

Characterization of Flavor Profiles of Cigar Tobacco Leaves Grown in China via Headspace–Gas Chromatography–Ion Mobility Spectrometry Coupled with Multivariate Analysis and Sensory Evaluation

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Cite This: *ACS Omega* 2024, 9, 15996–16005



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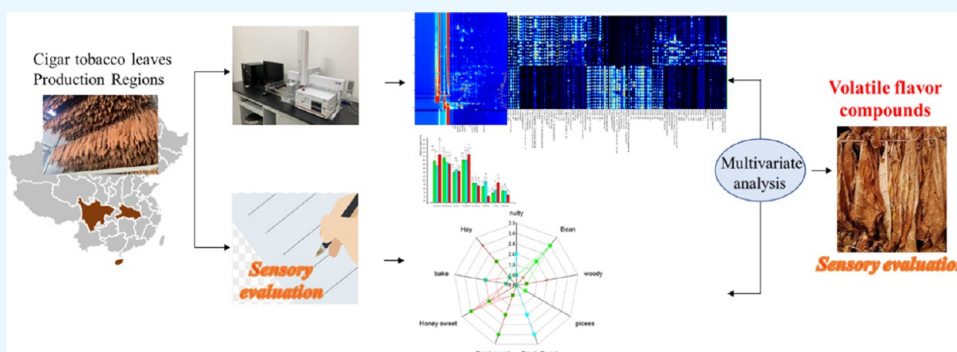
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ABSTRACT: Although cigar tobacco leaves (CTLs) have a high economic value, research regarding the flavor characteristics of CTLs is currently limited. A comprehensive study of the flavor characteristics of CTLs from different regions of China was conducted by identifying their volatile-flavor-containing compounds (VFCs) and flavors. The samples were analyzed via gas chromatography–ion mobility spectrometry (GC–IMS) and sensory evaluation. Results revealed considerable differences in the VFC contents of CTLs from different regions of China, suggesting that the VFLs of CTLs could be influenced by geographical origin. Mainly, phenols, pyrazines, and aldehydes were present in the CTLs from Sichuan. High contents of esters and pyrazines were present in the CTLs from Hubei, while esters were the major components of the CTLs from Hainan. Multivariate analysis results showed the effective differentiation of samples from different geographical origins based on the GC–IMS results. Sensory evaluation revealed that the flavors of CTLs from different geographical origins were different. 1,8-Pinene, 3-methyl-3-butene-1-ol, 2,3-dimethyl-5-ethylpyrazine, 4-methyl-3-penten-2-one, and (*E*)-2-pentenal might serve as geographical marker compounds, indicating the geographical origin of CTLs based on the results of GC–IMS and sensory evaluation. This study may be beneficial for the trade of CTLs and the development of cigar products.

1. INTRODUCTION

Cigars are famous for their rich aroma, plump taste, and high smoke concentration.^{1,2} Cigar tobacco leaves (CTLs) are obtained from tobacco plants, which originated from Latin America and are presently grown primarily in tropical and subtropical countries such as Cuba, Indonesia, Dominica, Brazil, Nicaragua, and China.³ CTLs are highly economic and play an important role in the development of agriculture and culture.⁴ The consumer market of cigars continues to expand with the development of the global economy and culture. Recent reports revealed that the global cigar market size was ~\$13.308 billion in 2021 and that in 2019, ~8.7 million adults in the United States of America frequently smoked cigars.^{5,6} Additionally, the cigar market in China increased from 2 million to 30 million in the last 5 years.

Cigars are completely rolled using CTLs, unlike traditional cigarettes.⁷ Thus, the flavor and aroma of CTLs determine the flavor characteristics of cigars, which is an important factor for cigar quality.⁸ Cigar flavor depends on the combination of volatile-flavor-containing compounds (VFCs), which can be detected via sensors. Cigar flavor directly affects sensory characteristics, quality, and consumer purchase intention. It is

Received: November 28, 2023

Revised: March 5, 2024

Accepted: March 14, 2024

Published: March 26, 2024



affected by the source, type, and processing of CTLs.⁹ VFCs such as alcohols, alkenes, ketones, heterocycles, phenols, esters, and acids were detected in CTLs. Alcohols are the main VFCs in CTLs as revealed via gas chromatography–mass spectrometry (GC–MS) results.^{8,10,11} Solid-phase microextraction (SPME)-based GC–MS studies have shown that 3-methylvaleric acid, acetic acid, 3-nonenene acetylpyridine, dienicotine, and dihydroactinidiolide are the main compounds present in CTLs.^{11–14}

Gas chromatography–ion mobility spectrometry (GC–IMS) is a detection technology that combines the high separation ability of GC and the rapid response of IMS.^{15,16} The technique boasts high sensitivity and accuracy, detects samples with low concentrations, does not require sample pretreatment, and retains the original flavor of the sample to the maximum extent. It is a new method to characterize the flavor or detect VFCs in agricultural products to identify different regions from which they originate.^{17,18} The difference in the Molixiang table grapes grown in different geographical regions of China was distinguished accurately by a combination of GC–IMS and principal component analysis (PCA).¹⁹ GC–IMS and statistical methods were used to identify fu brick tea,²⁰ white tea, and oolong tea²¹ from different regions. The VFCs of coffee were analyzed via GC–IMS and chemometrics analysis.²² GC–IMS was used to identify Chinese yellow wine,²³ white wine,²⁴ *Ophiopogon Radix*,²⁵ and *Polygonati Rhizoma*.²⁶ However, GC–IMS of VFCs combined with multivariate analysis has not been used to establish a methodology for identifying and analyzing CTLs.

The flavor profiles of CTLs from Sichuan (SC) (SC-CTLs), Hainan (HN) (HN-CTLs), and Hubei (HB) (HB-CTLs) were comprehensively analyzed and compared by using GC–IMS combined with multivariate analysis in the present study. The sensory evaluation method was adapted to provide reliable data regarding the flavor characteristics of the CTLs. Our results facilitate a better understanding of the flavor characteristics of CTLs and provide a novel strategy for identifying the geographical origin of CTLs.

