RESEARCH ARTICLE

Analysis of the relationship between carotenoid degradation product and quality of different parts of flue-cured tobacco

Xiaodong Gu[†], Si-ang Chen[†], Bing Liu, Yanxu Guo, Dong Zhou, Wu Wen, Minchang Liu, Junxi Wang, Xin Zheng, Zongze Geng, Wei Du, Yaping Ma, Jie Yang, Rongya Zhang[*], Yuxiang Deng[*]

Technology Center, China Tobacco Sichuan Industrial Co., Ltd., Chengdu, Sichuan, China.

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Carotenoid degradation products significantly influence the smoking quality of flue-cured tobacco. However, the relationship between these degradation products and the intrinsic quality of tobacco leaves remains unclear. This study investigated carotenoid degradation products in 167 aged tobacco leaf samples from different parts and provinces of China, using descriptive statistics, simple correlation analysis, regression analysis, canonical correlation analysis, and principal component analysis to explore their relationship with quality indicators. The results showed that the average content of carotenoid degradation products was the highest in upper leaves (66.59 μg/g) and the lowest in lower leaves (55.72 μg/g) with significant differences ($P < 0.05$). These degradation products were associated with conventional chemical components (e.g., positively correlated with potassium, and negatively correlated with reducing sugars and total sugars) as well as sensory evaluation indicators. Upper and lower leaves' degradation products positively correlated with smoke characteristics, while middle leaves' degradation products negatively correlated with taste characteristics. Canonical correlation analysis highlighted the impact of specific degradation products on aroma quality and strength. Principal component analysis indicated that these products significantly influenced taste characteristics across different leaf parts. This study provided a scientific basis for optimizing the formula ratio in digital module threshing-aided design for cigarette industrial enterprises.

Keywords: flue-cured tobacco; carotenoid; degradation product; chemical quality; sensory evaluation.

[*]**Corresponding authors:** Rongya Zhang and Yuxiang Deng, Technology Center, China Tobacco Sichuan Industrial Co., Ltd., Section 2, Chenglong Avenue, Longquanyi District, Chengdu, Sichuan 610100, China. Email: zhangry@sctobacco.com (Zhang R), yuxiangdeng@foxmail.com (Deng Y).

[†]These authors contributed equally to this work.

Introduction

Carotenoids are ubiquitously distributed in plant tissues [1, 2]. Carotenoid degradation products are also the core constituents of neutral aroma compounds in flue-cured tobacco, and their abundance and profile directly determine the leaf's aromatic quality and industrial usability [3]. These metabolites constitute the primary chemical determinants of floral, fruity, and sweet sensory attributes in plant-derived matrices [4, 5]. During post-harvest processing, these tetraterpenoid pigments are highly susceptible to the synergistic action of thermal, oxidative, and enzymatic stimuli, which trigger a cascade of structural cleavages. Consequently, a spectrum of low-odor-threshold C13-norisoprenoid volatiles including β-ionone, β-damascone, and

dihydroactinidiolide are produced [6, 7]. During leaf maturation, curing, aging, and combustion, carotenoids are cleaved into more than eighty isoprenoid aroma compounds whose structures depend on the precise sites of bond scission [8-10]. These products not only underpin the characteristic floral, fruity, and sweet base notes of flue-cured tobacco but also markedly influence smoke mellow-ness and aftertaste comfort [11, 12]. Among these, megastigmatrienone, dihydroactinidiolide, and β-damascone significantly enhance aroma quality and volume, whereas β-dihydrodamascone, geranylacetone, and β-cyclocitral exert pronounced negative effects [13, 14].

Previous studies have demonstrated that, when the carotenoid content in cured tobacco leaves is maintained within the optimal range of 30 – 40 mg per 100 g dry weight, the abundance of their degradation products exhibits a significant positive correlation with aroma volume [15, 16]. Moreover, employing low-temperature slow curing and other precisely controlled curing techniques can selectively enhance the proportion of beneficial degradation products, thereby holistically improving the intrinsic quality of the tobacco leaves [17, 18]. Nevertheless, existing investigations have predominantly adopted the "whole-plant leaf" scale, thereby overlooking the substantial inter-position variations in baseline carotenoid content and the corresponding degradation-product spectra [19, 20], even though positional effects constitute a pivotal determinant of industrial utilization preferences.

This research aimed to provide a theoretical basis for stalk-specific leaf selection and formulation optimization in cigarette manufacturing by using flue-cured tobacco samples collected from twelve major producing provinces of China after 12 months of aging. The abundance of carotenoid-derived volatiles in upper, middle, and lower stalk positions was systematically quantified, and these data with both chemical quality indices and sensory scores were integrated. Multiple statistical approaches including Pearson correlation, stepwise regression, canonical correlation, and principal component analysis were applied to quantitatively elucidate how stalk position modulated the "carotenoid degradation products — leaf quality" nexus. This research provided the potential to enhance the understanding of how different leaf positions affected carotenoid degradation and, consequently, the sensory and chemical quality of tobacco leaves, which could contribute to more targeted and efficient tobacco processing and product development strategies.

Materials and methods

Experimental materials

A total of 167 samples of aged tobacco leaves from different positions sourced from 12 provinces of China including Yunnan, Guangdong, Jiangxi, Sichuan, Hunan, Fujian, Heilongjiang, Guizhou, Chongqing, Inner Mongolia, Henan, and Shanxi were provided by Sichuan China National Tobacco Corporation (Chengdu, Sichuan, China). Among them, there were 52 upper leaf samples, 83 middle leaf samples, and 32 lower leaf samples.

