aroma compounds in TS, such as pigments, polyphenols and volatile aroma components (Peng et al., 2007; Zhao et al., 2016).

Flavour precursors, also known as precursors of flavour components, are substances that have no aroma or are not obvious but can be degraded to produce an aroma in the process of ageing or combustion (Yue et al., 2015; Wang et al., 2015). Latent aroma substances in tobacco accumulate, transform and degrade during the process of tobacco growth, modulation, purification and combustion, which can directly affect the aroma style and quality of tobacco, mainly including plasmatic pigments, polyphenols, organic acids, nitrogenous species and glycosides (Yue et al., 2015; Wang et al., 2015). Volatile aroma components are aromatic substances that have a low boiling point and are volatile in tobacco and smoke. According to the different aroma precursor substances, they can be divided into degraded carotene products, Maillard reaction products, degraded phenylalanine products and degraded cembrane products. In addition, the main aroma substances of TS include organic acids, carbonyl groups, hydroxyl groups, esters and lactones, and terpenes, which can be used as raw materials for the extraction of aroma substances. The aroma components of tobacco leaves change during maturing, drying, fermenting, processing and storage, and the variety, cultivation, altitude and climate are the key affecting factors (Banožić et al., 2020). Therefore, the analysis of flavour precursors and volatile aroma components in TS is of great significance to the flavouring and casing in the process of expanding cut stems, the development of low-tar cigarettes and the extraction of tobacco flavours and aroma components.

In recent years, few scholars have studied the fragrance components in TS. The investigation indicated that the aroma components in the leaves were significantly higher than those of TS. In contrast, the total amount of aldehydes in TS was higher than that in the leaves (Zhao et al., 2016). The content of the main volatile and semivolatile organic acids was the highest in the upper stems and the lowest in the lower stems for Dali TS of the Yunyan 85 variety (Peng et al., 2007). The analysis of volatile aroma substances in three main varieties (C3F) in Zhaotong showed that they had no significant difference in the total amount of volatile aroma components between different varieties. Meanwhile, the total amount of ketones, phenols, aldehydes, acids, heterocycles and olefins differed among different varieties (Li et al., 2012). The content variation of volatile aroma components is significantly different in TS from leaf base to tip in the main vein, and the total amount of volatile organic acids and volatile aroma substances first decreases and then increases (Liu et al., 2017). The volatile aroma components are also significantly different in different lengths of expanded cut stems, and the full content presented an increasing trend with the decrease in cut stem length (Qi et al., 2019). Overall, the natural conditions are very suitable for flue-cured tobacco production in Yunnan Province, and the total tobacco output from Yunnan Province accounted for 43.80% in China. Its tobacco leaves have the characteristics of good maturity, loose tissue structure, better colour and oiliness, and full aroma and have been favoured at home and abroad by tobacco companies (Fan et al., 2020). Accordingly, the TS produced by Yunnan also has good quality characteristics and is widely used in the production of cigarettes. However, as far as we know, no previous research has systematically investigated the differences between flavour precursors and volatile aroma components in TS from typical tobacco-growing areas.

In this context, we studied the difference in flavour precursors and volatile aroma components in TS from typical tobacco-growing areas in Yunnan and evaluated the contribution to improving the quality of cigarettes accordingly. The purpose is to provide a theoretical reference for the industrial value-added applicability of WTS in the aspects of flavour extraction and cigarette preparation.

2. Materials and methods

2.1. Materials

A total of 98 samples were selected from the different threshing and redrying factories in typical tobacco-growing areas of Yunnan Province. Meanwhile, five samples were chosen to further evaluate their contributions to the product quality of cigarette formula ingredients for application evaluation. The samples are distributed in the three regions of Diandong, Dianzhong and Dianxi (Shi et al., 2011). Diandong includes Qujing and Wenshan, Dianzhong includes Kunming, Chuxiong and Honghe, and Dianxi includes Dali, Baoshan, Lincang and Pu'er. The distribution of the 9 producing areas is shown in Figure 1, and Table 1 shows the distribution of the TS in growing areas, varieties, and parts.

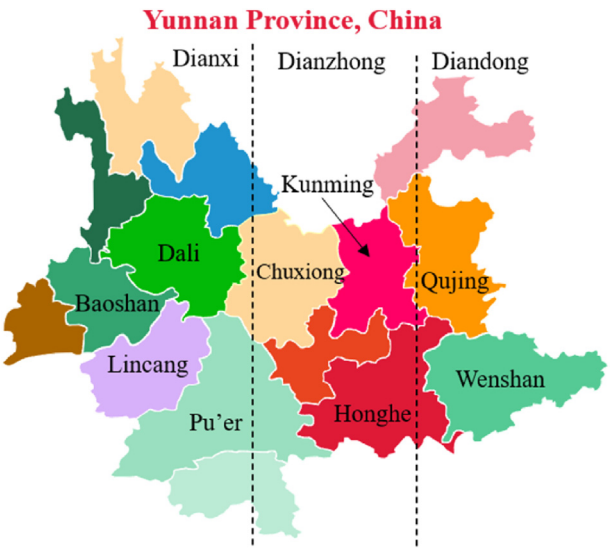

Figure 1. Distribution of the producing areas.

2.2. Analysis methods

2.2.1. Pigment and polyphenol compounds analysis

The pigment and polyphenol compounds of TS were measured using high-performance liquid chromatography (HPLC) (Periche et al., 2016; Long et al., 2017). First, the experimental samples were crushed to a size smaller than 0.35mm and balanced for 24 h at a constant temperature and humidity chamber at a temperature of 22 ± 1 °C and 60 ± 3% relative humidity, respectively. Then, each sample was weighed 0.100 and 0.500 g for the pigment and polyphenol analysis, respectively. The weighed sample was placed in an Erlenmeyer flask and 20 mL of 50% methanol was added to extracted for 30 min and held for 10 min via sonication. Finally, the supernatant was analysed by HPLC. The chromatographic columns were Waters Xterra RP-C18 (3.9 × 150 mm^2, 5 μm) and Waters μBondapark TM C18 (10 μm - 3.9 × 300 mm), and the column temperature was 30 °C. The flow rate was 1.0 mL/min. The sample volumes were 10 and 20 μL, and the 340 nm and 450 nm wavelengths were used to measure the contents of pigment and polyphenol, respectively.

2.2.2. Volatile aroma components

The volatile compounds of TS were determined by using a gas chromatography-mass spectrometer (GC–MS) (Moreno et al., 2015). The GC–MS system was consisted of an Agilent 6890N GC (Agilent, USA) and an Agilent 5975 mass spectrometer (Agilent, USA), and the GC device had a fused capillary column coated with HP5-MS (30 m × 0.25 mm i.d, 0.25 μm film thickness). Before testing, the experimental samples were prepared according to the same conditions as the test of pigment and polyphenol compounds. Then, the simultaneous distillation and extraction was used to extract the volatile components of samples. The extraction was further concentrated to 1.0 mL, and the 0.5 μL concentrate was injected into the GC–MS system to measure the volatile components based on the corresponding operation process (Long et al., 2017). Finally, the components were identified by comparing the electronic impact mass spectra with published data or reference compounds using the retention index (NIST05 and Wiley 275) (Zi et al., 2013b; Moreno et al., 2015; Long et al., 2017). The formula for calculating the content of volatile substances is followed the *Eq.*1:

$$M = \frac{M_1 \times S_1 \times 1000}{M_2 \times S_2} \qquad Eq.\ 1$$

where M is the volatile substance content (μg/g), M_1 is the mass of the internal standard substance (μg/g), S_1 is the peak area of the volatile substance (cm^2), M_2 is the mass of TS (μg/g) and S_2 is the peak area of the internal standard substance (cm^2).

2.3. Sensory evaluation of products

To further evaluate the contributions of TS to product quality, five samples consisting of upper and middle stems of Dali, upper stems of Kunming, and middle stems of Honghe and Qujing, were selected as formula ingredients of cigarette products. Their varieties are the same as Hongda. They were prepared into cut stems first and then added to the same cigarette products at a proportion of 1:10 between cut stems and formula cut tobacco leaves. Second, the prepared cigarette samples were balanced for 48 h at a constant temperature and humidity chamber at a temperature of 22 ± 1 °C and 60 ± 3% relative humidity. Finally, five smokers who underwent professional training were selected to perform the sensory evaluation of the experimental samples. The evaluated descriptors included three characteristics: aroma, smoke and taste. Among them, the aroma characteristics included aroma quality, aroma intensity and offensive odour. The smoke characteristics included smoke concentration, physiological strength, smoothness, glometaration and irritancy. The taste characteristics included dryness, aftertaste and sweetness. The maximum score of physiological strength and aftertaste was 5 points each, while the other indicator was 10 points, and the total score was 100 points (Qi et al., 2022). The performed sensory experiments received approval from an ethics committee by Yunnan Normal University.

2.4. Statistical analysis

The analysis of differences in flavour precursors and volatile aroma components values started verifying the ANOVA assumptions: normality distribution of the residuals and homoscedasticity. All the variables have a normal distribution of residuals. The statistical analyses were performed with SPSS 26.0 software.