2. EXPERIMENTAL METHODS

2.1. Reference Substances and Reagents. N-Ketone C4–C9 standard mix for headspace (HS)–GC–IMS was purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China).

2.2. CTL Samples. Thirty-two species of CTL samples were collected from three regions of SC, HB, and HN in China in 2021. Great Wall Cigar Factory provided SC1–SC8, HB1–HB8, and HN1–HN16 from SC, HB, and HN, respectively. The samples were stored at $-20\text{ }^{\circ}\text{C}$. The samples were weighed at room temperature in tightly closed glass bottles.

2.3. HS–GC–IMS Analysis. The experiment was analyzed using an HS–GC–IMS apparatus (Flavourspec, G.A.S., Dortmund, Germany) with an autosampler (CTC Analytics AG, Zwingen, Switzerland). We optimized the chromatographic column [MXT-5 (15 m \times 0.53 mm \times 1.0 μm) and SE-54 (30 m \times 0.32 mm \times 0.25 μm)], headspace incubation time (10, 20, and 30 min), temperature (60, 70, and 80 $^{\circ}\text{C}$), programmed carrier gas (Table 1), and injection volume (250, 500, and 1000 μL) and evaluated the repeatability of the method (6 parallel samples). The samples were thinly grated, while the dried samples were crushed into powder and separated through a 40-mesh sieve. The sample (0.5000 g) was taken in a 20 mL headspace glass sampling bottle (Zhejiang

Table 1. Programmed Carrier Gas

program 1		program 2		program 3	
time (min)	flow rate (mL min ⁻¹)	time (min)	flow rate (mL min ⁻¹)	time (min)	flow rate (mL min ⁻¹)
0	2	0	2	0	2
2	2	2	2	2	2
15	40	20	40	15	40
20	80	30	80	40	80
50	80	59	80	59	80

HAMAG Technology, Ningbo, China) and incubated at 80 $^{\circ}\text{C}$ for 30 min by rotating at a speed of 500 rpm. Headspace samples (500 μL) were automatically injected into an MXT-5 capillary column using an injector (85 $^{\circ}\text{C}$) with a splitless mode at 45 $^{\circ}\text{C}$ under isothermal conditions in a nitrogen of 99.99% purity. The programmed flow rate was program 3 (Table 1). The flow rate of purified nitrogen was maintained at a rate of 150 mL min⁻¹ as the drift gas. The analytes obtained after GC separation and elution at 75 $^{\circ}\text{C}$ were directed into the IMS ionization chamber. The analytes were ionized using a 3H ionization source under a positive-ion mode. An N-ketone C4–C9 (2-butanone, 2-pentanone, 2-hexanone, 2-heptanone, 2-octanone, and 2-nonanone) standard mix was used to calculate the retention index (RI) of each compound as an external reference. The qualitative analysis of VFCs is based on the RI and relative migration time of IMS.

2.4. Sensory Evaluation. The sensory quality evaluation was performed following the standard evaluation form provided by the Great Wall Cigar Factory (Sichuan, China). We considered CTL samples from three regions of SC, HB, and HN for the sensory quality evaluation. The samples were evaluated by a trained panel comprising two female and three male members of an average age of 38 years. The members with more than 5 years of sensory evaluation experience were formally trained for more than 6 months. CTL samples were rolled by professional rollers to make cigars before sensory evaluation and maintained at 65% relative humidity for 72 h. The flavor characteristics were evaluated on a 5-point scale. A high score indicated strong flavor characteristics. The evaluation score system of each sample is consistently recognized by all panelists.

2.5. Statistical Analysis. Results were analyzed via SPSS version 25.0 (SPSS Inc., Chicago, IL) with one-way analysis of variance (ANOVA) and Tukey's test to evaluate the difference. $P \leq 0.05$ was regarded as a significant value. The spectra and fingerprints were processed using the Reporter plugin and Gallery Plot plugin, respectively. PCA, partial least-squares discriminant analysis (PLS-DA), and orthogonal PLS-DA, were performed using Sim-ca. software (v14.0). Differential VFCs were determined according to a variable importance in projection (VIP) > 1 , $P \leq 0.05$, and fingerprints.

3. RESULTS AND DISCUSSION

3.1. Conditions for GC–IMS Analysis. The results of the optimized method are shown in Figure 1. The optimized condition for the analysis of VFCs in CTLs is as mentioned: 0.5000 g sample weight, 30 min incubation time, temperature 80 $^{\circ}\text{C}$, MXT-5 chromatographic column, carrier gas program 3, and 500 μL injection volume.

3.2. Analysis of the Topographic Plots of VFCs in CTLs via GC–IMS. GC–IMS was used to analyze the VFCs of the CTLs from different geographical regions. The two-