Determination of conventional chemical components in tobacco leaves

The Antaris II model of a Fourier Transform Near-Infrared (FT-NIR) analysis system (Nicolet Instrument Corp., Madison, Wisconsin, USA) was used to determine the contents of total alkaloids, reducing sugars, total sugars, potassium, chlorine, and total nitrogen in tobacco leaves based on the near-infrared analysis system platform for tobacco developed by the Zhengzhou Tobacco Research Institute. Briefly, after warming up the FT-NIR system for at least 30 minutes, performing system self-checks, and setting instrument parameters with a scanning range of 10,000/cm to 4,000/cm and a resolution of 8/cm, samples were allowed to stabilize at room temperature for over 2 hours before spectral collection. A background spectrum was collected initially and every 20 minutes

thereafter. Tobacco samples were prepared by pressing them into a sampling cup and collecting near-infrared spectra. For chemical component prediction, one spectrum was collected, while for quality style prediction or model building, two spectra were collected with a required 99.99% consistency match. The sampling cup was cleaned after each analysis.

Determination of carotenoid degradation products in tobacco leaves

An Agilent 8890-5977B gas chromatography–mass spectrometer (GC-MS) (Agilent Technologies, Santa Clara, California, USA) was utilized for the determination of carotenoid degradation products. The tobacco samples were initially destemmed, shredded, and dried at 40°C until they could be easily crushed by hand. The dried samples were then ground and passed through a 40-mesh sieve with the resulting powder stored in sealed bags for analysis. The GC-MS analysis involved a detailed sample preparation process where 2 g of tobacco powder was extracted with 7.5 mL of dichloromethane solution and 200 µL of an internal standard solution (1 mg/mL benzyl acetate in dichloromethane). After sonication and filtration, the GC-MS was set with a HP-5MS capillary column and operated under an injection volume of 1 µL at 290 ℃, a carrier gas flow rate of 1.5 mL/min, and a temperature program starting at 60 ℃ and ramping to 290℃. The mass spectrometer was set to scan from 29 to 400 amu with a solvent delay of 6 minutes, ensuring precise detection of carotenoid degradation products.

Determination of sensory evaluation indicators

1 g of the sample was destemmed and cut into shreds with a width of 0.8 mm. The shredded tobacco was then conditioned for 48 hours in a Binder KBF 720 temperature- and humidity-controlled chamber (Binder Inc., Bohemia, NY, USA) at 22 ± 1°C and 60 ± 2% relative humidity to equilibrate moisture content. The cigarettes rolled from the conditioned tobacco were stored in a refrigerator at -6°C for subsequent sensory evaluation. The sensory evaluation was conducted by professional assessors from Sichuan China National Tobacco Corporation following the basic requirements for single-component tobacco evaluation and the sensory quality evaluation standard (YC/T 138—1998). The scores for each sensory evaluation indicator were recorded as the average value.

Data statistical analysis

SPSS version 24.0 (IBM, Armonk, New York, USA) was employed in this research for statistical data analysis with *P* value less than 0.05 as statistically significant difference and *P* value less than 0.01 as highly significant difference.

Results and discussion

Descriptive statistical analysis of carotenoid degradation products in flue-cured tobacco from different parts

The content of carotenoid degr

adation products in tobacco leaves from different positions exhibited significant or highly significant differences. Among them, the average content of macrocyclic trione B was the highest, while the average content of β-ionone was the lowest. The coefficient of variation (CV) for geranyl acetone content in middle leaves was the highest (57.14%), indicating a large degree of variation. The CVs for geranyl acetone content in upper and lower leaves were 50.49% and 34.70%, respectively. In upper leaves, the CV for dihydroactinidiolide content was the highest at 57.12%, while the CV for β-damascenone content was the lowest at 22.62%. The CV for dihydroactinidiolone content in middle leaves was the lowest at 23.21%. In lower leaves, the CV for dihydroactinidiolide content was the highest at 45.20%, while the CV for geranyl acetone content was the lowest at 12.78% (Table 1). The total content of carotenoid degradation products generally followed the order of upper leaves > middle leaves > lower leaves. The difference in total carotenoid degradation products between upper and lower leaves was highly significant.

Tabel 1. Descriptive statistical results of carotenoids and their degradation products in different parts of flue-cured tobacco.

