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Research Article

Unraveling the Difference in the Composition/Content of the Aroma Compounds in Different Tobacco Leaves: For Better Use

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The composition/content of aroma compounds in tobacco leaves in the different producing areas varies too much, and it is very meaningful to develop advanced analysis techniques to investigate the composition/content-producing area correlation. Here, gas chromatography-ion mobility spectroscopy (GC-IMS) was used to analyze the composition/content of aroma compounds in tobacco samples from eight different producing areas. With this technique, ion mobility spectrum, differential ion mobility spectrum, and fingerprint spectrum were constructed for two-dimensional analysis. Then, the principal component analysis (PCA) and similarity analysis were performed for the eight tobacco samples. The results showed that the GC-IMS can detect 197 volatile aroma compounds in tobacco leaves and 75 of them are well identified. The fingerprint spectrum directly showed the difference in the composition/content of volatile aroma compounds in tobacco leaves from different producing areas. PCA and similarity analysis can clearly distinguish tobacco samples from different producing areas. This work demonstrated that the application of GC-IMS in analyzing the composition/content of aroma compounds in tobacco leaves is efficient. GC-IMS is a very powerful tool to give a direct and visual comparison of the composition/content of aroma compounds in tobacco leaves from different producing areas. The relationship between the composition/content of aroma compounds and producing areas could be established by this advanced technology. This work offers the possibility of planting or grading tobacco with different taste and smell more precisely in the future.

1. Introduction

As an industrial crop, tobacco is widely planted in more than 120 countries and regions in the world, which contributed too much to the local economy [1–3]. As the raw material of cigarettes, tobacco leaves contain very rich aroma compounds including alcohols, lipids, carbonyl groups, nitrogen heterocyclic groups, phenols, and acids, and many of these aroma compounds are closely related to the taste and smell of cigarettes [4–6]. For example, phenethyl alcohol endows the cigarette with the scent of flowers and acids can reduce the offensive smell of cigarette smoke. Therefore, identifying the composition/content of the aroma compounds in tobacco leaves and the corresponding influence factor is very important. To date, studies focused on the effect of the maturation stage and stalk position on the aroma compounds' composition/content in tobacco leaves have been reported [7–9]. Since the climate and soil conditions of each producing area varies a lot, the impact of producing areas on the composition/content of these aroma compounds in tobacco leaves should be investigated carefully. Indeed, producing areas are an essential factor in the flavor of tobacco leaves [7, 10–13]. However, this kind of study is rare and not deep enough. Significantly, precisely detecting the composition/content of aroma compounds in tobacco leaves will offer basic data and will be further used, from the

molecular level, in the producing areas-flavor of tobacco leaves study. Undoubtedly, the study will be essential for planting and efficiently utilizing tobacco. To achieve this, advanced analytical equipment and technique are the prerequisites to comprehensively understand the relationship between producing areas and the taste/smell of cigarettes.

Till now, many analytical facilities which were extensively used in organic chemistry have been utilized to study the composition of aroma compounds in tobacco leaves. For instance, two-dimensional gas chromatography-time-of-flight mass spectrometry (GC × GC-TOF-MS) combined with solvent extraction has been demonstrated to quickly and accurately determine free and bound aroma compounds in tobacco [14], Fourier transform near-infrared spectroscopy (FT-IR) combined with partial least squares regression can be effectively used to analyze the chemical components in tobacco [15, 16]. In addition, ultraperformance liquid chromatography (UPLC) [17, 18], ion chromatography (IC) [19], and nuclear magnetic resonance technology (NMR) [20, 21] are also applied to the detection and identification of tobacco chemicals. Although these methods have their characteristics, there are still some limitations of the present analytical facilities and methods. For example, tedious sample preparation, long analysis time, costly, and sophisticated data analysis hindered their practical applications in aroma compounds' detection. Not only that, these traditional methods cannot offer a direct and visual comparison between tobacco samples, lacking analytical capacity. Hence, it is necessary to develop more powerful technologies to meet the requirement of detecting all aroma compounds in tobacco leaves effectively and precisely with a single testing equipment.