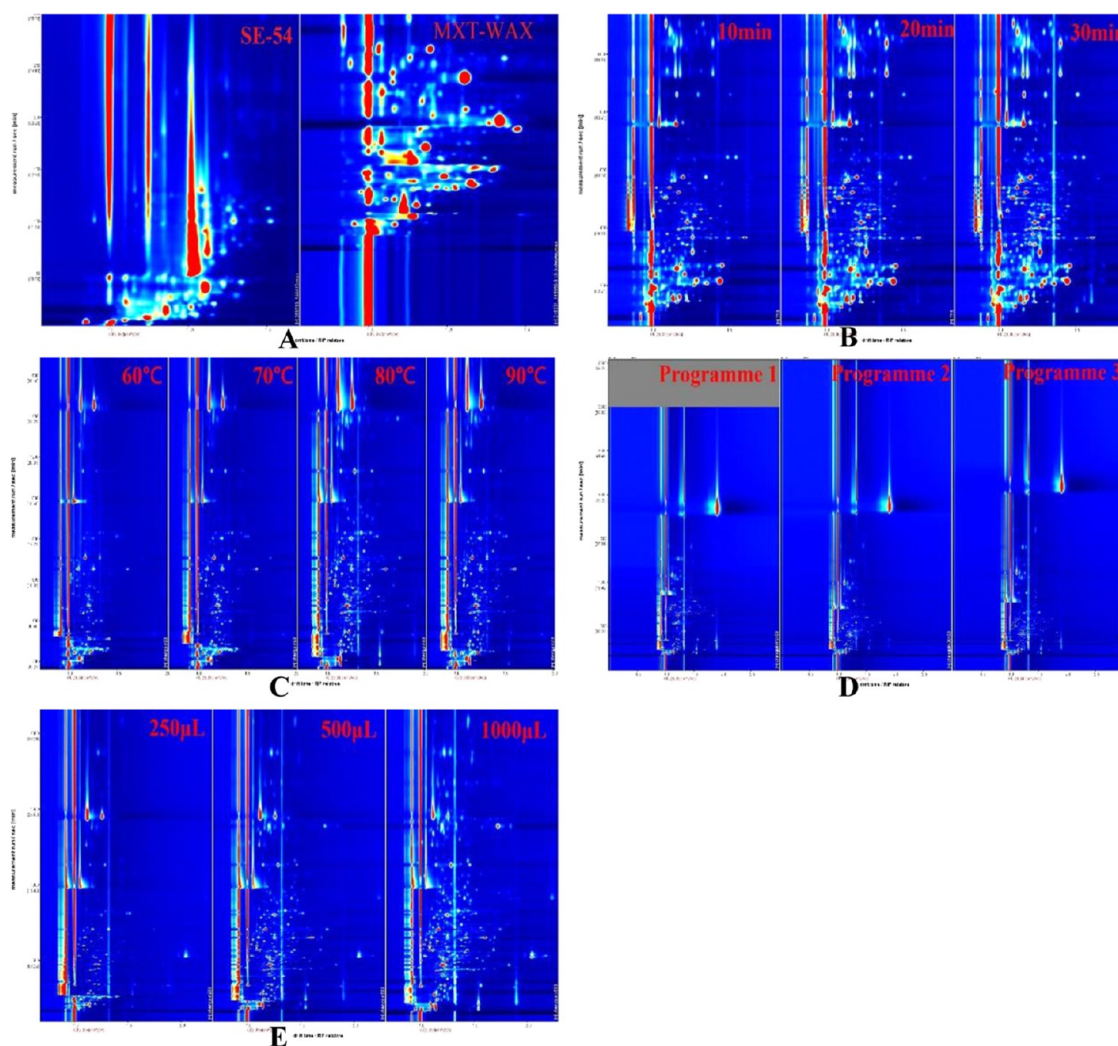


Figure 1. 2D-topographic plots under different GC–IMS conditions: (A) chromatographic column, (B) headspace incubation time, (C) temperature, (D) programmed carrier gas, and (E) injection volume.

dimensional (2D) top view of the GC–IMS (Figure 2A) recorded image of VFCs can be used to visually compare the composition of volatile substances among different samples. Y represents the retention time of the gas chromatograph, and X represents the ion migration time.²⁷ Figure 2A shows that the CTL samples are rich in VFCs. Peak signals are observed in the range of the retention time of 200–1200 s and drift time of 1.0–1.5 ms. The spectral map of a sample from the SC region was selected as a reference to obtain the 2D top-view plot of the samples produced in other areas (Figure 2B). Figure 2B shows white dots representing SC-CTL samples, while red and blue dots represent HB- and HN-CTL samples. A significant difference in the molecular structure and VFC concentrations is observed among SC-, HB-, and HN-CTLs. This might be due to the different regional climate conditions, which have an important effect on the flavor of CTLs.

3.3. Fingerprints of VFCs in CTLs. Fingerprints visually represent the results of the flavor measurement of samples. The VFC contents of CTLs can be compared visually and quantitatively via fingerprint analysis.^{28,29} Figure 3 shows the qualitative identification of 129 compounds. VFC contents are different in the CTLs of each region. Region I representing SC-CTLs comprised the compounds including tetrahydrofuran, 3-methylbutyl acetate, 1-butanol, pyridine, acetaldehyde, 2-

methyl-1-butanol, 1,8-pinene, and 3-methyl-3-buten-1-ol. Region II representing HN-CTLs comprised the compounds including methyl 3-(methylthio)propionate, 4-methyl-3-penten-2-one, ethyl butyrate, (*E*)-pentalen, 4-methylthiazole, 1-propanethiol, methyl *tert*-butyl ether, 2,3-pentanedione trimethylamine, and isoamyl formate. Region III representing HB-CTLs comprised the compounds including 2,3-dimethyl-5-ethylpyrazine 1-penten-3-ol, 2-methylbutanol, benzaldehyde, isoamyl formate, *N*-methylpyrrolidone, α -pinene, methylthio-propanal, and ethyl octanoate.

3.4. Difference of VFCs in CTLs Analyzed via GC–IMS.

Seventy-four compounds were identified based on the results of the GC–IMS library and NIST database comprising 14 aldehydes, 12 alcohols, 14 esters, 12 ketones, 2 acids, 4 pyrazines, 4 olefins, 2 phenols, and 10 other compounds (Table 2). The relative standard deviation (RSD) value is $\leq 13.329\%$, indicating good repeatability of the method with reliable data. ANOVA analysis shows that 68 VFCs have significant differences. Monomers (M) and dimers (D) are detected, and unidentified peaks are numbered.