3. Results and discussion

3.1. Analysis of flavour precursors and volatile aroma components differences

3.1.1. Analysis of flavour precursors

The smoking quality of expanded cut stems is affected by volatile aroma components, which are usually generated from the degradation of flavour precursors in the processing of tobacco materials, such as pigments and polyphenols. The pigments primarily include lutein and β-carotene, and the polyphenols primarily include chlorogenic acid, scopoletin and rutin. The overall distribution of flavour precursors in TS from typical tobacco-growing areas in Yunnan is shown in Table 2.

Table 2 shows that the proportions of lutein and β-carotene in carotenoids are 59.73% and 40.20%, respectively. Accordingly, the proportions of chlorogenic acid, scopoletin and rutin in polyphenols were

Table 1. Sample information of WTS from typical tobacco-growing areas in Yunnan.

TS classification	Growing areas			Varieties			Parts		
	Diandong	Dianxi	Dianzhong	Hongda	K326	Yun 87	upper	middle	lower
Sample number	31	21	46	28	32	38	27	58	13

Table 2. Overall status of flavour precursors in TS of Yunnan.

Flavour precursors (μg/g)	min	max	mean	CV (%)	skewness	kurtosis
lutein	1.49	12.25	4.78 ± 2.35	49.14	1.37	1.71
β-carotene	1.80	7.63	3.84 ± 1.32	34.27	1.12	1.07
chlorogenic acid ($\times10^3$)	0.39	1.05	0.61 ± 0.14	23.39	0.92	0.67
scopoletin ($\times10^3$)	0.13	0.50	0.30 ± 0.07	23.88	0.35	0.37
rutin ($\times10^3$)	0.06	0.61	0.21 ± 0.11	51.68	1.42	2.02

54.46%, 26.79% and 18.75%, respectively. The coefficient of variation (CV) values was between 23.39% and 51.68% for the six kinds of flavour precursors, indicating that there were significant fluctuations for each flavour precursor of TS, which were due to the considerable difference between the minimum value and the maximum value. In addition, the values of skewness and kurtosis are both greater than 0, showing that the distribution of the six kinds of flavour precursors is skewed to the right. At the same time, it is relatively concentrated overall.

Table 3 shows the difference in flavour precursor components in TS. Overall, the CV values are between 13.04% and 61.77% for the contents of flavour precursors in TS. The CV values are greater than 30% for the contents of lutein and rutin in different growing areas, varieties and parts, indicating that there are large fluctuations for the flavour precursors between different growing areas, varieties and parts.

The order was Dianxi > Dianzhong > Diandong for the contents of lutein, chlorogenic acid, scopoletin and rutin between the different growing areas. The contents of β-carotene decreased in the order Dianxi > Diandong > Dianzhong. Each flavour precursor in Dianxi was significantly higher than that in Dianzhong and Diandong ($P < 0.05$). Compared with those in Diandong and Dianzhong, the contents of lutein, β-carotene, chlorogenic acid, scopoletin and rutin in Dianxi were approximately 105.63%, 66.23%, 35.08%, 20.69% and 33.33% higher, respectively. In addition, there was a significant difference in the rutin contents in Dianzhong and Diandong. This is mainly due to the sunshine and light intensity. The plastid pigments are sensitive to light and increase significantly with the weakening of light intensity, and the weaker the light is, the greater the promotion effect (Yang et al., 2007). The contents of polyphenols were significantly affected by light (Wen et al., 2002), which increased with increasing sunlight duration. Dianxi has longer sunshine duration and weaker light intensity. Therefore, it leads to higher pigment and polyphenol substance contents than Dianzhong and Diandong.

The contents of lutein and β-carotene were Yun87 > K326 > Hongda between the different varieties, and Yun87 was approximately 51.42% higher than Hongda. The chlorogenic acid content was Yun87 > Hongda > K326, and Yun87 was approximately 22.64% higher than K326. The content of scopoletin was Yun87 > K326 > Hongda, with a significant difference between them ($P < 0.05$). The rutin contents were Yun87 > K326 > Hongda, and there was no significant difference between Hongda and K326, but both were approximately 27.00% lower than Yun87. The total contents of various flavour precursors were highest in Yun 87 and lowest in Hongda. This is because the unique climatic environment and geographical factors of Yunnan are also important factors affecting the pigment and polyphenol content in the stems due to the genetic decisions of different varieties of roasted tobacco (Zhu et al., 2017). In addition, most areas in Yunnan are dominated by Yunnan 87, which leads to the high pigment and polyphenol content of Yunnan 87.

Similarly, the contents of lutein, chlorogenic acid and scopoletin were in the order of upper > lower > middle between the different parts, and the β-carotene and rutin contents were in the order of upper > middle > lower. The contents of each flavour precursor in the upper part were higher than those in the middle and lower parts. The chlorogenic acid content was approximately 19.30% higher than that of the middle part. The contents of scopoletin and rutin in the middle and lower parts were approximately 21.62% and 50.00% lower than those in the upper parts, respectively. However, there were no significant differences in some flavour precursors between the different parts, which was mainly due to the large fluctuation of precursor substances in each part. Studies have shown that the maturity of tobacco leaves has a greater effect on pigments and polyphenols. With increasing maturity of the tobacco leaves, the pigment and polyphenol contents of the tobacco leaves increased accordingly (Liu et al., 2019b). However, the lower and middle tobacco leaves are picked first and the upper leaves are picked last. Therefore, the upper tobacco leaves were more mature, resulting in higher pigment and polyphenol contents in the upper TS.

In summary, the pigments and polyphenols are significantly higher in Dianxi than in Dianzhong and Diandong, and the content of rutin is significantly higher in Dianzhong than in Diandong. The content of flavour precursors is highest in Yunnan 87 and lowest in K326 among different varieties, and the content of flavour precursors is highest in the upper part and lowest in the lower part among different parts. These results indicate the flavour precursors in TS from different regions of Yunnan have obvious regional characteristics.

3.1.2. Analysis of volatile aroma components

Sixty-seven volatile compounds were detected using GC–MS analysis for TS. According to functional groups, it can be divided into 23 ketones, 14 aldehydes, 9 alcohols, 7 esters, 4 acids, 3 phenols, 6 heterocycles and 1 olefin. The proportion of each kind of compound was 16.65%, 11.07%, 19.58%, 23.03%, 1.85%, 0.88%, 1.24% and 25.69% in the total volatile aroma components, respectively. Ketones are mainly composed of 10.00% 1-(1H-pyrrol-2-yl)-ethanone, 21.89% solanone, 10.21% β-damascenone, 9.49% solanastone and 11.77% farnesyl. Furfural and phenylacetaldehyde are the main aldehydes, and they are approximately

Table 3. Analysis of flavour precursor components in TS.

Flavour precursors (μg/g)	growing areas			Varieties			Parts		
	Dianxi	Dianzhong	Diandong	Hongda	K326	Yun 87	Upper	middle	lower
lutein	8.04 ± 2.57^a	3.91 ± 1.14^b	3.87 ± 1.42^b	3.58 ± 1.13^a	4.59 ± 2.45^{ab}	5.83 ± 2.50^b	5.94 ± 3.31^a	4.29 ± 1.33^a	4.57 ± 2.87^a
β-carotene	5.42 ± 1.53^a	3.38 ± 0.79^b	3.46 ± 0.93^b	3.12 ± 0.70^a	3.79 ± 1.34^{ab}	4.42 ± 1.36^b	4.47 ± 1.87^a	3.61 ± 0.80^a	3.56 ± 1.49^a
chlorogenic acid ($\times10^3$)	0.77 ± 0.16^a	0.57 ± 0.09^b	0.56 ± 0.10^b	0.64 ± 0.12^a	0.53 ± 0.11^b	0.65 ± 0.16^a	0.68 ± 0.16^a	0.57 ± 0.12^b	0.64 ± 0.13^a
scopoletin ($\times10^3$)	0.35 ± 0.10^a	0.29 ± 0.04^b	0.27 ± 0.06^b	0.25 ± 0.05^a	0.30 ± 0.06^b	0.33 ± 0.07^c	0.37 ± 0.07^a	0.27 ± 0.05^b	0.29 ± 0.04^b
rutin ($\times10^3$)	0.28 ± 0.14^a	0.21 ± 0.11^b	0.17 ± 0.07^c	0.18 ± 0.08^a	0.19 ± 0.09^a	0.25 ± 0.13^b	0.34 ± 0.11^a	0.17 ± 0.06^b	0.15 ± 0.05^b

Notes: Table values are the measured mean ± standard deviation. The uppercase letters "a, b and c" stand for the significant differences between samples (Tukey's LSD test, $p < 0.05$).