In recent years, a technology integrating the advantages of gas chromatography and ion mobility spectroscopy technology (GC-IMS) attracted our attention because of its high sensitivity, short analysis time, high selectivity, and no need for sample pretreatment detecting ability [22]. Especially, GC-IMS can perform orthogonal two-dimensional analysis. Due to these merits, this technology has been widely used in food detection [23], explosives detection [24], biomedical analysis [25], and environmental monitoring [26]. We assume that the composition/content of tobacco leaves will be effectively analyzed by this technology, resulting in a deep understanding of the relationship between the composition/content of aroma compounds and producing areas. Herein, as a proof-of-concept experiment, GC-IMS was applied to obtain a direct and visual comparison of the composition/content of aroma compounds in tobacco leaves from different producing areas. This kind of detailed comparison is very helpful to build a relationship between the composition/content of aroma compounds and producing areas, then to distinguish samples accurately and clearly from different production regions. More importantly, it is of significance for tobacco flavor control and tobacco leaves grade.

2. Materials and Methods

2.1. Analyzing Materials. The C3F grade tobaccos from QujingMalong, Yunnan province (YN-QJML-C3F), Chuxiong, Yunnan province (YN-CHX-C3F), Zhaotong, Yunnan province (YN-ZHT-C3F), Guangyuan, Sichuan province (SCH-GY-C3F), Liangshanhuidong, Sichuan province (SCH-LSHHD-C3F), Liangshanhuili, Sichuan province (SCH-LSHHL-C3F), Zunyi, Guizhou province (GZH-ZY-C3F), and Qianxi'nan, Guizhou province (GZH-QXXN-C3F), were selected as testing materials.

2.2. Analytical Instruments and Methods. Flavour Spec GC-IMS was purchased from Gesellschaft für analytische Sensorsysteme mbH (Germany). Aroma compounds in tobacco samples from different regions were analyzed by GC-IMS in a positive ion mode. The GC-IMS was equipped with an automatic headspace sampler and a nonpolar chromatographic capillary column FS-SE-54-CB-0.5. The column length and internal diameter are 15 m and 0.53 mm, respectively. Initially, a certain amount of tobacco leave samples was randomly chosen from a place and ground into powder. Then, 1.0 g of the above-prepared tobacco powder was taken and placed in a sample inlet bottle (20 ml) for further injection, which does not require the pretreatment of tobacco samples. After incubation at 80°C for 15 min, 500 μL of the headspace was automatically injected using a heated syringe (80°C) into the heated injector (85°C) of the GC-IMS equipment. The volatile compounds were separated through the GC column at 60°C. The carrier gas (N_2, purity 99.999%) is set at 0–2 mL/min, 2 mL/min, 2–10 mL/min, 2–10 mL/min, 10–20 mL/min, 20–25 mL/min, 10–100 mL/min, and 100–150 mL/min in succession. The drift tube was operated at a constant voltage of 400 V cm^{-1}, a temperature of 45°C, and the drift gas (N_2, purity 99.999%) rate was set at 150 mL/min. The output data were collected in a special software tool that comes with the IMS instrument.

3. Results and Discussion

3.1. The Ion Mobility Spectrogram of the Tobacco Samples. Initially, the aroma compounds of the eight different tobacco samples were detected by carrying out GC-IMS analysis. As shown in Figure 1, a high density of reactive ion peak (RIP) points was marked in the coordinate system. The position of these peak points was determined by the retention time and drift time of the detected aroma compounds during the GC-IMS analysis. In the ion mobility spectrum, one peak point represents one aroma compound. In a selected tobacco sample, as high as 197 peak points could be counted. Moreover, the concentration of certain aroma compound was displayed by the colorized difference method. The red area indicated that the concentration of the aroma compound is high, the deeper the color, the higher concentration it had, and the blue area was the opposite. Based on this rule, the aroma compounds' distribution and concentration information in a tobacco sample were obtained visually. In addition, the difference among the eight tobacco samples can also be reflected through the direct comparison. By comparing the rectangular area in Figure 1, it was found that the difference in concentrations of some aroma compounds in tobacco is significant in different provinces. By comparing