VFCs in CTLs from different regions are the same, but the proportion of different types of compounds varies. The relative contents of VFCs in SC-, HB-, and HN-CTLs are shown in Figure 4. Ketones, aldehydes, esters, and alcohols are the main

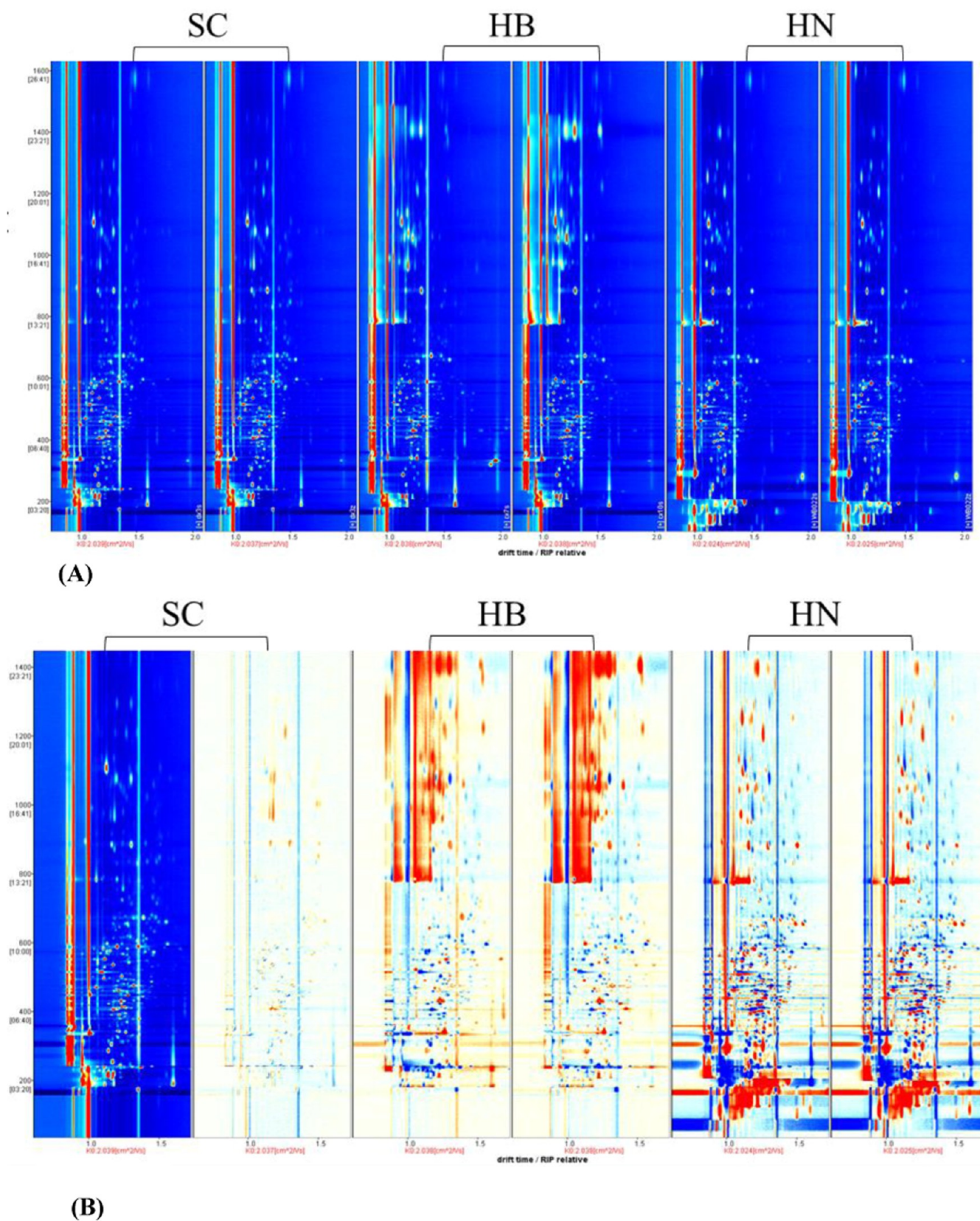


Figure 2. GC-IMS analysis of CTLs of three regions: (A) 2D-topographic plots and (B) comparison of topographic plots.

compounds in these CTLs, consistent with the results reported by Hu et al.¹⁰ The carbonyl groups of aldehydes and ketones are responsible for the aroma. The compounds with carbonyl groups have a beautiful aroma.¹¹ Aldehydes with hay, nuts,

candy, or dried cheese have a low threshold aroma value.³⁰ Ester compounds provide tobacco leaves with a sweet, fruity, and wine-like aroma.³¹ Alcohols also enhance the floral and fruity aroma of CTLs.³² The contents of ketones (22.87%),

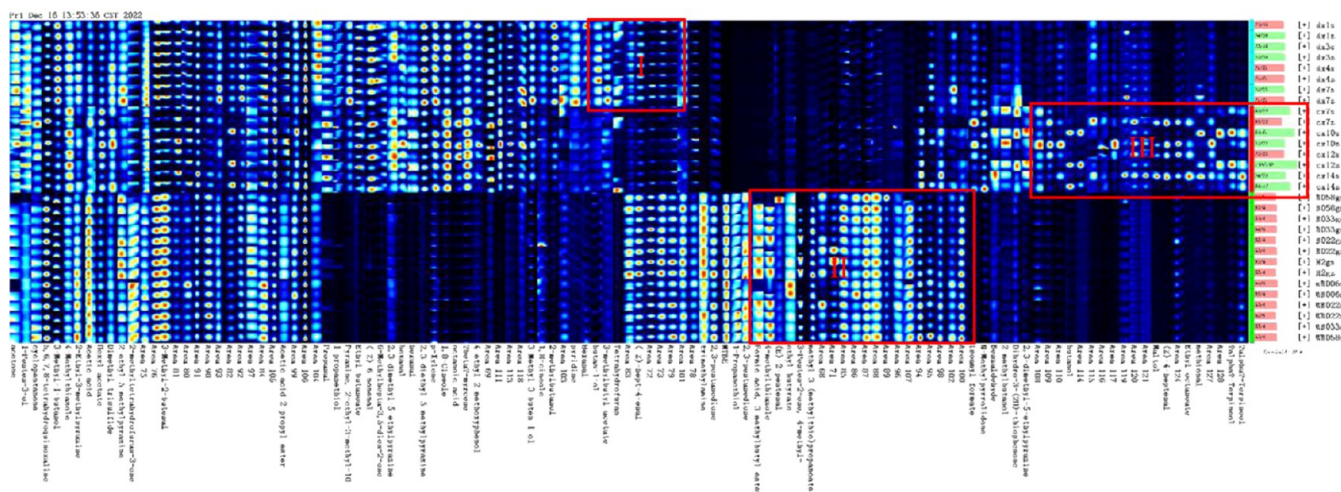


Figure 3. Fingerprints of VOCs in CTLs from different regions.