25.65% and 57.71%. Alcohols are mainly composed of 32.10% inbetol, 29.43% phytol, 21.44% cembratriene-diol and 7.52% benzyl alcohol. Esters are mainly composed of 9.96% methyl palmitate, 10.86% ethyl palmitate and 70.58% methyl linolenate. Acids are mainly composed of 18.38% 2-methylbutyric acid, 61.43% palmitic acid and 17.58% furoic acid. Phenols are mainly composed of 15.68% 2-methoxy-phenol, 30.12% benzo[b]thiophene and 54.20% 2-methoxy-4-vinylphenol. Similarly, heterocycles are mainly composed of 47.01% pyridine, 11.78% butylated hydroxytoluene and 11.88% anthracene. Li et al. (2012) studied the content of aroma-causing components in Yunnan Zhaotong Yun87 TS. The total amounts of aldehydes, alcohols, acids, phenols, esters, heterocyclic compounds, olefins and aroma components were 8.37, 17.74, 17.23, 14.61, 1.19, 24.24, 2.09, 31.70 and 117.16 μg/g, respectively. These results are consistent with the results of this paper.

The classification analysis results of volatile aroma components are shown in Table 4. The order of the total amounts of phenols and acids was Diandong > Dianxi > Dianzhong between the different growing areas, and the total phenols of Diandong were significantly higher than those of Dianxi and Dianzhong. The order of the total amount of ketones and heterocycles was Dianxi > Dianzhong > Diandong, the order of the total amount of alcohol and esters was Dianxi > Diandong > Dianzhong, and the content of Dianxi was significantly higher than that of Diandong and Dianzhong. The order of the total amount of aldehydes was Diandong > Dianzhong > Dianxi, with significant differences between them. The olefins amount followed the order Dianxi > Diandong > Dianzhong, and the content of Dianxi was significantly higher than that of Diandong and Dianzhong, which was approximately 24.91% and 45.39% higher, respectively. Similarly, the total aroma components were ranked as Dianxi > Diandong > Dianzhong, and Dianxi was significantly higher by 26.55% and 34.16% than Diandong and Dianzhong, respectively.

The total amount of phenols, ketones, esters, and heterocycles was Yun 87 > K326 > Hongda between different varieties, the alcohols amount was Yun 87 > Hongda > K326, and the contents of Yun87 were significantly higher than those of K326 and Hongda, except heterocycles. The acid amount decreased in the order Hongda > Yun 87 > K326, while there was no significant difference among the three varieties. The aldehydes amount was in the order Hongda > K326 > Yun 87, and the contents of K326 and Hongda were significantly higher than that of Yun87. The total aroma component was higher by approximately 17.97% and 19.03%, respectively, while there was no significant difference between K326 and Hongda. The total olefin amount and aroma components were shown to be Yun87 > K326 > Hongda, and the contents of Yun87 were significantly higher than those of K326 and Hongda. Compared with K326 and Hongda, the total aroma components were higher by approximately 17.98% and 19.04%, and the olefin amount was higher by approximately 27.12% and 29.05%, respectively.

Similarly, the phenols amount was middle > upper > lower, the ketones amount was upper > middle > lower, the total amount of alcohols, acids and aldehydes was middle > lower > upper, and the esters amount was lower > middle > upper among different parts. The total amount of heterocycles, olefins and aroma components showed the order of upper > lower > middle. Ketones were significantly higher in the upper part than in the middle and lower parts. Aldehydes were significantly higher in the middle and lower parts than in the upper part. Phenols were significantly higher in the upper and middle parts than in the lower part.

3.1.3. Analysis of degradation products

To further explore the changes and distribution of volatile aroma components, they were classified and analysed according to the degradation products, as shown in Table 4 and Figure 2. The results show that the volatile aroma components can be mainly divided into four categories of degradation products, namely, carotene, Maillard reaction, phenylalanine and cembrane.

Figure 2a shows that the content of degraded carotene products is Dianxi > Dianzhong > Diandong among the different growing areas. Moreover, it is significantly higher in Dianxi than in Dianzhong and Diandong ($P < 0.05$), which is approximately 66.55% and 59.90% higher, respectively. The content of Maillard reaction products is in the order of Diandong > Dianzhong > Dianxi. Its content is approximately 19.66% and 40.59% higher in Diandong than in Dianzhong and Dianxi, respectively, and its content in Dianzhong is approximately 17.49% higher than that in Dianxi, and there are significant differences in the three regions. The content of degraded phenylalanine products is in the order of Diandong > Dianzhong > Dianxi, while there is no significant difference in different regions. The content of degraded cembrane products is in the order of Dianxi > Diandong > Dianzhong, and its content is significantly higher in Dianxi than in Diandong and Dianzhong, which are higher by approximately 41.69%. The trends of the four degradation products are not consistent among different regions, mainly due to the different effects of climatic factors, cultivation conditions, etc., on the different degradation products. Sunshine duration and precipitation are not directly related to the formation of the Maillard reaction products (Li et al., 2015). Both soil drought and excessive soil moisture can reduce the degraded cembrane products and degraded carotene products in flue-cured tobacco. In addition, cypressane terpenoids are mainly found in the cuticle of tobacco leaves and are the main components of the secretion of tobacco glandular hairs, which are synthesized by the glandular head cells of glandular hairs (Li et al., 2015). The content of degraded cembrane products in Dianxi is significantly higher than that in the flue-cured tobacco growing areas of Dianzhong and Diandong due to the climate in this region, which is mainly in the south and middle subtropics and is the most heat-rich region in Yunnan Province, with abundant rainfall during the growing period of roasted tobacco fields and a high content of tobacco glandular hair secretions (Shi et al., 2011).

Among the different varieties in Figure 2b, the content of degraded carotene products shows the order Yun87 > K326 > Hongda, and the content of the Yun 87 variety is significantly higher by approximately 30.24% than that of Hongda. The degraded product contents of carotene and phenylalanine were in the order of Hongda > K326 > Yun87 and K326 > Hongda > Yun87, respectively, and there were no significant differences among the three varieties. The contents of degraded cembrane products are all shown as Yun87 > K326 > Hongda, and the contents of Yun87 are significantly higher than those of K326 and Hongda. Compared with K326 and Hongda, the degraded cembrane products are higher by approximately 34.80% and 43.92%, respectively. This indicates that the aroma substance of TS is related to the variety characteristics of flue-cured tobacco leaves, while the ecological environment of cultivation is also an important factor for flue-cured tobacco.

Similarly, the content of degraded carotene products is upper > lower > middle among different parts in Figure 2c, and the upper is significantly higher by approximately 60.81% and 49.27% than the middle and lower parts, respectively, while there is no significant difference between the middle and the lower parts. The contents of the Maillard reaction and degraded phenylalanine products are in the order of middle > lower > upper. Both the middle and the lower parts are significantly higher than the upper part, and they are correspondingly higher by approximately 16.71% and 10.22%, respectively. The content of degraded cembrane products is in the order of upper > middle > lower, and there are significant differences among the three parts. The upper part is significantly higher by approximately 18.40% and 56.88% than the middle and the lower parts, respectively, and the middle part is significantly higher by approximately 32.50% than the lower part.

In conclusion, the results are consistent with the analysis of flavour precursors in TS, which have obvious regional characteristics. The contents of most degradation products are higher in Dianxi areas and the upper and middle parts for the volatile aroma components in TS according to the previous analysis. These results imply that the TS of the upper and middle parts can be preferred and classified according to the typical tobacco growing areas in eastern, central and western Yunnan, which is beneficial for improving the use value of TS in the cigarette industry.

Table 4. Analysis of volatile aroma components in TS.