Carotenoid degradation products	Upper leaves			Middle leaves			Lower leaves		
	Average content (μg/g)	Range	Coefficient of variation (%)	Average content (μg/g)	Range	Coefficient of variation (%)	Average content (μg/g)	Range	Coefficient of variation (%)
β-Damascenone	11.2 ± 2.53^{b}	5.2-18.36	22.62	11.71 ± 3.03^{ab}	5.61-19.71	25.85	12.65 ± 2.11^{a}	7.72-17.76	16.65
Dihydrodamascenone	4.8 ± 2.74^{a}	0.71-12.57	57.12	4.44 ± 1.95^{ab}	0.53-8.91	43.99	3.68 ± 1.66^{b}	0.35-5.95	45.20
6-Methyl-5-hepten-2-one	0.56 ± 0.14^{aA}	0.27-0.93	25.00	0.46 ± 0.13^{bB}	0.24-0.86	28.84	0.37 ± 0.09^{cC}	0.25-0.68	25.34
β-Ionone	0.52 ± 0.25^{a}	0.12-1.28	48.38	0.42 ± 0.17^{b}	0.1-0.96	40.45	0.42 ± 0.13^{b}	0.16-0.72	31.24
Geranyl acetone	2.83 ± 1.07^{aA}	1.18-5.75	37.96	2.8 ± 1.14^{aA}	1.26-6.82	40.74	2.03 ± 0.26^{bB}	1.63-2.54	12.78
Dihydroactinidiolide	4.92 ± 1.15^{b}	2.86-8.32	23.41	5.5 ± 1.28^{a}	3.08-10.08	23.21	5.19 ± 1.21^{ab}	2.51-8.52	23.27
α-Damascone	2.83 ± 1.23^{aA}	1.03-6.89	43.54	2.15 ± 0.66^{bB}	1.19-4.13	30.86	2.32 ± 0.67^{bAB}	1.15-3.69	28.78
β-Damascone	14.42 ± 6.41^{aA}	5.01-32.66	44.48	10.81 ± 3.47^{bB}	6.21-22.19	32.05	11.19 ± 3.18^{bB}	5.86-18.29	28.38
γ-Damascone	1.66 ± 0.76^{aA}	0.53-3.97	45.71	1.28 ± 0.42^{bB}	0.6-2.92	32.86	1.36 ± 0.36^{bAB}	0.72-2.13	26.66
δ-Damascone	11.06 ± 5.17^{aA}	3.69-25.38	46.79	8.09 ± 2.84^{bB}	4.08-17.55	35.15	8.26 ± 2.43^{bB}	4.34-13.3	29.43
Farnesyl acetone	11.8 ± 5.96^{aAB}	3.48-31.09	50.49	12.17 ± 6.96^{aA}	3.49-35.83	57.14	8.24 ± 2.86^{bB}	5.03-19.04	34.70
Total	66.59 ± 21.85^{aA}	32.09-134.62	32.81	59.85 ± 15.57^{abAB}	33.87-107.26	26.02	55.72 ± 9.58^{bB}	36.55-71.44	17.20

Note: Different lowercase letters following the average content of the same row indicated significant differences ($P < 0.05$), while different uppercase letters indicated highly significant differences ($P < 0.01$).

Correlation analysis between carotenoid degradation products in different tobacco leaf positions and chemical quality indices

Correlation analysis was conducted between the content of carotenoid degradation products in different tobacco leaf positions and chemical quality indices. In the upper tobacco leaves, except for the non-significant correlation between the content of 6-methyl-5-hepten-2-one and total alkaloids, all other substances showed highly significant positive correlations. Except for the non-significant correlation between the content of β-damascenone and the contents of reducing sugar and total sugar, all other degradation products showed highly significant negative correlations with reducing sugar and total sugar. The contents of β-damascenone, 6-methyl-5-hepten-2-one, β-ionone, α-damascone, and geranyl acetone showed significant negative correlations with chloride content. Except for the non-significant correlation between the content of β-damascenone and potassium content, all other products showed significant or highly significant positive correlations with potassium content. The contents of β-damascenone and α-damascone products showed significant or highly significant positive correlations with total nitrogen content. The total content of carotenoid degradation products in the upper tobacco leaves showed significant or highly significant positive correlations with total alkaloids, potassium, and total nitrogen, and significant or highly significant negative correlations with reducing sugar, total sugar, and chloride content. In the middle tobacco leaves, dihydrodamascenone, 6-methyl-5-hepten-2-one, β-ionone, and damascene products all showed highly significant positive correlations with total alkaloids. Except for the non-significant correlation between the content of β-damascenone and the contents of reducing sugar and total sugar, all other products showed highly significant negative correlations

Table 2. Correlation analysis between contents of main carotenoids and their degradation products in different parts of flue-cured tobacco and conventional chemical components.