alcohols (22.98%), and acids (8.94%) of CTLs are the highest in HN-CTLs with the acid content significantly higher than those of SC- and HB-CTLs (Figure 4). Although acids have no direct effect on flavor, they can modulate the pH of smoke and improve mellowness and smoke concentration, thereby indirectly affecting the flavor characteristics of CTLs. The aldehyde (21.27%) and phenol (5.03%) contents of SC-CTLs are the highest, which are significantly higher than those of HN-CTLs (Figure 4). The ester (15.40%) and olefin (9.54%) contents of HB-CTLs are the highest, which are significantly higher than those of HN- and SC-CTLs. The pyrazine contents of HB- and SC-CTLs are significantly higher than those of HN-CTLs (Figure 4). The VOCs in CTLs are different owing to the influence of the tobacco genotype, origin environment, agronomic measures, and modulation process.¹¹ Our study indicates that the ecological conditions of the CTL origin influence the VOCs of CTLs.

3.5. Multivariate Analysis of VOCs in CTLs. The difference between the HN-, HB-, and SC-CTLs was analyzed by using PCA, PLS-DA, and OPLS-DA. Figure 5A shows a difference between the samples from different production regions, indicating that SC-, HN-, and HB-CTLs are different. The clustered SC-, HN-, and HB-CTL samples are separated from other samples via GC-IMS, indicating that this technique can effectively distinguish CTLs from different regions, consistent with the results of PLS-DA and OPLS-DA (Figure 5B,C). The score plot R2X of the model is ≥ 0.638 , suggesting that the PCA, PLS-DA, and OPLS-DA models have good adaptability and predictability. Figure 5D shows R2 and Q2 intercept values as (0, 0.32) and (0, -0.557), respectively, indicating that the OPLS-DA model is robust, reliable, and free from overfitting.

The VIP value of the OPLS-DA model was used to screen the key chemical components and highlight the key aroma components of CTLs from different regions, as shown in Figure 6. Twenty-one components (aldehydes, ketones, phenols, alcohols, etc) of HN-, HB-, and SC-CTLs have VIP values >1 . This result indicates that these 21 components can distinguish the CTLs from different regions. This finding is similar to the result obtained from the fingerprint spectrum and one-way ANOVA method.

3.6. Identification of Chemical Markers. The potential chemical markers of CTL samples from different regions were

identified based on the RI and IMS library search. The OPLS-DA model was designed by using the Sim-ca. software. The interested potential biomarkers could be extracted from the loading and VIP plots of that model.

An ANOVA value of $P \leq 0.05$, GC-IMS fingerprint, and VIP value of >1 were set as the restriction conditions to select the characteristic VOCs. Ten chemical markers in the samples from different regions were identified (Table 3). The characteristic VOCs of (i) SC-CTLs are 1,8-pinene and 3-methyl-3-buten-1-ol, which can impart a herbal and sweet fruit aroma to CTLs,^{33–35} (ii) HN-CTLs are MTBE, 1-propanethiol, 4-methyl-3-penten-2-one, trimethylamine, 4-methylthiazole, and (*E*)-2-pentenal, where MTBE, 4-methyl-3-penten-2-one, and 4-methylthiazole endow CTLs with terpenes, honey, and nut flavors, respectively,^{36–38} and (iii) HB-CTLs are *N*-methylpyrrolidone and 2, 3-dimethyl-5-ethylpyrazine, which endow CTLs with coffee and nut flavors, respectively.^{39,40}

3.7. Sensory Evaluation. Figure 7 shows the sensory evaluation results. The nine sensory descriptions of the CTL samples of three regions were evaluated. CTLs collected from three different regions possess different flavor characteristics. The highest score of SC-CTLs is for burnt sweet, bean, bake, honey-sweet, and hay aroma (Figure 7), which is probably associated with the high concentration of phenols (smoky aroma, burnt sweet, and pieces), pyrazines (bake, nuts, and coffee),⁴¹ and aldehydes (hay, nuts, candy, and dried cheese)³⁰ (Figure 4). A high concentration of 3-methyl-3-buten-1-ol probably contributes to the sweet aroma of SC-CTLs.⁴² The GC-IMS results (Table 3) show that the hay aroma score is positively correlated to 1,8-pinene identified in SC-CTLs.⁴³ The fresh sweet, baked, and nutty flavor of HB-CTLs is predominant based on the sensory evaluation analysis (Figure 7), probably associated with the high concentration of esters (fresh sweet)³² and pyrazines (bake and nutty)⁴¹ (Figure 4). Combined with the GC-IMS results (Table 3), the bake and nutty score is positively correlated with 2,3-dimethyl-5-ethylpyrazine identified in HB-CTLs.⁴⁴ The pyrazine compounds are identified as strong key volatile compounds owing to their low odor threshold value.⁴⁵ HN-CTLs possess honey-sweet, hay, burnt sweet, and woody flavor characteristics (Figure 7), attributed to esters that provide sweet and fruity aroma³² (Figure 4). Table 3 shows that the concentrations of 4-methyl-3-penten-2-one and (*E*)-2-pentenal are high in HN-