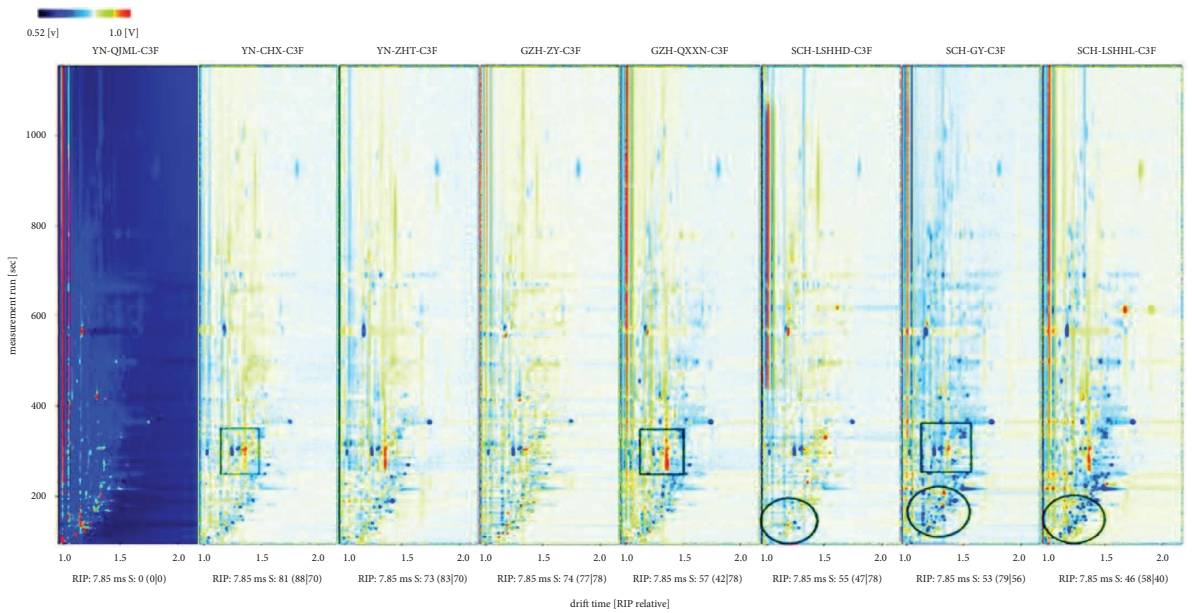

Figure 1: The ion mobility spectrogram of the eight tobacco samples. The red area represents a high concentration of aroma compound; the deeper the color, the higher concentration it had, and the blue area was the opposite.

the circular area in Figure 1, obvious differences were also found that the concentrations of some aroma compound in tobacco samples from different cities in the same province.

3.2. Qualitative Analysis of Volatile Compounds in Tobacco. The qualitative analysis of aroma compounds in tobacco is the first step to understand the relationship between origins and tobacco taste. As discussed above, taking the YN-QJML-C3F as the reference sample, 197 peak points could be obtained in the ion mobility spectrum (Figure 2). In the figure, the x-axis represents drift time, the y-axis is the retention time. As is displayed, 75 of the peak points could be qualitatively analyzed, including alcohols, ketones, aldehydes, acids, lipids, furans, phenylalanine degradation products, and browning products. To be noted, some peak points correspond to the same compound because of the existence of monomers and dimers. This could be explained by the high content of the compound in the sample. The details of the identified volatile compounds are summarized in Table 1. In the table, the name of specific compounds and their corresponding peak positions in the ion mobility spectrum were revealed. The determination of the peak position and drift time of a certain compound can act as the reference and facilitate qualitative analysis of a tobacco leaf sample from an unknown producing area. Moreover, the odors of each compound are also clearly listed for reference [27]. These compounds presented different degrees of plant aroma. For example, some compounds presented pungent odors, and some presented tobacco odors, which are associated with the rich aroma compounds of tobacco. Aroma acids in tobacco can directly volatilize into smoke, which has a direct impact on taste and aroma compounds. Alcohol compounds in tobacco have a great influence on the quality of flue gas, such as benzyl alcohol and phenethyl alcohol, which can increase the fragrance of flowers in roasted tobacco [28, 29]. Carbonyl aroma compounds, including aldehydes, ketones, and quinones, have an important impact on the quality and flavor of tobacco [29]. Benzaldehyde can increase almond flavor in smoke, and 3-hydroxy-2-butanone can increase cream flavor in smoke. Lipid aroma compounds in tobacco also have important effects on aroma and taste. Amyl acetate can increase the banana smell in smoke, and ethyl valerate can increase the apple smell in smoke [30]. Overall, all of these aroma compounds together consist of the thick scent of the aroma of tobacco samples.