Position	Carotenoid Degradation Products	Total Alkaloids	Reducing Sugar	Chlorine	Potassium	Total Sugar	Total Sugar
Upper tobacco leaves	β-Damascenone	0.395**	-0.095	-0.281*	0.085	-0.083	0.349*
	Dihydrodamascenone	0.435**	-0.566**	-0.208	0.557**	-0.571**	0.196
	6-Methyl-5-hepten-2-one	0.233	-0.501**	-0.347*	0.547**	-0.496**	0.007
	β-Ionone	0.682**	-0.576**	-0.300*	0.627**	-0.585**	0.213
	Geranyl acetone	0.505**	-0.662**	-0.116	0.771**	-0.675**	-0.101
	Dihydroactinidiolide	0.431**	-0.518**	-0.088	0.294*	-0.531**	0.158
	α-Damascone	0.673**	-0.758**	-0.285*	0.621**	-0.752**	0.466**
	β-Damascone	0.649**	-0.787**	-0.262	0.676**	-0.789**	0.379**
	γ-Damascone	0.691**	-0.725**	-0.257	0.585**	-0.723**	0.480**
	δ-Damascone	0.629**	-0.775**	-0.260	0.646**	-0.777**	0.402**
	Farnesyl acetone	0.514**	-0.429**	-0.289*	0.367**	-0.409**	0.107
	Total	0.699**	-0.751**	-0.317*	0.650**	-0.747**	0.350*
Middle tobacco leaves	β-Damascenone	0.149	0.088	-0.357**	0.502**	0.118	0.237*
	Dihydrodamascenone	0.326**	-0.054	-0.167	0.340**	-0.047	-0.018
	6-Methyl-5-hepten-2-one	0.394**	-0.607**	0.329**	0.026	-0.614**	0.234*
	β-Ionone	0.351**	-0.050	-0.297**	0.316**	-0.052	0.252*
	Geranyl acetone	0.151	-0.566**	0.618**	-0.201	-0.552**	0.090
	Dihydroactinidiolide	0.136	-0.399**	0.142	0.075	-0.412**	0.238*
	α-Damascone	0.522**	-0.388**	-0.346**	0.462**	-0.390**	0.336**
	β-Damascone	0.485**	-0.423**	-0.348**	0.510**	-0.429**	0.271*
	γ-Damascone	0.486**	-0.389**	-0.366**	0.458**	-0.379**	0.340**
	δ-Damascone	0.520**	-0.445**	-0.362**	0.491**	-0.448**	0.301**
	Farnesyl acetone	0.213	-0.357**	0.140	0.092	-0.361**	0.195
	Total	0.433**	-0.431**	-0.140	0.411**	-0.428**	0.300**
Lower tobacco leaves	β-Damascenone	-0.147	-0.302	0.060	0.617**	-0.280	-0.137
	Dihydrodamascenone	0.236	0.396	0.034	-0.310	0.377	0.029
	6-Methyl-5-hepten-2-one	0.072	-0.147	-0.005	-0.164	-0.119	0.094
	β-Ionone	0.201	-0.433*	-0.006	0.383	-0.416	0.215
	Geranyl acetone	0.019	-0.240	-0.060	-0.017	-0.238	0.019
	Dihydroactinidiolide	0.110	-0.341	-0.152	0.180	-0.316	0.327
	α-Damascone	-0.081	-0.739**	0.027	0.660**	-0.692**	0.153
	β-Damascone	0.074	-0.864**	-0.076	0.709**	-0.842**	0.348
	γ-Damascone	-0.010	-0.833**	-0.078	0.583**	-0.794**	0.279
	δ-Damascone	0.196	-0.848**	-0.102	0.654**	-0.847**	0.498*
	Farnesyl acetone	0.345	-0.187	-0.305	0.233	-0.213	0.133
	Total	0.198	-0.695**	-0.145	0.647**	-0.685**	0.323

Notes: *: $P < 0.05$. **: $P < 0.01$.

with reducing sugar and total sugar. The contents of β-damascenone, β-ionone, and damascene products showed highly significant negative correlations with chloride content, while the contents of 6-methyl-5-hepten-2-one and geranyl acetone showed highly significant positive correlations with chloride content. Except for the non-significant correlation between the contents of 6-methyl-5-hepten-2-one, geranyl acetone, dihydroactinidiolide, farnesyl acetone and potassium content, all other products showed highly significant positive correlations with potassium content. Further, except for the non-significant correlation between the contents of dihydrodamascenone, geranyl acetone, farnesyl acetone and total nitrogen content, all other products showed significant or highly significant positive correlations with total nitrogen content. The total content of carotenoid degradation products in the middle tobacco leaves showed significant or highly significant positive correlations with total alkaloids, potassium, and total nitrogen, and

highly significant negative correlations with reducing sugar and total sugar. In the lower tobacco leaves, total alkaloids and chloride content showed no significant correlation with the carotenoid degradation products. The contents of β-ionone and damascene products were significantly or highly significantly negatively correlated with reducing sugar content. The content of damascene products was highly significantly positively correlated with potassium content and highly significantly negatively correlated with total sugar content. The content of δ-damascone was significantly positively correlated with total nitrogen content. The total content of carotenoid degradation products in the lower tobacco leaves was significantly negatively correlated with reducing sugar and total sugar content, and highly significantly positively correlated with potassium content (Table 2). The results showed that the total content of carotenoid degradation products in different positions of flue-cured tobacco was highly significantly positively correlated with potassium content and highly significantly negatively correlated with reducing sugar and total sugar content. The total content of carotenoid degradation products in upper and middle tobacco leaves was significantly or highly significantly positively correlated with total alkaloids and total nitrogen content.

Simple correlation analysis between the content of carotenoid degradation products in different positions of flue-cured tobacco and sensory evaluation indicators

The seven sensory evaluation indicators were categorized into three groups including aroma quality, aroma intensity, and off-flavors defined as aroma characteristics, strength and concentration defined as smoke characteristics, aftertaste and irritation defined as taste characteristics. Subsequently, a simple correlation analysis was conducted between the content of carotenoid degradation products and these three characteristics. In the upper tobacco leaves, the contents of dihydrodamascenone, β-ionone, dihydroactinidiolide, and damascone compounds were significantly or highly significantly positively correlated with smoke characteristic indicators. The content of 6-methyl-5-hepten-2-one was significantly negatively correlated with off-flavors. The content of geranyl acetone was significantly or highly significantly positively correlated with aroma intensity, strength, and concentration. The content of farnesyl acetone was significantly positively correlated with strength. In the middle tobacco leaves, the content of β-damascenone was significantly positively correlated with concentration, while the content of dihydrodamascenone was highly significantly positively correlated with aroma quality and concentration. The contents of 6-methyl-5-hepten-2-one and farnesyl acetone were significantly or highly significantly negatively correlated with aroma quality, aftertaste, and irritation, while the content of β-ionone was significantly positively correlated with aroma intensity and concentration, and the content of geranyl acetone was highly significantly negatively correlated with aroma quality and aftertaste. The content of dihydroactinidiolide was highly significantly negatively correlated with aftertaste. The contents of damascene compounds were significantly or highly significantly positively correlated with aroma intensity and concentration, and significantly or highly significantly negatively correlated with irritation. In the lower tobacco leaves, the contents of dihydrodamascenone, β-ionone, and farnesyl acetone were significantly positively correlated with aroma quality. The contents of β-damascenone and β-ionone were significantly or highly significantly positively correlated with aroma intensity. The contents of β-ionone and δ-damascone were significantly positively correlated with strength, while the contents of β-ionone and δ-damascone were significantly positively correlated with concentration (Table 3). The total content of carotenoid degradation products in the upper tobacco leaves was highly significantly positively correlated with smoke characteristics. In the middle tobacco leaves, the total content of carotenoid degradation products was significantly positively correlated with

Table 3. Correlation analysis between the contents of main carotenoids and their degradation products in different parts of flue-cured tobacco and sensory evaluation indexes.