Table 2. GC–IMS Integration Parameters of CTLs

no.	compound	CAS no.	formula	MW	RI	R _t [s]	D _t [au]	P	RSD (%)
1	acetone	C67641	C3H6O	58.1	791.6	187.812	1.11524	0.000	2.073
2	propanal	C123386	C3H6O	58.1	831.5	207.57	1.0545	0.000	1.560
3	ethyl acetate	C141786	C4H8O2	88.1	911.3	253.445	1.10029	0.009	0.816
4	butanal	C123728	C4H8O	72.1	896.6	244.315	1.11164	0.000	2.239
5	1-propanethiol	C107039	C3H8S	76.2	851.3	218.117	1.17198	0.000	5.718
6	butan-2-one	C78933	C4H8O	72.1	912.5	254.232	1.24489	0.078	6.132
7	3-methylthiopropional	C3268493	C4H8OS	104.2	907.9	251.324	1.39574	0.068	2.410
8	ethyl propanoate	C105373	C5H10O2	102.1	898.8	245.655	1.44805	0.000	3.763
9	3-methylbutanal	C590863	C5H10O	86.1	917.8	257.596	1.16863	0.000	5.165
10	2-ethyl-3-methyl-pyrazine	C15707230	C7H10N2	122.2	987.7	306.876	1.16349	0.000	4.165
11	ethyl butanoate	C105544	C6H12O2	116.2	1032.9	344.452	1.21232	0.000	0.940
12	N-methylpyrrolidone	C872504	C5H9NO	99.1	1029.7	341.6	1.26255	0.000	3.971
13	β-pinene	C127913	C10H16	136.2	1083.2	391.86	1.21803	0.000	2.586
14	hexanal	C66251	C6H12O	100.2	1078.3	386.892	1.25711	0.000	5.808
15	isoamyl formate	C110452	C6H12O2	116.2	1061.4	370.537	1.27154	0.000	2.328
16	2,3-pentanedione	C600146	C5H8O2	100.1	1071.1	379.847	1.22285	0.000	5.593
17	(Z)-6-nonenal	C2277192	C9H16O	140.2	1099	407.792	1.17166	0.000	7.049
18	6-methylhepta-3,5-dien-2-one	C1604280	C8H12O	124.2	1099	407.792	1.2012	0.000	3.929
19	2,3-dimethyl-5-ethylpyrazine	C15707343	C8H12N2	136.2	1105.1	413.472	1.23151	0.000	1.759
20	hexanal	C66251	C6H12O	100.2	1078.3	386.892	1.45711	0.000	1.739
21	5-nonanone	C502567	C9H18O	142.2	1086.4	395.094	1.35042	0.000	1.713
22	diethyl trisulfide	C3600246	C4H10S3	154.3	1124.4	431.826	1.2288	0.051	8.602
23	hexanal	C66251	C6H12O	100.2	1078.3	386.892	1.65711	0.000	1.420
24	cyclopentanone	C120923	C5H8O	84.1	1154.4	462.227	1.10076	0.632	7.861
25	butan-1-ol	C71363	C4H10O	74.1	1153	460.701	1.18079	0.000	10.387
26	p-xylene	C106423	C8H10	106.2	1162.9	471.192	1.05532	0.000	2.909
27	pyridine	C110861	C5H5N	79.1	1172.1	481.022	1.25057	0.000	1.271
28	1,8-cineole	C470826	C10H18O	154.3	1168	476.64	1.31838	0.000	1.875
29	1-penten-3-ol	C616251	C5H10O	86.1	1166.9	475.42	1.35084	0.000	1.675
30	octanoic acid	C124072	C8H16O2	144.2	1178.2	487.704	1.4459	0.000	8.188
31	β-myrcene	C123353	C10H16	136.2	1154.2	461.948	1.21347	0.000	3.047
32	2-methylbutanol	C137326	C5H12O	88.1	1192.3	503.523	1.23414	0.000	1.172
33	3-methyl-3-buten-1-ol	C763326	C5H10O	86.1	1220.9	537.121	1.17452	0.000	2.545
34	5,6,7,8-tetrahydroquinoxaline	C34413359	C8H10N2	134.2	1262.1	589.568	1.19879	0.000	13.329
35	4-ethyl-2-methoxyphenol	C2785899	C9H12O2	152.2	1268.8	598.526	1.23592	0.000	2.368
36	4-methylthiazole	C693958	C4H5NS	99.2	1264.3	592.452	1.35077	0.000	2.463
37	hexyl acetate	C142927	C8H16O2	144.2	1320.2	674.117	1.38934	0.000	3.443
38	acetic acid	C64197	C2H4O2	60.1	1384.5	785.232	1.05428	0.000	2.484
39	2-ethyl-3-methylpyrazine	C15707230	C7H10N2	122.2	1435.9	886.961	1.16379	0.001	1.683
40	dimethyl trisulfide	C3658808	C2H6S3	126.3	1436.5	888.129	1.30296	0.026	1.602
41	2-ethyl-5-methylpyrazine	C13360640	C7H10N2	122.2	1384.8	785.689	1.20053	0.000	2.602
42	tetrahydrofuran	C109999	C4H8O	72.1	902	247.609	1.06254	0.001	2.373
43	(Z)-hept-4-enal	C6728310	C7H12O	112.2	1253.4	577.998	1.15246	0.000	4.146
44	allyl isothiocyanate	C57067	C4H5NS	99.2	895.4	243.6	1.37222	0.005	1.472
45	propan-2-ol	C67630	C3H8O	60.1	910.5	252.93	1.21253	0.031	1.233
46	ethyl pentanoate	C539822	C7H14O2	130.2	910	252.624	1.27386	0.068	3.426
47	tert-butanol	C75650	C4H10O	74.1	904.7	249.345	1.32081	0.045	3.825
48	trimethylamine	C75503	C3H9N	59.1	581.7	111.02	1.11226	0.000	1.818
49	tert-butyl ether (MTBE)	C1634044	C5H12O	88.1	678.8	141.585	1.13478	0.000	1.640
50	1-propanethiol	C107039	C3H8S	76.2	851.3	218.117	1.37198	0.000	1.572
51	2,3-pentanedione	C600146	C5H8O2	100.1	1078.3	386.892	1.55711	0.000	2.247
52	propanethiol	C107039	C3H8S	76.2	811.9	197.595	1.16678	0.000	3.414
53	2-hexanone	C591786	C6H12O	100.2	798.5	191.075	1.18446	0.571	2.121
54	formic acid, 3-methylbutyl ester	C110452	C6H12O2	116.2	805.2	194.337	1.27178	0.000	3.007
55	4-methylthiazole	C693958	C4H5NS	99.2	1264.3	592.452	1.15077	0.000	0.807
56	(E)-2-pentenal	C1576870	C5H8O	84.1	757.4	172.394	1.36887	0.000	2.215
57	acetic acid 2-propyl ester	C108214	C5H10O2	102.1	905	249.485	1.16036	0.001	2.629
58	benzaldehyde	C100527	C7H6O	106.1	1511	1059.922	1.15528	0.000	1.292
59	2-methylbutanol	C137326	C5H12O	88.1	1192.3	503.523	1.43414	0.000	3.441
60	dihydro-3-(2H)-thiophenone	C1003049	C4H6OS	102.2	1540.9	1137.685	1.18116	0.007	1.831
61	2,3-dimethyl-5-ethylpyrazine	C15707343	C8H12N2	136.2	1105.1	413.472	1.53151	0.000	2.575