volatile aroma components (μg/g)	growing areas			positions			varieties		
	Dianxi	Dianzhong	Diandong	upper	middle	lower	Hongda	K326	Yun 87
Ketones									
1-penten-3-one	0.67 ± 0.42^{a}	0.63 ± 0.38^{a}	0.49 ± 0.30^{a}	0.70 ± 0.43^{a}	0.50 ± 0.32^{b}	0.78 ± 0.35^{a}	0.62 ± 0.42^{a}	0.63 ± 0.34^{a}	0.54 ± 0.36^{a}
3-hydroxy-2-butanone	0.66 ± 0.19^{a}	0.98 ± 0.33^{b}	0.75 ± 0.25^{a}	0.87 ± 0.41^{a}	0.84 ± 0.25^{a}	0.80 ± 0.32^{a}	0.90 ± 0.33^{a}	0.89 ± 0.35^{a}	0.75 ± 0.24^{a}
2-cyclopentene-1,4-dione①	0.46 ± 0.14^{a}	0.46 ± 0.17^{a}	0.57 ± 0.12^{b}	0.38 ± 0.14^{a}	0.55 ± 0.15^{b}	0.50 ± 0.12^{b}	0.51 ± 0.18^{a}	0.48 ± 0.17^{a}	0.50 ± 0.14^{a}
2-methyl tetrahydro furan-3-one②	0.55 ± 0.23^{a}	0.67 ± 0.22^{b}	0.83 ± 0.22^{c}	0.55 ± 0.24^{a}	0.73 ± 0.23^{b}	0.86 ± 0.14^{c}	0.77 ± 0.20^{a}	0.74 ± 0.20^{ab}	0.60 ± 0.28^{b}
1-(2-furyl)-ethanone②	0.17 ± 0.08^{a}	0.24 ± 0.09^{b}	0.29 ± 0.09^{c}	0.17 ± 0.08^{a}	0.26 ± 0.10^{b}	0.30 ± 0.07^{b}	0.26 ± 0.10^{a}	0.26 ± 0.09^{a}	0.21 ± 0.10^{b}
6-Methyl-5-hepten-2-one	0.15 ± 0.04^{a}	0.09 ± 0.03^{b}	0.08 ± 0.04^{b}	0.14 ± 0.05^{a}	0.09 ± 0.03^{b}	0.08 ± 0.03^{b}	0.08 ± 0.02^{a}	0.09 ± 0.03^{a}	0.13 ± 0.05^{b}
1-(1H-pyrrol-2-yl)-ethanone②	1.18 ± 0.29^{a}	1.47 ± 0.37^{b}	1.84 ± 0.51^{c}	1.18 ± 0.38^{a}	1.68 ± 0.47^{b}	1.55 ± 0.18^{b}	1.67 ± 0.50^{a}	1.50 ± 0.40^{ab}	1.43 ± 0.49^{b}
1-(3-pyridyl)-ethanone②	0.11 ± 0.05^{a}	0.08 ± 0.03^{a}	0.06 ± 0.02^{b}	0.11 ± 0.04^{a}	0.07 ± 0.03^{b}	0.09 ± 0.04^{a}	0.07 ± 0.03^{a}	0.08 ± 0.04^{a}	0.09 ± 0.04^{a}
keto isophorone①	0.76 ± 0.68^{a}	0.41 ± 0.40^{a}	0.21 ± 0.18^{b}	0.71 ± 0.63^{a}	0.25 ± 0.25^{b}	0.57 ± 0.51^{a}	0.35 ± 0.32^{a}	0.47 ± 0.56^{a}	0.43 ± 0.48^{a}
pulegone②	0.05 ± 0.04^{a}	0.05 ± 0.03^{a}	0.07 ± 0.03^{a}	0.05 ± 0.04^{ab}	0.06 ± 0.03^{a}	0.03 ± 0.03^{b}	0.05 ± 0.03^{a}	0.05 ± 0.03^{a}	0.06 ± 0.04^{a}
solanone③	3.61 ± 3.04^{a}	3.18 ± 2.07^{a}	3.40 ± 2.21^{a}	4.37 ± 3.52^{a}	3.23 ± 1.48^{b}	1.71 ± 1.15^{c}	2.51 ± 1.32^{a}	2.66 ± 1.64^{a}	4.52 ± 2.89^{a}
β-damascenone①	2.94 ± 2.83^{a}	1.36 ± 1.04^{a}	0.92 ± 0.42^{b}	2.65 ± 2.59^{a}	1.02 ± 0.65^{b}	1.68 ± 1.30^{b}	1.16 ± 0.70^{a}	1.69 ± 1.64^{a}	1.74 ± 2.12^{a}
β-damascone①	0.55 ± 0.47^{a}	0.30 ± 0.31^{a}	0.16 ± 0.14^{b}	0.48 ± 0.41^{a}	0.19 ± 0.20^{b}	0.49 ± 0.46^{a}	0.28 ± 0.28^{a}	0.31 ± 0.36^{a}	0.33 ± 0.37^{a}
6,10-dimethylundeca-5,9-dien-2-one①	0.16 ± 0.06^{a}	0.12 ± 0.06^{b}	0.10 ± 0.05^{b}	0.18 ± 0.06^{a}	0.10 ± 0.04^{b}	0.10 ± 0.05^{b}	0.10 ± 0.03^{a}	0.11 ± 0.05^{a}	0.15 ± 0.07^{b}
β-ionone①	0.47 ± 0.34^{a}	0.44 ± 0.28^{a}	0.51 ± 0.29^{a}	0.54 ± 0.40^{a}	0.49 ± 0.23^{a}	0.25 ± 0.18^{b}	0.38 ± 0.23^{a}	0.40 ± 0.25^{a}	0.59 ± 0.33^{b}
megastigmatrienone A①	0.21 ± 0.09^{a}	0.16 ± 0.09^{a}	0.12 ± 0.06^{a}	0.23 ± 0.08^{a}	0.12 ± 0.05^{b}	0.17 ± 0.12^{c}	0.15 ± 0.08^{a}	0.14 ± 0.09^{a}	0.17 ± 0.09^{a}
megastigmatrienone B①	0.36 ± 0.23^{a}	0.26 ± 0.15^{a}	0.32 ± 0.12^{a}	0.41 ± 0.23^{a}	0.28 ± 0.10^{b}	0.17 ± 0.09^{c}	0.24 ± 0.13^{a}	0.25 ± 0.11^{a}	0.38 ± 0.19^{b}
megastigmatrienone C①	0.43 ± 0.39^{a}	0.15 ± 0.07^{b}	0.13 ± 0.06^{b}	0.35 ± 0.36^{a}	0.13 ± 0.06^{b}	0.20 ± 0.19^{c}	0.14 ± 0.05^{a}	0.24 ± 0.32^{a}	0.22 ± 0.20^{a}
megastigmatrienone D①	0.42 ± 0.30^{a}	0.34 ± 0.19^{a}	0.44 ± 0.15^{a}	0.49 ± 0.31^{a}	0.38 ± 0.14^{b}	0.22 ± 0.13^{c}	0.32 ± 0.17^{a}	0.34 ± 0.16^{a}	0.48 ± 0.24^{a}
2,3,6-trimethyl-1,4-naphthalenedione	0.16 ± 0.06^{a}	0.12 ± 0.07^{b}	0.09 ± 0.03^{c}	0.14 ± 0.05^{a}	0.11 ± 0.07^{a}	0.11 ± 0.05^{a}	0.10 ± 0.05^{a}	0.14 ± 0.07^{a}	0.12 ± 0.06^{ab}
solanastone③	1.59 ± 0.90^{a}	1.48 ± 0.82^{a}	1.30 ± 0.79^{a}	1.79 ± 0.91^{a}	1.37 ± 0.81^{b}	1.10 ± 0.44^{b}	1.20 ± 0.80^{a}	1.85 ± 0.89^{b}	1.29 ± 0.67^{a}
farnesylacetone A①	0.83 ± 0.18^{a}	0.69 ± 0.20^{b}	0.65 ± 0.23^{b}	0.91 ± 0.16^{a}	0.63 ± 0.18^{b}	0.63 ± 0.20^{b}	0.61 ± 0.14^{a}	0.69 ± 0.24^{ab}	0.79 ± 0.21^{b}
farnesylacetone B①	1.31 ± 0.52^{a}	0.91 ± 0.49^{b}	1.20 ± 0.92^{ab}	1.12 ± 0.54^{a}	1.15 ± 0.77^{a}	0.74 ± 0.30^{a}	1.05 ± 0.73^{a}	1.09 ± 0.84^{a}	1.11 ± 0.46^{a}
total ketones	17.79 ± 3.24^{a}	14.6 ± 3.85^{b}	14.52 ± 3.85^{b}	18.53 ± 3.10^{a}	14.21 ± 2.52^{b}	13.12 ± 3.10^{b}	13.54 ± 2.05^{a}	15.11 ± 3.52^{ab}	16.64 ± 2.05^{b}
Aldehydes									
3-methyl-2-butenal	0.08 ± 0.02^{a}	0.07 ± 0.02^{b}	0.05 ± 0.03^{c}	0.08 ± 0.03^{a}	0.06 ± 0.03^{b}	0.07 ± 0.02^{ab}	0.06 ± 0.03^{a}	0.07 ± 0.03^{a}	0.07 ± 0.03^{a}
hexanal②	0.15 ± 0.04^{a}	0.15 ± 0.05^{a}	0.12 ± 0.03^{b}	0.18 ± 0.05^{a}	0.13 ± 0.03^{b}	0.13 ± 0.02^{b}	0.15 ± 0.05^{a}	0.14 ± 0.04^{a}	0.14 ± 0.04^{a}
furfural②	2.21 ± 0.36^{a}	2.54 ± 0.42^{b}	2.95 ± 0.51^{c}	2.21 ± 0.42^{a}	2.77 ± 0.49^{b}	2.66 ± 0.33^{b}	2.77 ± 0.46^{a}	2.55 ± 0.52^{b}	2.51 ± 0.52^{b}
2-pyridine carboxaldehyde②	0.11 ± 0.03^{a}	0.11 ± 0.02^{a}	0.13 ± 0.02^{b}	0.10 ± 0.02^{a}	0.12 ± 0.02^{b}	0.12 ± 0.02^{b}	0.12 ± 0.03^{a}	0.11 ± 0.02^{a}	0.12 ± 0.02^{a}
benzaldehyde④	0.19 ± 0.05^{a}	0.17 ± 0.03^{b}	0.16 ± 0.02^{b}	0.18 ± 0.03^{a}	0.16 ± 0.03^{b}	0.19 ± 0.03^{a}	0.16 ± 0.03^{a}	0.17 ± 0.03^{ab}	0.18 ± 0.04^{b}
5-methylfurfural②	0.14 ± 0.02^{a}	0.17 ± 0.04^{b}	0.22 ± 0.06^{c}	0.14 ± 0.04^{a}	0.20 ± 0.05^{b}	0.18 ± 0.03^{b}	0.19 ± 0.05^{a}	0.18 ± 0.05^{ab}	0.17 ± 0.05^{b}
2,4-heptadienal A	0.06 ± 0.02^{a}	0.05 ± 0.02^{ab}	0.04 ± 0.02^{b}	0.06 ± 0.02^{a}	0.05 ± 0.02^{b}	0.04 ± 0.01^{b}	0.05 ± 0.02^{ab}	0.04 ± 0.01^{a}	0.06 ± 0.02^{b}