Position	Carotenoid Degradation Products	Aroma Characteristics			Smoke Characteristics		Taste Characteristics	
		Aroma Quality	Aroma Intensity	Off-flavors	Strength	Concentration	Aftertaste	Irritation
Upper tobacco leaves	β-Damascenone	-0.050	-0.063	-0.236	0.155	0.150	-0.141	-0.218
	Dihydrodamascenone	0.070	0.160	-0.093	0.490**	0.305*	0.074	-0.033
	6-Methyl-5-hepten-2-one	-0.246	-0.033	-0.304*	0.095	0.041	-0.264	-0.228
	β-Ionone	0.054	0.221	-0.001	0.456**	0.383**	-0.009	-0.007
	Geranyl acetone	-0.018	0.349*	-0.080	0.533**	0.450**	-0.049	-0.028
	Dihydroactinidiolide	0.154	0.187	0.225	0.322*	0.366**	0.215	0.099
	α-Damascone	-0.067	0.057	-0.188	0.554**	0.367**	-0.114	-0.227
	β-Damascone	-0.028	0.125	-0.132	0.589**	0.400**	-0.067	-0.132
	γ-Damascone	-0.067	0.045	-0.156	0.517**	0.364**	-0.085	-0.156
	δ-Damascone	-0.033	0.091	-0.140	0.560**	0.384**	-0.058	-0.148
	Farnesyl acetone	-0.187	0.035	-0.247	0.275*	0.210	-0.305*	-0.341*
	Total	-0.064	0.115	-0.188	0.558**	0.401**	-0.126	-0.212
Middle tobacco leaves	β-Damascenone	0.108	0.188	-0.054	-0.053	0.232*	0.004	-0.102
	Dihydrodamascenone	0.319**	0.383**	0.104	0.107	0.362**	0.148	-0.024
	6-Methyl-5-hepten-2-one	-0.434**	-0.100	-0.153	-0.042	-0.015	-0.556**	-0.383**
	β-Ionone	0.165	0.263*	-0.026	0.001	0.235*	0.024	-0.178
	Geranyl acetone	-0.388**	-0.175	-0.177	-0.054	-0.068	-0.462**	-0.199
	Dihydroactinidiolide	-0.140	-0.051	-0.152	-0.123	-0.137	-0.289**	-0.185
	α-Damascone	0.102	0.265*	-0.120	0.017	0.293**	-0.055	-0.280*
	β-Damascone	0.102	0.259*	-0.094	-0.004	0.300**	-0.043	-0.257*
	γ-Damascone	0.115	0.273*	-0.101	-0.013	0.293**	-0.090	-0.290**
	δ-Damascone	0.114	0.274*	-0.085	0.001	0.293**	-0.065	-0.266*
	Farnesyl acetone	-0.253*	0.007	-0.180	-0.099	0.037	-0.406**	-0.266*
	Total	-0.043	0.199	-0.149	-0.056	0.234*	-0.250*	-0.302**
Lower tobacco leaves	β-Damascenone	0.319	0.505*	0.339	0.196	0.233	0.366	0.321
	Dihydrodamascenone	0.437*	0.198	0.108	0.167	0.253	0.326	0.331
	6-Methyl-5-hepten-2-one	0.122	0.035	-0.041	0.216	-0.269	0.034	-0.137
	β-Ionone	0.454*	0.597**	0.278	0.403	0.518*	0.243	0.174
	Geranyl acetone	0.139	-0.171	-0.072	0.188	-0.008	-0.081	-0.037
	Dihydroactinidiolide	0.383	0.284	-0.047	0.370	0.269	0.171	0.023
	α-Damascone	0.171	0.253	-0.043	0.247	0.211	0.046	-0.085
	β-Damascone	0.095	0.256	-0.089	0.461*	0.396	0.050	-0.159
	γ-Damascone	0.039	0.270	-0.116	0.339	0.168	-0.040	-0.298
	δ-Damascone	0.094	0.326	-0.090	0.524*	0.472*	0.092	-0.125
	Farnesyl acetone	0.444*	0.359	-0.147	0.370	0.332	-0.105	0.164
	Total	0.407	0.488*	-0.015	0.558**	0.504*	0.171	0.078

Notes: *: $P < 0.05$. **: $P < 0.01$.

concentration and significantly or highly significantly negatively correlated with taste characteristics. In the lower tobacco leaves, the total content of carotenoid degradation products was significantly positively correlated with aroma intensity and significantly or highly significantly positively correlated with smoke characteristics.