Table 2. continued

no.	compound	CAS no.	formula	MW	RI	R _t [s]	D _t [au]	P	RSD (%)
62	3-penten-2-one, 4-methyl	C141797	C6H10O	98.1	818.4	200.847	1.43875	0.002	2.087
63	ethyl butyrate	C105544	C6H12O2	116.2	985.8	305.429	1.20665	0.000	6.416
64	2-methyltetrahydrofuran-3-one	C3188009	C5H8O2	100.1	777.8	181.437	1.0693	0.000	2.028
65	methyl 3-(methylthio)propanoate	C13532188	C5H10O2S	134.2	1025.1	337.616	1.14497	0.000	4.935
66	3-methyl-2-butenal	C107868	C5H8O	84.1	1198	510.017	1.09672	0.000	2.369
67	butanol	C71363	C4H10O	74.1	1116.8	424.546	1.37757	0.000	3.013
68	(Z)-4-heptenal	C6728310	C7H12O	112.2	1219.7	535.719	1.61919	0.001	9.749
69	maltol	C118718	C6H6O3	126.1	1205.3	518.55	1.61072	0.000	2.588
70	ethyl octanoate	C106321	C10H20O2	172.3	1464.9	950.049	1.47647	0.001	10.225
71	methional	C3268493	C4H8OS	104.2	1510.6	1058.923	1.40297	0.001	6.874
72	α -terpineol	C98555	C10H18O	154.3	1630.6	1407.401	1.2267	0.000	3.531
73	2-heptanone	C110430	C7H14O	114.2	885.8	237.809	1.72344	0.010	6.147
74	3-methylbutyl acetate	C123922	C7H14O2	130.2	1101	409.633	1.30624	0.000	2.277

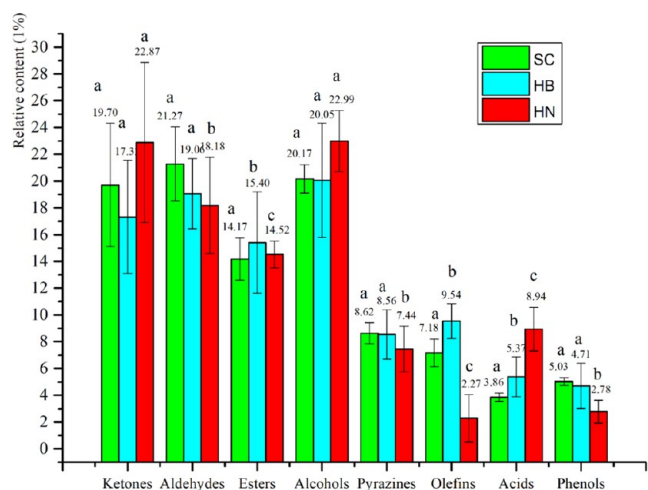


Figure 4. Volatile compound proportion of CTLs identified via GC–IMS from different regions. Note: a, b, and c. The same letter indicates that the proportion of volatile compounds of CTLs from different regions is not statistically significant ($P > 0.05$), while different letters indicate that they are statistically significant ($P \leq 0.05$).

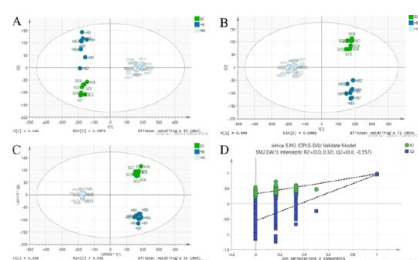


Figure 5. Multivariate analysis of VOCs in CTLs from three regions: (A) principal component analysis (PCA), (B) partial least-squares discriminant analysis (PLS-DA), and (C) orthogonal PLS-DA score plots based on flavor profiles. (D) The corresponding OPLS-DA validation plots.

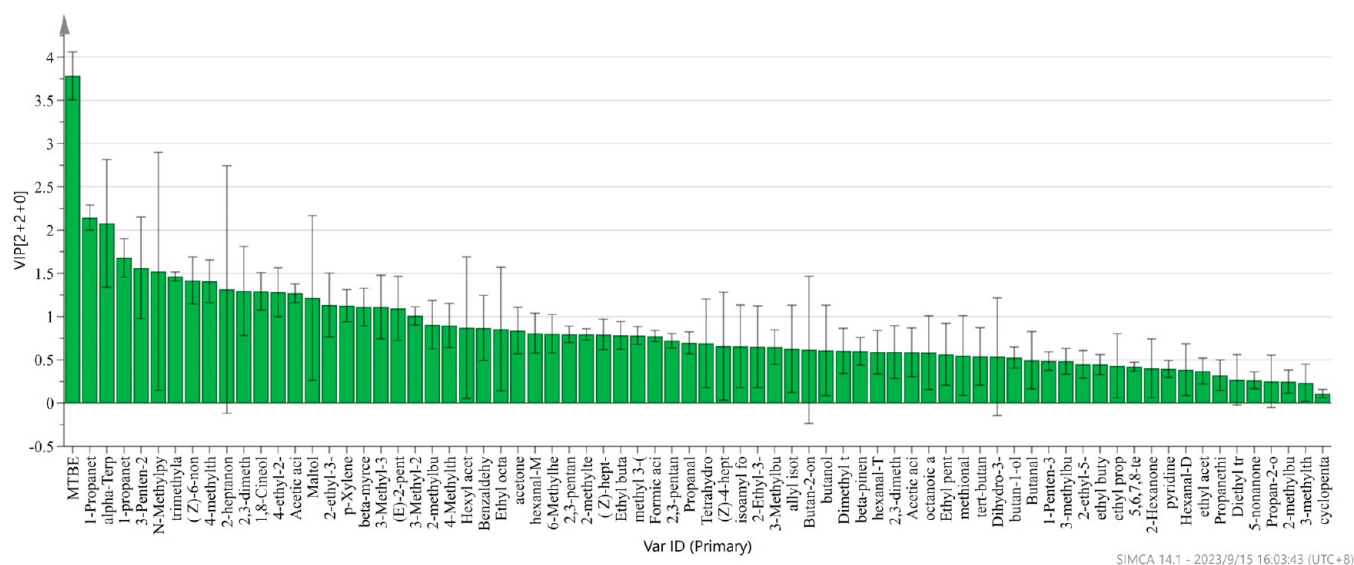
CTLs, which play an important role in providing the honey-sweet and hay aroma. The flavor characteristics of CTLs are affected by the concentration of volatile compounds and the odor threshold of each compound. Further, some unidentified volatile compounds might play an important role in aroma contribution.