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Table 4 (*continued*)

volatile aroma components (μg/g)	growing areas			positions			varieties		
	Dianxi	Dianzhong	Diandong	upper	middle	lower	Hongda	K326	Yun 87
4-pyridine carboxaldehyde②	0.05 ± 0.01^{a}	0.05 ± 0.02^{a}	0.07 ± 0.02^{b}	0.05 ± 0.01^{a}	0.06 ± 0.02^{a}	0.06 ± 0.02^{a}	0.06 ± 0.02^{a}	0.06 ± 0.02^{a}	0.05 ± 0.02^{a}
2,4-heptadienal B	0.18 ± 0.07^{a}	0.18 ± 0.08^{a}	0.12 ± 0.06^{b}	0.23 ± 0.08^{a}	0.14 ± 0.06^{b}	0.11 ± 0.04^{c}	0.16 ± 0.07^{ab}	0.14 ± 0.05^{a}	0.19 ± 0.09^{b}
phenylacetaldehyde④	4.94 ± 0.65^{a}	5.80 ± 1.11^{b}	6.54 ± 1.85^{c}	4.61 ± 1.40^{a}	6.31 ± 1.16^{b}	6.39 ± 1.02^{b}	6.24 ± 1.31^{a}	6.01 ± 1.09^{a}	5.43 ± 1.67^{b}
Nonanal	0.12 ± 0.05^{a}	0.16 ± 0.06^{b}	0.19 ± 0.04^{c}	0.15 ± 0.06^{a}	0.17 ± 0.05^{b}	0.14 ± 0.07^{a}	0.15 ± 0.05^{a}	0.17 ± 0.06^{a}	0.17 ± 0.05^{a}
2,6-nonadienal	0.10 ± 0.05^{a}	0.12 ± 0.08^{a}	0.07 ± 0.05^{b}	0.13 ± 0.09^{a}	0.08 ± 0.05^{b}	0.12 ± 0.07^{a}	0.10 ± 0.08^{a}	0.10 ± 0.08^{a}	0.09 ± 0.05^{a}
crocetin④	0.24 ± 0.08^{ab}	0.27 ± 0.08^{a}	0.23 ± 0.08^{b}	0.24 ± 0.09^{a}	0.25 ± 0.08^{a}	0.25 ± 0.10^{a}	0.26 ± 0.09^{a}	0.26 ± 0.09^{a}	0.23 ± 0.07^{a}
fourteen aldehyde	0.17 ± 0.17^{a}	0.27 ± 0.21^{b}	0.26 ± 0.12^{b}	0.20 ± 0.18^{a}	0.28 ± 0.17^{b}	0.15 ± 0.16^{a}	0.21 ± 0.16^{a}	0.28 ± 0.20^{a}	0.24 ± 0.17^{a}
total aldehydes	8.76 ± 0.94^{a}	10.09 ± 2.15^{b}	11.15 ± 2.15^{c}	8.55 ± 1.67^{a}	10.78 ± 1.50^{b}	10.61 ± 1.67^{b}	10.66 ± 1.60^{a}	10.26 ± 1.55^{a}	9.65 ± 1.60^{b}
Alcohols									
3-methyl-1-butanol	0.18 ± 0.06^{a}	0.17 ± 0.04^{b}	0.19 ± 0.05^{a}	0.18 ± 0.05^{a}	0.18 ± 0.05^{a}	0.19 ± 0.06^{a}	0.17 ± 0.04^{a}	0.19 ± 0.05^{a}	0.17 ± 0.05^{a}
furfuryl alcohol②	0.46 ± 0.08^{a}	0.50 ± 0.07^{b}	0.63 ± 0.10^{c}	0.50 ± 0.07^{a}	0.55 ± 0.12^{b}	0.51 ± 0.05^{a}	0.54 ± 0.12^{a}	0.53 ± 0.09^{a}	0.53 ± 0.11^{a}
benzyl alcohol④	1.89 ± 0.57^{a}	1.24 ± 0.32^{b}	1.15 ± 0.48^{b}	1.65 ± 0.57^{a}	1.23 ± 0.43^{b}	1.26 ± 0.52^{b}	1.08 ± 0.34^{a}	1.28 ± 0.49^{b}	1.61 ± 0.54^{c}
Linalool①	0.09 ± 0.04^{a}	0.07 ± 0.02^{b}	0.07 ± 0.01^{b}	0.08 ± 0.04^{a}	0.07 ± 0.01^{a}	0.08 ± 0.03^{a}	0.07 ± 0.02^{a}	0.08 ± 0.03^{a}	0.08 ± 0.02^{a}
phenylethanol④	0.75 ± 0.68^{a}	0.78 ± 0.47^{a}	0.87 ± 0.41^{b}	0.85 ± 0.68^{a}	0.85 ± 0.39^{a}	0.47 ± 0.43^{b}	0.64 ± 0.40^{a}	0.76 ± 0.50^{ab}	0.95 ± 0.53^{b}
3-oxo-α-ionol①	0.37 ± 0.48^{a}	0.08 ± 0.14^{b}	0.02 ± 0.09^{b}	0.28 ± 0.44^{a}	0.04 ± 0.11^{b}	0.19 ± 0.25^{a}	0.07 ± 0.12^{a}	0.16 ± 0.34^{a}	0.13 ± 0.31^{a}
Inbetol	7.30 ± 2.51^{a}	5.48 ± 2.37^{b}	5.13 ± 1.84^{b}	4.67 ± 1.32^{a}	6.09 ± 2.44^{b}	6.55 ± 3.10^{b}	5.75 ± 2.60^{ab}	5.11 ± 2.03^{a}	6.32 ± 2.39^{b}
Phytol	6.75 ± 1.89^{a}	4.69 ± 1.62^{b}	5.17 ± 2.09^{b}	4.91 ± 1.51^{a}	5.49 ± 2.10^{a}	5.14 ± 2.35^{a}	5.02 ± 2.10^{ab}	4.88 ± 2.18^{a}	5.81 ± 1.63^{b}
cembratriene-diol③	6.05 ± 1.51^{a}	3.25 ± 1.54^{b}	3.23 ± 1.83^{b}	3.88 ± 1.63^{a}	3.89 ± 2.15^{a}	3.59 ± 2.00^{a}	3.53 ± 2.10^{a}	3.22 ± 1.91^{a}	4.61 ± 1.74^{b}
total alcohol	23.83 ± 5.30^{a}	16.26 ± 6.00^{b}	16.46 ± 6.00^{b}	17.00 ± 4.73^{a}	18.38 ± 6.68^{a}	17.97 ± 4.73^{a}	16.87 ± 6.68^{a}	16.21 ± 6.43^{a}	20.21 ± 6.68^{b}
Esters									
butyrolactone	0.40 ± 0.12^{a}	0.46 ± 0.10^{b}	0.47 ± 0.11^{b}	0.39 ± 0.07^{a}	0.46 ± 0.11^{b}	0.52 ± 0.08^{c}	0.48 ± 0.12^{a}	0.44 ± 0.07^{b}	0.44 ± 0.12^{b}
dihydro actinidiolide①	0.24 ± 0.21^{a}	0.27 ± 0.19^{a}	0.35 ± 0.13^{b}	0.33 ± 0.24^{a}	0.30 ± 0.14^{a}	0.14 ± 0.13^{b}	0.28 ± 0.17^{ab}	0.24 ± 0.17^{a}	0.34 ± 0.18^{b}
methyl myristate	0.24 ± 0.16^{a}	0.20 ± 0.16^{a}	0.13 ± 0.07^{b}	0.26 ± 0.17^{a}	0.14 ± 0.08^{b}	0.27 ± 0.19^{a}	0.19 ± 0.15^{a}	0.21 ± 0.15^{a}	0.17 ± 0.11^{a}
dibutyl phthalate④	0.82 ± 0.24^{a}	0.97 ± 0.50^{a}	0.81 ± 0.28^{a}	0.94 ± 0.50^{ab}	0.82 ± 0.32^{a}	1.05 ± 0.40^{b}	0.97 ± 0.49^{a}	0.96 ± 0.43^{a}	0.77 ± 0.23^{b}
methyl palmitate	3.04 ± 0.84^{a}	1.82 ± 0.46^{b}	1.88 ± 0.51^{b}	2.44 ± 0.99^{a}	2.02 ± 0.56^{b}	1.77 ± 0.74^{b}	1.67 ± 0.47^{a}	2.10 ± 0.85^{b}	2.42 ± 0.68^{c}
ethyl palmitate	3.22 ± 2.45^{a}	2.06 ± 1.01^{b}	2.00 ± 1.06^{b}	1.67 ± 0.32^{a}	2.46 ± 1.26^{b}	2.80 ± 3.08^{b}	2.11 ± 1.12^{ab}	1.98 ± 1.01^{a}	2.69 ± 1.99^{b}
methyl linolenate	20.66 ± 3.89^{a}	13.15 ± 3.54^{b}	13.59 ± 4.07^{b}	13.92 ± 4.47^{a}	15.19 ± 4.67^{a}	15.66 ± 6.16^{a}	13.61 ± 4.09^{a}	13.49 ± 4.5^{a}	17.04 ± 4.9^{b}
total esters	28.62 ± 6.42^{a}	18.94 ± 5.53^{b}	19.24 ± 5.53^{b}	19.95 ± 5.34^{a}	21.40 ± 6.24^{a}	22.21 ± 5.34^{a}	19.30 ± 5.30^{a}	19.42 ± 5.98^{a}	23.86 ± 5.30^{b}
Acids									
2-methylbutyric acid	0.39 ± 0.10^{a}	0.29 ± 0.05^{b}	0.29 ± 0.08^{b}	0.33 ± 0.12^{a}	0.30 ± 0.06^{a}	0.32 ± 0.07^{b}	0.28 ± 0.04^{a}	0.32 ± 0.09^{b}	0.33 ± 0.09^{b}
palmitic acid	0.98 ± 0.43^{a}	1.04 ± 1.67^{a}	1.10 ± 1.70^{a}	0.67 ± 0.19^{a}	1.28 ± 1.91^{a}	0.78 ± 0.29^{a}	1.21 ± 2.07^{a}	0.90 ± 1.09^{a}	1.04 ± 1.30^{a}
furoic acid②	0.26 ± 0.08^{a}	0.26 ± 0.09^{a}	0.38 ± 0.11^{b}	0.22 ± 0.06^{a}	0.34 ± 0.11^{b}	0.27 ± 0.04^{c}	0.31 ± 0.12^{a}	0.30 ± 0.10^{a}	0.29 ± 0.10^{a}
myristic acid	0.04 ± 0.01^{a}	0.04 ± 0.02^{a}	0.05 ± 0.02^{a}	0.06 ± 0.02^{a}	0.04 ± 0.01^{b}	0.04 ± 0.02^{b}	0.04 ± 0.01^{a}	0.05 ± 0.02^{a}	0.05 ± 0.02^{a}
total acid	1.67 ± 0.45^{a}	1.63 ± 1.77^{a}	1.82 ± 1.77^{a}	1.28 ± 0.29^{a}	1.96 ± 1.96^{a}	1.41 ± 0.29^{a}	1.84 ± 2.13^{a}	1.57 ± 1.15^{b}	1.71 ± 2.13^{a}