Regression analysis of the total content of carotenoid degradation products in different positions of flue-cured tobacco with sensory evaluation indicators

The total content of carotenoid degradation products in different positions of flue-cured tobacco was grouped according to the principle of sequence distribution with a class interval of 1.00. The average value of the total carotenoid degradation products and the average score of the strongly correlated sensory evaluation indicators were calculated for each group. Subsequently, regression analysis was conducted

for each position and its corresponding sensory evaluation indicators. The total content of carotenoid degradation products in each position exhibited a quadratic regression relationship with the corresponding sensory evaluation indicators. The results showed that the scores of smoke characteristics in upper tobacco leaves first increased and then decreased with the increase in the total content of carotenoid degradation products. The scores of taste characteristics in middle tobacco leaves decreased with the increase in the total content of carotenoid degradation products. The scores of smoke characteristics in lower tobacco leaves gradually increased with the increase in the total content of carotenoid degradation products (Figure 1).

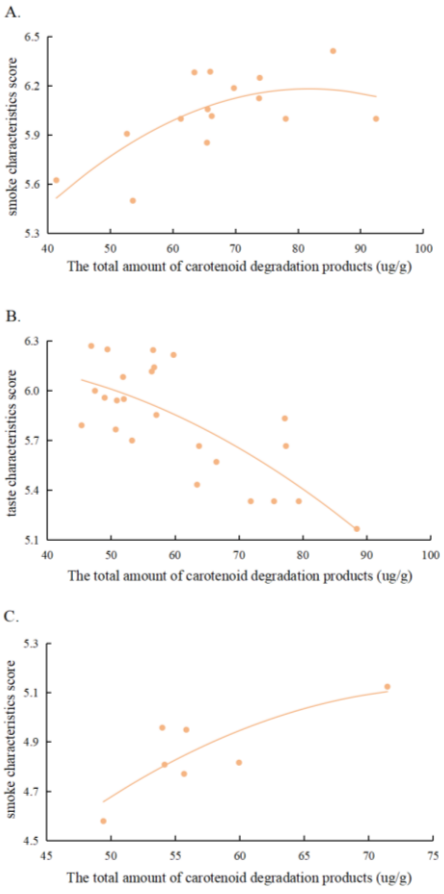

Figure 1. Regression analysis between the total carotenoid degradation products and sensory evaluation scores in different stalk positions of flue-cured tobacco. **A.** Upper leaves. **B.** Middle leaves. **C.** Lower leaves.

Analysis of the relationship between the total content of carotenoid degradation products in different positions of flue-cured tobacco and tobacco leaf quality

The total content of carotenoid degradation products in the upper and middle tobacco leaves, which included β-damascenone (X1), dihydrodamascenone (X2), 6-methyl-5-hepten-2-one (X3), β-ionone (X4), geranyl acetone (X5), dihydroactinidiolide (X6), α-damascone (X7), β-damascone (X8), γ-damascone (X9), δ-damascone (X10), farnesyl acetone (X11), aroma quality (Y1), aroma intensity (Y2), off-flavors (Y3), strength (Y4), concentration (Y5), aftertaste (Y6), and irritation (Y7)], was subjected to canonical correlation analysis. The results showed that the first group of upper leaves and the first two groups of middle leaves had larger canonical correlation coefficients with $P < 0.05$ (Table 4). The linear expression of the first canonical variable for the upper leaves was composed as follows.

$$U_1 = -0.0573X_1 - 0.359X_2 + 0.292X_3 + 0.685X_4 - 0.745X_5 + 0.002X_6 + 0.550X_7 - 5.456X_8 - 0.443X_9 + 4.895X_{10} - 0.002X_{11}$$

$$V_1 = 0.061Y_1 - 0.319Y_2 + 0.208Y_3 - 0.770Y_4 - 0.173Y_5 + 0.438Y_6 - 0.321Y_7$$

From the first canonical variable, among the carotenoid degradation products, the loadings of geranyl acetone content (-0.745), β-damascone content (-5.456), and δ-damascone content (4.895) were relatively large. Among the sensory evaluation indicators of flue-cured tobacco, the loadings of strength (-0.770) and aftertaste (0.438) were also relatively large. These variables played a dominant role in the canonical variable. Therefore, the contents of geranyl acetone, β-damascone, and δ-damascone had a dominant influence on the sensory indicators of strength and aftertaste. Specifically, the contents of geranyl acetone and β-damascone had a positive effect on strength but a negative effect on aftertaste. The content of δ-damascone had a negative effect on strength but a positive effect on aftertaste.

Table 4. Canonical correlation analysis between carotenoids and their degradation products in upper and middle leaves of flue-cured tobacco and smoking evaluation indexes.

Position	Canonical variable	Canonical correlation coefficient	*P*
Upper Leaves	1	0.806	0.012
	2	0.720	0.306
	3	0.513	0.859
	4	0.471	0.898
	5	0.368	0.939
	6	0.290	0.916
	7	0.236	0.796
Middle Leaves	1	0.734	0.000
	2	0.599	0.026
	3	0.504	0.250
	4	0.448	0.580
	5	0.360	0.888
	6	0.177	0.991
	7	0.127	0.946

The linear expression of the first canonical variable for the middle leaves was composed as below.

$U_1 = -0.334X_1 + 0.265X_2 - 0.383X_3 + 0.132X_4 - 0.378X_5 + 0.167X_6 + 0.542X_7 + 0.700X_8 + 0.172X_9 - 1.529X_{10} - 0.466X_{11}$

$V_1 = 0.382Y_1 - 0.466Y_2 - 0.030Y_3 + 0.462Y_4 - 0.512Y_5 + 0.943Y_6 - 0.105Y_7$

From the first canonical variable, among the carotenoid degradation products, the loadings of α-damascone content (0.542), β-damascone content (0.700), and δ-damascone content (-1.529) were relatively large. Among the sensory evaluation indicators of flue-cured tobacco, the loadings of concentration (-0.512) and aftertaste (0.943) were also relatively large. These variables played a dominant role in the canonical variable. Therefore, the contents of α-damascone, β-damascone, and δ-damascone had a dominant influence on the sensory indicators of concentration and aftertaste. Specifically, α-damascone and β-damascone had a negative effect on concentration but a positive effect on aftertaste. δ-damascone had a positive effect on concentration but a negative effect on aftertaste. The linear expression of the second canonical variable was composed as follows.