Basic chemical measurements, such as total nitrogen, total alkaloids, reducing sugars, total sugars, and polyphenols, are

the most frequently used parameters to determine the quality of CTLs that could provide important information for quality measurement and control.⁴⁶ However, there are limitations to using these parameters for quality evaluation as they have shown a poor correlation with sensory perceptions.^{8,11} The sensory quality is difficult to measure objectively. The sensory evaluation result of this study coincides with GC–IMS analysis, suggesting that GC–IMS is an efficient technology for determining the authenticity and quality of CTLs.

4. CONCLUSIONS

Flavor profiles are an important factor for CTL quality assessment that can be affected by the different geographical origins of CTLs. The flavor profiles of CTLs obtained from three different areas of China were analyzed via HS–GC–IMS and sensory evaluation in this study. The GC–IMS spectrogram revealed differences among the SC-, HB-, and HN-CTL samples. A total of 129 compounds were detected, and 74 VOCs were identified in CTLs such as 14 esters, 12 alcohols, 14 aldehydes, 12 ketones, 4 pyrazines, 3 olefins, 3 acids, 2 phenols, and several other volatile compounds. The multivariate analysis results indicated that differences in VOCs among the samples from different producing areas were evident. Therefore, GC–IMS combined with multivariate analysis is an efficient and alternative method for the identification of CTLs from different geographical areas. Furthermore, the sensory analysis revealed that SC-CTLs exhibited more burnt sweet, baked bean, honey-sweet, and hay aroma, mainly attributed to the high concentration of phenols, pyrazines, and aldehydes than those of HN-CTLs. 1,8-Pinene and 3-methyl-3-butene-1-ol were not detected in HB- and HN-CTLs. HB-CTLs had a fresh sweet, baked, and nutty flavor, which was probably owing to the presence of higher concentrations of esters (fresh sweet) than those in SC- and HN-CTLs and pyrazines (baked and nutty) higher than those in SC- and HN-CTLs. 2,3-Dimethyl-5-ethylpyrazine identified in HB-CTLs was not detected in SC- and HN-CTLs. HN-CTLs with relatively more honey-sweet, hay, burnt sweet, and woody aromas owing to higher ester concentrations than those of SC-CTLs. 4-Methyl-3-penten-2-one and (E) 2-pentenal present in HN-CTLs were not detected in SC- and HB-CTLs. Hence, these compounds could probably be used as geographical marker compounds to determine the origin of the CTLs. The above results can provide a theoretical basis for CTL trade and cigar formulation research. However, further investigation should be conducted to explore the mechanism of



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Figure 6. Variable importance in the projection (VIP) value of the OPLS-DA model.

Table 3. Identification of Chemical Markers

no.	region	compound	VIP	P
1	HN	MTBE	3.78366	0.000
2	HN	1-propanethiol	2.14488	0.000
3	HN	4-methyl-3-penten-2-one	1.56311	0.002
4	HB	N-methylpyrrolidone	1.52241	0.000
5	HN	trimethylamine	1.46496	0.000
6	HN	4-methylthiazole	1.40765	0.000
7	HB	2,3-dimethyl-5-ethylpyrazine	1.29552	0.000
8	SC	1,8-pinene	1.29176	0.000
9	SC	3-methyl-3-butene-1-ol	1.11059	0.000
10	HN	(E)-2-pentenal	1.09383	0.000

Appendix Table 2. The data sets of VCFs. (XLSX)

Appendix Table 3. Sensory evaluation. (XLSX)

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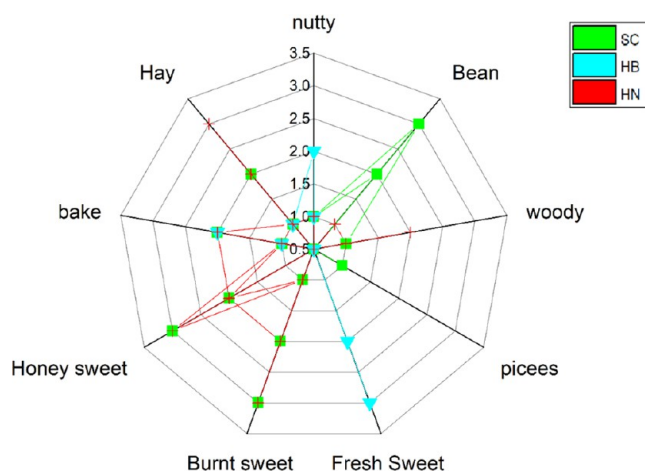


Figure 7. Sensory evaluation profiles of CTL samples.

variation in aroma volatile accumulation under different geographical origins.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.3c09499>.

Appendix Table 1. The data sets of repeatability. (XLSX)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors are grateful for the fund support from the Major Science and Technology Program of China National Tobacco Corporation [110202201033(XJ-04), 110202101066(XJ-15), and 110202101060] and China Tobacco Sichuan Industrial Co., Ltd. [hx202002]. They also thank ACS authoring services for language editing of this manuscript.

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