3.3. Fingerprint Analysis. Although a large number of aroma compounds were qualitatively identified and also their odor taste was clarified in the tobacco sample, it is still not easy to know how much contribution of each aroma compound to the whole flavor of tobacco leaves, which further led to some difficulties in the comparative analysis of the influence of producing areas. To investigate the content of certain aroma compounds more comprehensively in a tobacco sample, the gallery plot plug-in of LAV software developed by G. A. S. was used to select all the peaks in the ion mobility spectrum and automatically generate the fingerprint spectrum. The fingerprint spectrum enables easy and fast comparisons of different samples, differences in concentrations or presence/absence of marker peaks can be easily seen. The fingerprint spectrum of the sixteen batches of tobacco samples is shown in Figure 3, where the X-axis is the type of aroma compounds, and the Y-axis is the tobacco samples. In the Y-axis, two parallel samples from the same producing area were selected for the comparative analysis. In addition to the fingerprint spectrum, the specific data of the peak position and the area of identified compounds in the fingerprint spectrum were also revealed for the comparison

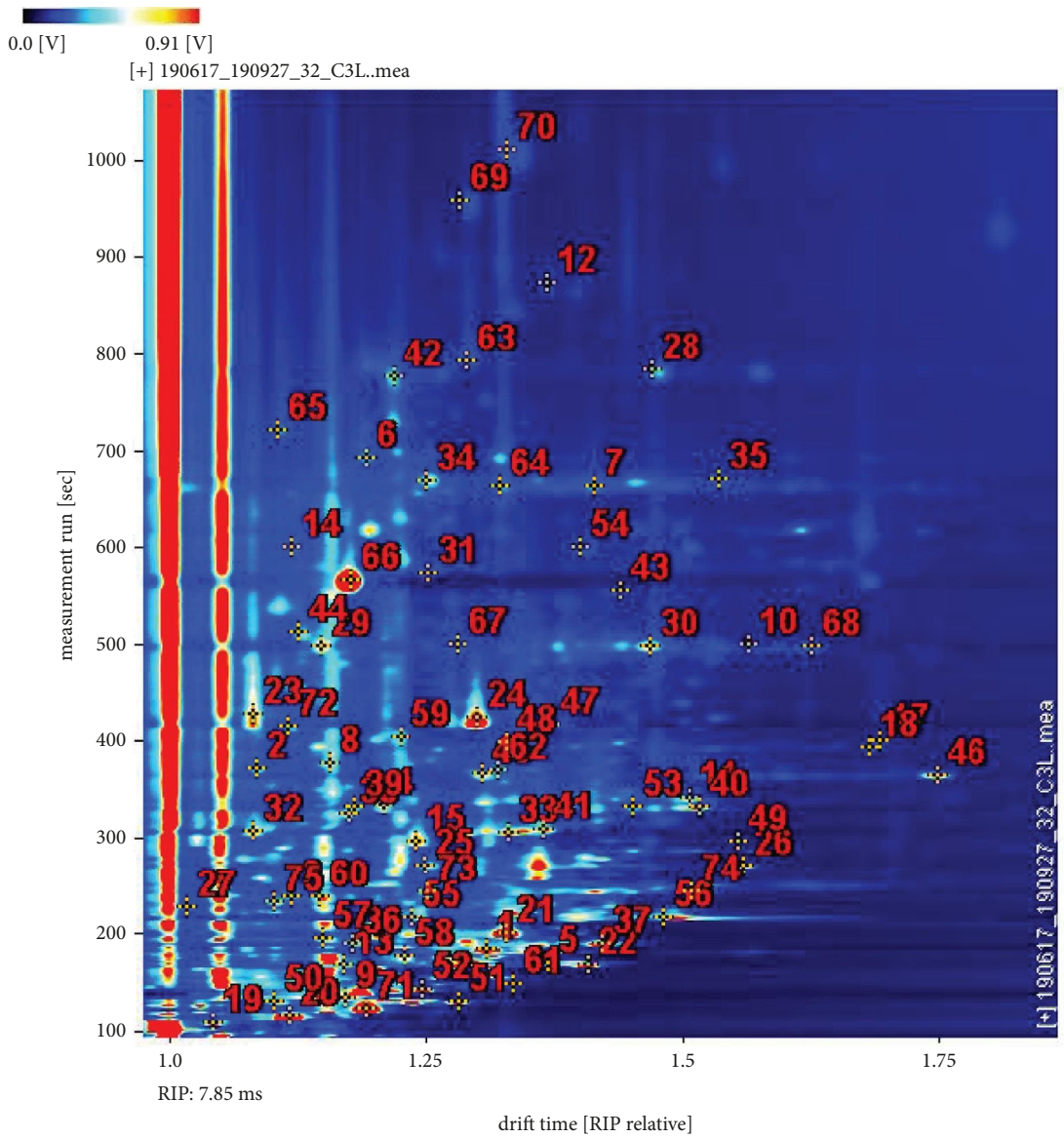

Figure 2: 75 marked peaks were qualitatively identified in the ion mobility spectrum of sample YN-QJML-C3F.