$U_2 = -0.246X_1 + 0.471X_2 - 0.171X_3 + 0.232X_4 + 0.151X_5 - 1.026X_6 - 0.069X_7 - 0.001X_8 + 0.329X_9 + 0.533X_{10} + 0.427X_{11}$

$V_2 = 0.248Y_1 - 0.001Y_2 - 0.145Y_3 + 0.232Y_4 + 0.722Y_5 - 0.065Y_6 - 0.306Y_7$

From the second canonical variable, among the carotenoid degradation products, the loadings of dihydrodamascenone content (0.471), dihydroactinidiolide content (-1.026), and δ-damascone content (0.533) were relatively large. Among the sensory evaluation indicators of flue-cured tobacco, the loading of concentration (0.722) was also relatively large. These variables played a dominant role in the canonical variable. Therefore, the contents of dihydrodamascenone, dihydroactinidiolide, and δ-damascone had a dominant influence on the sensory indicator of concentration. Specifically, dihydrodamascenone and δ-damascone had a positive effect on concentration, while dihydroactinidiolide had a negative effect on concentration. Canonical correlation analysis was conducted between the total content of carotenoid degradation products in tobacco leaves from three positions and the sensory evaluation indicators with the results showed that the first two canonical groups had significant difference ($P < 0.05$) and relatively large correlation coefficients. Therefore, these

Table 5. Canonical correlation analysis between total carotenoids and their degradation products and smoking evaluation indexes in tobacco leaves.

Canonical Variable	Canonical correlation coefficient	*P*
1	0.742	0.000
2	0.556	0.000
3	0.424	0.262
4	0.290	0.918
5	0.208	0.991
6	0.109	0.999
7	0.059	0.992

two groups were selected for analysis (Table 5). The linear expression of the first canonical variable was composed as follows.

$U_1 = 0.232X_1 + 0.091X_2 - 0.418X_3 - 0.068X_4 + 0.190X_5 + 0.634X_6 - 0.016X_7 + 0.054X_8 + 1.087X_9 - 1.835X_{10} - 0.503X_{11}$

$V_1 = 0.366Y_1 - 0.154Y_2 + 0.009Y_3 - 0.430Y_4 - 0.350Y_5 + 0.244Y_6 + 0.192Y_7$

From the first canonical variable, among the carotenoid degradation products, the loadings of dihydroactinidiolide content (0.634), γ-damascone content (1.087), and δ-damascone content (-1.835) were relatively large. Among the sensory evaluation indicators of flue-cured tobacco, the loadings of aroma quality (0.366) and strength (-0.430) were also relatively large, which indicated that the contents of dihydroactinidiolide, γ-damascone, and δ-damascone had a significant influence on aroma quality and strength in the sensory evaluation of flue-cured tobacco. Specifically, dihydroactinidiolide and γ-damascone had a positive effect on aroma quality but a negative effect on strength, while δ-damascone had a negative effect on aroma quality but a positive effect on strength. The linear expression of the second canonical variable was composed as below.

$U_2 = -0.175X_1 + 0.457X_2 - 0.413X_3 + 0.462X_4 - 0.186X_5 - 0.117X_6 + 0.515X_7 + 0.597X_8 - 1.093X_9 + 0.103X_{10} - 0.390X_{11}$

$V_2 = 0.310Y_1 - 0.580Y_2 + 0.017Y_3 + 1.049Y_4 - 0.259Y_5 + 0.711Y_6 + 0.047Y_7$

From the second canonical variable, among the carotenoid degradation products, the loadings of α-damascone content (0.515), β-damascone content (0.597), and γ-damascone content (-1.093) were relatively large. Among the sensory evaluation indicators of flue-cured tobacco, the loadings of strength (1.049) and aftertaste (0.711) were also relatively large, which indicated that the contents of α-damascone, β-damascone, and γ-damascone had a significant influence on strength and aftertaste in the sensory evaluation of flue-cured tobacco. Specifically, α-damascone and β-damascone had a positive effect on both strength and aftertaste, while γ-damascone had a negative effect on strength.

Principal component analysis of the relationship between total carotenoid degradation products in different positions of flue-cured tobacco and tobacco leaf quality

The results showed that the first three principal components of the upper tobacco leaves had eigenvalues greater than 1, and their cumulative variance contribution rate reached 61.25%, exceeding 60%, which met the standard for principal component extraction. Among them, the first principal component had a contribution rate of 28.54% and showed large positive coefficients with aftertaste, dihydrodamascenone, ionone, dihydroactinidiolide, α-damascone, β-damascone, γ-damascone, and δ-damascone,

Table 6. Eigenvalues and variance contribution rates of principal component analysis for upper leaves.