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Table 4 (*continued*)

volatile aroma components (μg/g)	growing areas			positions			varieties		
	Dianxi	Dianzhong	Diandong	upper	middle	lower	Hongda	K326	Yun 87
Phenols									
2-methoxy-phenol	0.17 ± 0.08^{a}	0.13 ± 0.10^{b}	0.10 ± 0.04^{c}	0.17 ± 0.12^{a}	0.10 ± 0.05^{b}	0.16 ± 0.09^{a}	0.12 ± 0.08^{a}	0.13 ± 0.10^{a}	0.13 ± 0.07^{a}
benzo[b]thiophene②	0.15 ± 0.16^{a}	0.22 ± 0.16^{b}	0.34 ± 0.13^{c}	0.18 ± 0.15^{a}	0.28 ± 0.15^{b}	0.19 ± 0.23^{a}	0.21 ± 0.16^{a}	0.26 ± 0.18^{a}	0.26 ± 0.17^{a}
2-methoxy-4-vinylphenol④	0.45 ± 0.29^{a}	0.41 ± 0.22^{a}	0.47 ± 0.15^{a}	0.46 ± 0.29^{a}	0.47 ± 0.18^{a}	0.28 ± 0.18^{b}	0.41 ± 0.23^{a}	0.39 ± 0.19^{a}	0.51 ± 0.23^{b}
total phenols	0.78 ± 0.37^{a}	0.76 ± 0.19^{a}	0.91 ± 0.19^{b}	0.81 ± 0.33^{a}	0.85 ± 0.24^{a}	0.63 ± 0.33^{b}	0.74 ± 0.28^{a}	0.78 ± 0.26^{a}	0.89 ± 0.28^{b}
Hterocyclics									
pyridine②	0.44 ± 0.08^{a}	0.57 ± 0.13^{b}	0.55 ± 0.10^{b}	0.54 ± 0.12^{a}	0.55 ± 0.13^{a}	0.46 ± 0.05^{b}	0.56 ± 0.12^{a}	0.56 ± 0.11^{a}	0.50 ± 0.12^{b}
2, 3-dihydrobenzofuran②	0.21 ± 0.07^{a}	0.20 ± 0.07^{a}	0.16 ± 0.05^{b}	0.23 ± 0.08^{a}	0.17 ± 0.05^{b}	0.22 ± 0.07^{a}	0.19 ± 0.08^{a}	0.20 ± 0.07^{a}	0.19 ± 0.06^{a}
indole④	0.10 ± 0.09^{a}	0.10 ± 0.07^{a}	0.12 ± 0.04^{a}	0.11 ± 0.09^{a}	0.12 ± 0.05^{a}	0.06 ± 0.06^{b}	0.10 ± 0.06^{a}	0.09 ± 0.06^{a}	0.13 ± 0.07^{b}
butylated hydroxytoluene④	0.33 ± 0.40^{a}	0.12 ± 0.22^{b}	0.02 ± 0.11^{c}	0.26 ± 0.37^{a}	0.05 ± 0.15^{b}	0.25 ± 0.31^{a}	0.10 ± 0.18^{a}	0.16 ± 0.28^{a}	0.14 ± 0.31^{a}
2,3-bipyridine②	0.03 ± 0.03^{a}	0.03 ± 0.02^{a}	0.04 ± 0.02^{a}	0.03 ± 0.02^{a}	0.04 ± 0.02^{b}	0.02 ± 0.02^{a}	0.03 ± 0.02^{a}	0.03 ± 0.02^{a}	0.04 ± 0.02^{a}
Anthracene	0.23 ± 0.08^{a}	0.12 ± 0.03^{b}	0.10 ± 0.04^{b}	0.16 ± 0.06^{a}	0.12 ± 0.05^{b}	0.16 ± 0.12^{a}	0.11 ± 0.03^{a}	0.12 ± 0.05^{a}	0.17 ± 0.08^{b}
total amount of heterocycles	1.34 ± 0.41^{a}	1.14 ± 0.21^{b}	1.00 ± 0.21^{c}	1.33 ± 0.36^{a}	1.04 ± 0.20^{b}	1.17 ± 0.36^{ab}	1.09 ± 0.24^{a}	1.15 ± 0.31^{a}	1.16 ± 0.24^{a}
Olefins									
neophytadiend	29.79 ± 6.30^{a}	20.49 ± 7.17^{b}	23.85 ± 7.55^{b}	21.07 ± 7.11^{a}	21.39 ± 8.05^{a}	27.19 ± 7.10^{b}	24.97 ± 8.36^{a}	23.06 ± 7.54^{a}	22.73 ± 8.79^{a}
Total aroma components	112.57 ± 16.38^{a}	83.91 ± 18.43^{b}	88.95 ± 19.98^{b}	85.11 ± 20.74^{a}	85.88 ± 21.93^{a}	101.31 ± 18.36^{b}	92.41 ± 19.08^{a}	91.69 ± 21.57^{a}	89.85 ± 27.03^{a}

Notes: Table values are the measured mean ± standard deviation. The uppercase letters "a, b and c" stand for the significant differences between samples (Tukey's LSD test, $p < 0.05$). ①, ②, ③, and ④ denotes the products of degraded carotene, Maillard reaction, degraded cembrane and degraded phenylalanine, respectively.

3.2. Analysis of affected factors

The flavour precursors in tobacco are essential and directly influence the chemical quality and appearance of cigarettes. The content of flavour precursors varies among different growing areas, parts and varieties, reflecting that the flavour precursors are affected by the factors of ecological conditions and cultivation techniques in different tobacco-planting areas on flue-cured tobacco (Yang et al., 2005).

The flavour precursors will undergo a series of complex chemical reactions, degrading and transforming into a large number of volatile aroma compounds in the process of maturation, alcoholization and combustion. These reactions are beneficial for enhancing the aroma richness of cigarettes and improving the balance and coordination of smoke during the smoking process. Therefore, the presence of β-carotene, lutein, chlorogenic acid, scopoletin and rutin in TS will also affect the aroma and colour of tobacco and thus the smoking quality of expanded cut stems. β-Carotene and lutein can degrade into dihydrokiwilactone (dihydroactinidiolide), β-violanone, geranyl acetone, macrotrienone, β-dihydrosemarone, β-semarone and other important volatile flavour components that affect the style characteristics of tobacco (Hu et al., 2007). The higher their content is, the more prominent the clean aroma type of flue-cured tobacco (Zhao et al., 2007). A variety of volatile aroma components that degrade due to polyphenols have important effects on physiological and biochemical activities, colour, fragrance and taste, and physiological strength (Li et al., 2008).

3.2.1. Factors of growing areas

Our previous analysis showed that the contents of most flavour precursors in TS gradually decreased from west to the east among different tobacco-growing areas in Yunnan, which were significantly higher in Dianxi than in Dianzhong and Diandong areas. These differences can be attributed to the differences in the growing areas. The contents of polyphenols, plastid pigments and their degradation products were significantly affected by light (Yang et al., 2007; Wen et al., 2002). Meanwhile, the pigment contents can also increase with increasing temperature and rainfall during the tobacco mature period (Zhang et al., 2006; Li et al., 2008). However, excessive flooding will reduce the chlorophyll content in tobacco and even damage the structure, resulting in a decrease in the neophytadiene content (Li et al., 2015). Previous studies also showed that both dry periods and long-term soil drought were beneficial for improving the contents of plastid pigments during the tobacco maturity stage (Li et al., 2015).

Flavonoids, e.g., chlorogenic acid, are "ultraviolet filters" for terrestrial plants that can protect plants from damage by ultraviolet radiation (Wen et al., 2002). While the proportion of ultraviolet radiation in the total solar radiation is particular (Li et al., 2015), the increase in the number of sunshine duration and radiation will lead to an increase in ultraviolet radiation, which in turn leads to an increase in the total amount of polyphenols in tobacco with the increase in light intensity and duration (Li et al., 2015). As a whole, the factors of light power, temperature and ultraviolet radiation gradually decrease from west to the east in Yunnan Province. This climate phenomenon is not suitable for the accumulation and degradation of pigments and polyphenols in eastern tobacco. This results in the contents of most flavour precursors being higher in the western areas and lower in the eastern part.

In addition, the contents of plastid pigments and polyphenols were also affected by altitude, fertilizer, soil type, cultivation technology and the variety of flue-cured tobacco. Correspondingly, these factors also affect the contents of the degraded products of the Maillard reaction, phenylalanine and the cembrane. Rainfall has the most significant impact on the content of degraded cembrane products in tobacco in the mature period, while excessive flooding or drought will inhibit the degraded

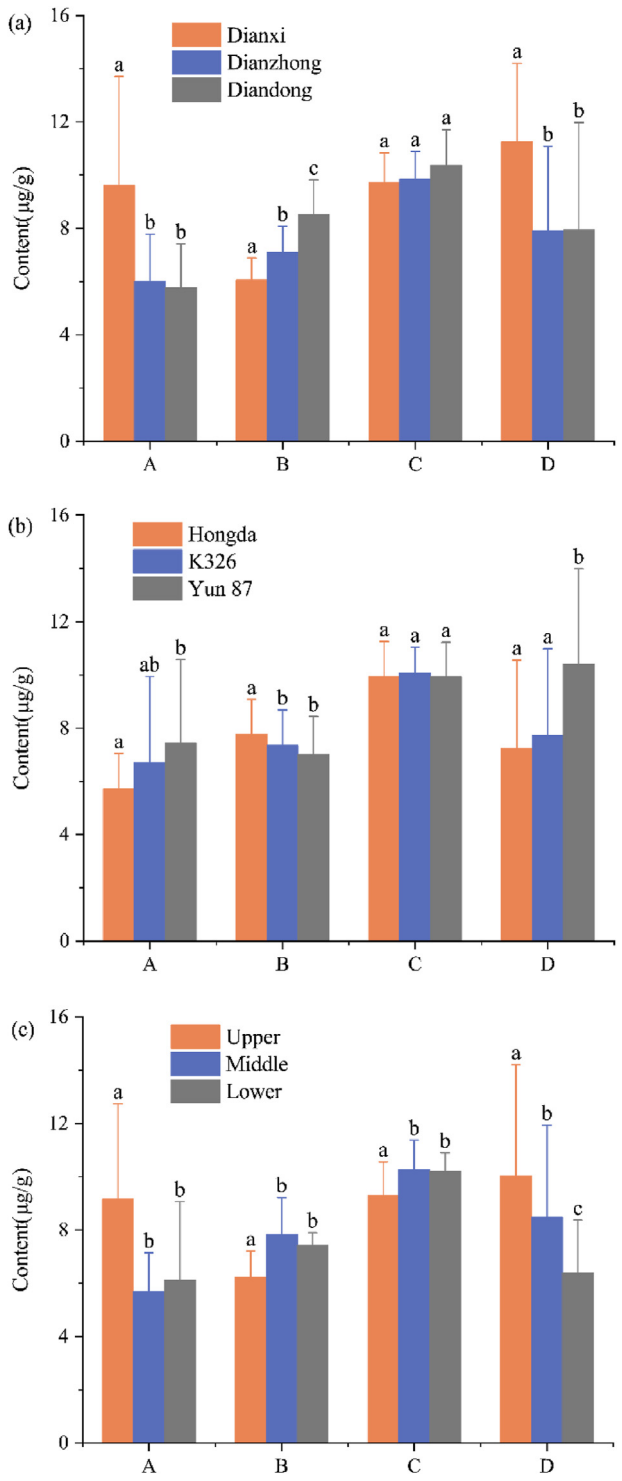

Figure 2. Degradation products of volatile aroma components in TS (A: degraded carotene products, B: Maillard reaction products, C: degraded phenylalanine products, D: degraded cembrane products; a: growing areas, b: varieties, c: parts). Different letters indicate significant differences at $p < 0.05$.

cembrane products (Li et al., 2015). The relative humidity, cloud cover and average temperature are the major climatic factors that affect the Maillard reaction products during the growth period of tobacco (Li et al., 2015). Short-term drought is beneficial to the accumulation of Maillard reaction products during the maturity period of tobacco, while drought is detrimental to the accumulation of Maillard reaction products during the mature period (Li et al., 2009). These results are mainly attributed to the conditions of complex geology and unique climate in Yunnan Province, resulting in the degraded product contents of the Maillard reaction and phenylalanine being the highest in Diandong and the lowest in Dianxi. These differences have also formed the unique style of different tobacco growing areas, further demonstrating the superiority of tobacco materials in Yunnan.

3.2.2. Factors of different parts

Our analysis showed that the contents of flavour precursors and degraded carotene products in TS among different parts were significantly higher than those of the lower and middle parts. The contents of lutein, β-carotene, polyphenols and degraded carotene products were the highest in the upper part, which are similar to those of tobacco leaves (Xi et al., 2011). These results are mainly due to the proportion of upper tobacco leaves that are approximately 40% of the whole plant. The part of upper leaves is greater with sufficient sun, which is beneficial to the accumulation of more small molecular compounds by photosynthesis, resulting in higher plastid pigments and degradation products in the upper part than those in middle and lower parts of tobacco leaves.

In addition, the secretion of glandular hairs in tobacco can promote the synthesis of aroma components on the surface of tobacco leaves. The number of glandular hairs is in the order of upper leaves > middle leaves > lower leaves, which correspondingly increases with increasing elevation (Zhao et al., 2010). Therefore, the volatile aroma components and their most degradation products were highest in the Dianxi area and upper part. The degraded-product contents of the Maillard reaction and phenylalanine are the highest in the middle part and the lowest in the upper part, mainly due to the influence of maturity, relative humidity and average temperature during tobacco maturity.

3.2.3. Factors of different varieties

The contents of flavour precursors and volatile aroma components in the Yun 87 variety were the highest among the different types. The reason is that the aroma substances in flue-cured tobacco depend not only on the cultivation technology and climatic conditions but also on the variety's genetic characteristics (Zhu et al., 2017). Previous studies have shown that the aroma components had large differences among different flue-cured tobacco varieties, indicating that the effects of the additive and nonadditive in flue-cured tobacco genes together determine the contents of flavour substances, and the widespread heritability was high (Liu et al., 2007).

Based on the comparative analysis of flavour precursors and volatile aroma components in TS from typical tobacco areas in Yunnan, we also found that these components have some differences among different growing areas, varieties and parts. Their variation rules of Diandong, Dianzhong, Dianxi, and the corresponding parts in TS were consistent with the quality characteristics of tobacco leaves. They show that the contents of flavour precursors and volatile aroma components were higher in the middle and upper TS. Therefore, the middle and upper TS can be classified according to the typical tobacco growing areas in eastern, central and western Yunnan, which have a better significance for promoting the comprehensive utilization of TS and the compatibility of cigarette raw materials.