Variable	First principal component	Second principal component	Third principal component
Aroma quality	0.198	0.006	0.675
Aroma intensity	-0.097	0.769	-0.265
Off-flavors	-0.271	0.409	0.642
Strength	0.034	0.832	-0.090
Concentration	-0.364	0.721	-0.007
Aftertaste	0.713	-0.051	-0.163
Irritation	0.083	0.121	0.601
β-damascenone	0.050	-0.063	-0.453
Dihydrodamascenone	0.840	0.284	-0.139
6-methyl-5-hepten-2-one	0.149	-0.419	-0.686
β-Ionone	0.836	-0.006	0.175
Geranyl acetone	-0.124	0.669	-0.205
Dihydroactinidiolide	0.670	0.310	-0.089
α-Damascone	0.884	-0.077	-0.046
β-Damascone	0.794	0.237	0.022
γ-Damascone	0.807	0.149	0.082
δ-Damascone	0.640	-0.060	0.244
Farnesyl acetone	-0.057	0.886	-0.205
Eigenvalue of principal component	5.137	3.666	2.221
Contribution rate of principal component (%)	28.540	20.368	12.342
Cumulative contribution rate of principal component (%)	28.540	48.908	61.250

which indicated that the first principal component reflected the taste characteristics of sensory quality and could be categorized as the taste factor. The second principal component had a contribution rate of 20.35% and showed large positive coefficients with aroma intensity, strength, concentration, geranyl acetone, and farnesyl acetone, which indicated that the second principal component reflected the smoke characteristics of sensory quality and could be categorized as the smoke factor. The third principal component had a contribution rate of 12.34% and showed large positive coefficients with aroma quality, off-flavors, irritation, and 6-methyl-5-hepten-2-one, which indicated that the third principal component reflected the aroma characteristics of sensory quality and could be categorized as the aroma factor (Table 6). The first three principal components of the middle tobacco leaves had eigenvalues greater than 1, and their cumulative variance contribution rate reached 64.91%, exceeding 60%, which met the standard for principal component extraction. Among them, the first principal component had a contribution rate of 46.67% and showed large positive coefficients with concentration, aftertaste, dihydrodamascenone, geranyl acetone, α-damascone, β-damascone, γ-damascone, δ-damascone, and farnesyl acetone, and a large negative coefficient with 6-methyl-5-hepten-2-one, which indicated that the first principal component reflected the taste characteristics of sensory quality and could be categorized as the taste factor. The second principal component had a contribution rate of 10.29% and showed a large positive coefficient with off-flavors and a large negative coefficient with dihydroactinidiolide, which indicated that the second principal component reflected the aroma characteristics of sensory quality and could be categorized as the aroma factor. The third principal component had a contribution

Table 7. Eigenvalues and variance contribution rates of principal component analysis for middle leaves.

Variable	1	2	3
Off-flavors	0.345	0.553	0.176
Strength	0.27	0.589	-0.647
Concentration	0.753	-0.111	0.033
Aftertaste	0.808	0.091	-0.106
Irritation	0.481	0.23	0.704
Dihydrodamascenone	0.886	-0.038	-0.02
6-methyl-5-hepten-2-one	-0.779	-0.069	0.276
Geranyl acetone	0.73	0.081	0.095
Dihydroactinidiolide	0.155	-0.795	-0.208
α-Damascone	0.783	-0.088	0.025
β-Damascone	0.773	-0.182	0.049
γ-Damascone	0.703	-0.018	-0.13
δ-Damascone	0.815	-0.112	-0.009
Farnesyl acetone	0.757	-0.123	0.098
Eigenvalue of principal component	6.534	1.441	1.117
Contribution rate of principal component (%)	46.671	10.291	7.978
Cumulative contribution rate of principal component (%)	46.671	56.962	64.941

Note: The numbers 1, 2, and 3 in the table represented the loadings of the respective variables on the first, second, and third principal components derived from the principal component analysis.

rate of 7.98% and showed large negative coefficients with strength, concentration, and irritation, which indicated that the third principal component reflected the smoke characteristics of sensory quality and could be categorized as the smoke factor (Table 7).

The present study demonstrated that carotenoid degradation products exhibited a pronounced vertical gradient within the tobacco plant with total amounts decreasing acropetally as upper leaves > middle leaves > lower leaves, and the difference between upper and lower leaves was highly significant [16]. This disparity was closely linked to the photosynthetic efficiency, diurnal temperature differential, and N–K partitioning pattern among leaf positions, which together dictated the accumulation level of precursor substances and their subsequent cleavage – conversion efficiency. Further correlation analyses revealed that these degradation products maintained robust positive relationships with reducing sugars, total sugars, potassium content, smoke fineness, and palate sweetness, indicating that they not only directly imparted aromatic notes but also might enhance overall smoking quality indirectly by improving burn rate and modulating acid–base balance. Consistent with the strong "aroma compound–potassium" coupling reported by Rong *et al*. [21], the highly significant positive correlation between carotenoids and aroma volume found by Tao *et al*. [22], the significant positive association between degradation products and aftertaste revealed by Nong *et al.* [23], and the finding that these products served as core chemical markers for distinguishing the fresh, full, and intermediate aroma styles of flue-cured tobacco [24], this study, at the leaf-position scale, once again confirmed the pivotal role of carotenoid degradation products in shaping tobacco quality. In production practice, a targeted regulation strategy combining "position-specific selection + fine-tuned processing" can therefore be established, employing measures such as low-temperature slow curing or staged temperature control to elevate the proportion of beneficial components in upper and middle leaves, thereby achieving localized quality optimization together with overall synergy. Future efforts should expand the range of flavor-style samples and leverage

integrative high-throughput metabolomics coupled with sensory omics to deeply mine the "chemical fingerprint–sensory attribute" mapping model, thereby furnishing cigarette manufacturers with more granular data support for flavor-formula design, raw-material grading and procurement, and the development of region-specific brands.

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