3.3. Application evaluation

The flavour components of tobacco ingredients have a significant contribution to cigarette products to some degree (Qi et al., 2022). Generally, sensory evaluation is an important means of assessing the quality of tobacco leaves and tobacco products (Ashley et al., 2012; Phat et al., 2016; Chen et al., 2021a). Fig. 3a, b illustrates the influences of five different TS on the sensory quality of cigarette products. As shown in Fig. 3a, b, the sensory scores were 89.33, 87.67, 88.33, 86.83 and 86.17 for different experimental cigarettes with TS of the Dali upper and middle parts, Kunming upper parts, Honghe middle parts and Qujing middle parts, respectively. The experimental cigarette product containing the

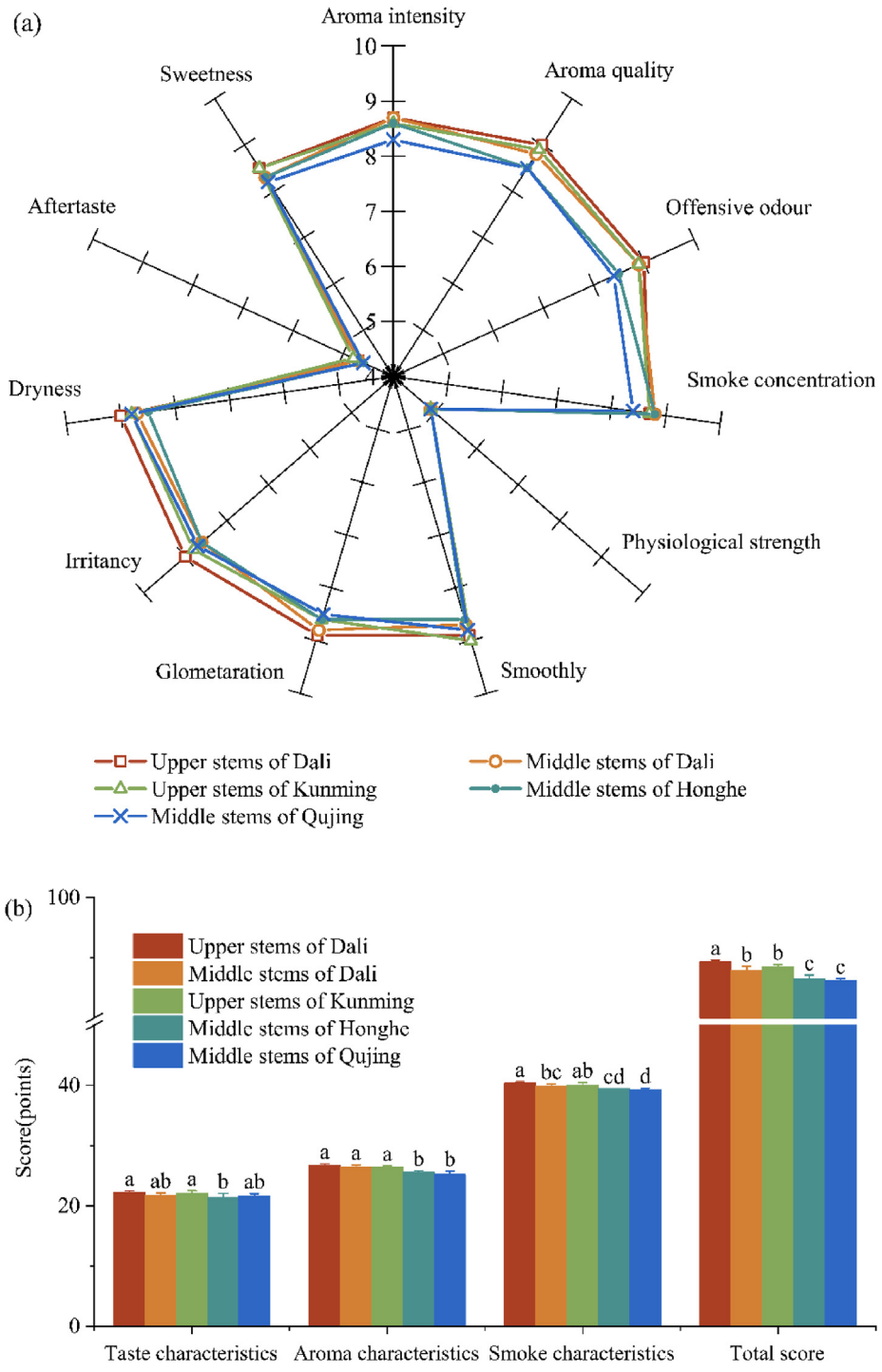

Figure 3. Application evaluation of sensory quality for TS added into cigarettes. (a: Sensory quality profile; b: Sensory characteristics histogram). Different letters indicate significant differences at $p < 0.05$.

upper stems of Dali had sufficient aroma quality and aroma intensity, smooth aroma, obvious sweetness, less irritancy and offensive odour. Therefore, this product has better sensory quality and coordination performance with a significantly higher sensory score of 89.33 than the others ($P < 0.05$). Similarly, the experimental cigarette products containing middle stems of Dali and Kunming also had sufficient aroma quality, aroma intensity and smoke concentration, less offensive odour, but poor glometaration, and more irritancy and dryness, so that the aroma characteristics were moderate, and the smoke characteristics were slightly worse. The experimental cigarette products containing middle stems of Qujing and Honghe had insufficient aroma quality and aroma intensity, obvious dryness, irritancy and offensive odour. As a result, the aroma characteristics and taste characteristics were slightly worse, and the total scores of sensory evaluation were significantly lower than other samples ($P < 0.05$).

The above sensory quality is mainly attributed to the difference in aroma components of different TS. Previous studies have shown that the contents of chlorogenic acid are positively correlated with aroma quality, aroma intensity, irritation and volatility, and a higher content of chlorogenic acid is beneficial to improve the sensory quality of cigarette products (Chen et al., 2021b). The content of aldehydes was negatively correlated with the total score of sensory quality, while the content of

olefins was positively correlated with the total sensory score (Chen et al., 2021b). Our research found that polyphenols, pigments, neophytadiend content and total aroma substances were significantly higher in Dianxi than in Dianzhong and Diandong, and the aldehydes were lower than in Dianzhong and Diandong. The polyphenol and pigment contents of the upper stems were higher than those of the middle and lower parts, and the aldehydes were lower than those of the middle and lower parts. Therefore, the sensory quality of experimental cigarettes containing TS of Dali had better aroma quality and aroma intensity, and the qualities of aroma, smoke and taste characteristics were better overall.

Furthermore, the sensory qualities of the experimental cigarettes containing TS from Kunming and Honghe were much better than those of Qujing overall. The main reason is also related to the difference in aroma components in TS. Our previous analysis suggested that the polyphenols were slightly higher in Dianzhong than in Diandong, and aldehydes were slightly lower than in Diandong. The content of aldehydes was significantly higher in Diandong than in Dianxi and Dianzhong, while the total amount of olefins and aromatic substances was slightly higher than in Dianzhong. As a result, the sensory qualities are much better for TS of Dianzhong than for those of Diandong.

Based on the above analysis, our study indicated that the higher flavour components in TS can also provide a contribution to improving the quality of cigarettes. Meanwhile, there are obvious regional characteristics of aroma components and sensory quality of TS in Yunnan. Overall, the use of middle and upper TS is preferred and used to classify according to the typical tobacco growing areas in eastern, central and western Yunnan. This is beneficial to promote the comprehensive utilization of TS and the compatibility of cigarette formula materials.

4. Conclusion

The value-added utilization of WTS has important scientific significance and social value. As a special smoking product, tobacco ingredients have many complex flavour components, which directly determine the quality characteristics of products. In this work, based on HPLC and GC–MS analyses, we systematically studied the differences in flavour precursors and volatile aroma components in WTS from typical tobacco-growing areas in Yunnan Province. The results show that the contents of flavour precursors, carotenoid and hexane degradation products, neophydiene, and total volatile aroma substances in TS are the highest in the Dianxi area, Yun 87 variety and upper part, while the contents of Maillard reaction products and phenylalanine degradation products are the highest in the Diandong area. The distribution patterns of flavour precursors and volatile aroma components of TS are similar to the quality characteristics of tobacco leaves. The products have better sensory quality and coordination performance for the Dianxi and Dianzhong areas. Overall, we concluded that the aroma components and sensory quality of TS have obvious regional characteristics in Yunnan. The higher flavour components can provide a better contribution to improving cigarette quality accordingly. The use of middle and upper TS should be preferred and classified to process according to the typical tobacco growing areas in eastern, central and western Yunnan, which has provided a theoretical reference for the industrial applicability of TS in flavour extraction and cigarette preparation.

Declarations

Author contribution statement

Xing Fan: Analyzed and interpreted the data; Wrote the paper.

Wenhua Zi: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Jincheng Ao, Boyu Li: Performed the experiments

Junfeng Qiao, Yong Wang, Yonghong Nong: Analyzed and interpreted the data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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