in Table S1. As shown in Figure 3, the similarity of the fingerprint spectrum of different batches of tobacco samples but the same producing area is high. This result verified that this analytic method is reproducible and reliable. For the tobacco samples from different producing areas, the variation in the content of aroma compounds is obvious. For instance, compounds 3-methylvaleric acid dimer, isoamyl acetate monomer, and 2-methylbutanol dimer are lower in samples SCH-GY-C3F with a peak area of ≈288 and SCH-LSHHL-C3F with a peak area of ≈269 in comparison to those from other places (peak area are more than 600). The sample YN-QJML-C3F showed a much higher content of ethyl 2-methylbutanoate and isoamyl acetate dimmer than other samples. Especially for the compound ethyl 2-methylbutanoate (peak area: ≈880), its highest content in the sample YN-QJML-C3F can act as an important indicator to distinguish it from other tobacco samples (most of their peak area is below 150). Samples SCH-LSHHD-C3F and GZH-ZY-C3F have more similarities in aroma compounds even though they are from different provinces. Both of them have a higher content of the compound Z-3-hexen-1-ol than other tobacco samples, these two samples can be distinguished from other ones easily. To further distinguish samples SCH-LSHHL-C3F from GZH-ZY-C3F, carefully checking the content of (E, Z)-2, 6-nonadienal in these two samples is helpful. According to the fingerprint spectrum, the sample has a higher content of (E, Z)-2, 6-nonadienal is SCH-LSHHD-C3F with a peak area of ≈180, the lower one is GZH-ZY-C3F with a peak area of ≈70. To be noted, even in the tobacco samples from the same province but different countries or cities, a distinct difference in the concentrations of aroma compounds between these samples also existed. For example, samples from Yunnan Province YN-QJML-C3F, YN-ZHT-C3F, and YN-CHX-C3F, each have their own

Table 1: Qualitative analysis results of aroma compounds of the sample YN-QJML-C3F.

Count	Compound	CAS#	Rt (sec)	Dt (RIPrel)	Odor
1	1-Hexanol	111-27-3	370.84	1.3225	Ethereal fusel oil fruity alcoholic sweet green
2	1-Hydroxy-2-propanone	116-09-6	176.879	1.2314	Pungent sweet caramelly ethereal
3	1-Pentanol dimer	71-41-0	243.274	1.5114	Fusel oil sweet balsam
4	1-Pentanol monomer	71-41-0	243.665	1.2525	Fusel oil sweet balsam
5	2, 3-Butandione	431-03-8	134.977	1.1733	Strong butter sweet creamy pungent caramel
6	2, 3-Butanediol	513-85-9	309.304	1.3672	Fruity creamy buttery
7	2-Acetylfuran	1192-62-7	414.86	1.1162	Sweet balsam almond cocoa caramel coffee
8	2-Acetylpyridine	1122-62-9	599.881	1.1209	Popcorn heavy corn chip fatty tobacco
9	2-Acetylpyrrole	1072-83-9	719.975	1.1059	Musty nut skin maraschino cherry coumarin licorice walnut bready
10	2-Butanone	78-93-3	142.74	1.2474	Acetone-like ethereal fruity camphor
11	2-Ethyl-1-hexanol	104-76-7	663.231	1.4153	Citrus fresh floral oily sweet
12	2-Ethylfuran	3208-16-0	185.481	1.3101	Sweet burnt earthy malty
13	2-Heptanol	543-49-7	416.435	1.3732	Fresh lemongrass herbal sweet floral-fruity green
14	2-Hexenol dimer	2305-21-7	331.76	1.5192	Fruity green leafy
15	2-Hexenol monomer	2305-21-7	332.608	1.1815	Fruity green leafy
16	2-Methyl-1-propanol	78-83-1	168.732	1.1712	Ethereal winey cortex
17	2-Methylbutanoic acid	116-53-0	334.057	1.2096	Pungent acid roquefort cheese
18	2-Methylbutanol dimer	137-32-6	216.955	1.4844	Roasted wine onion fruity fusel alcoholic whiskey
19	2-Methylbutanol monomer	137-32-6	217.327	1.2376	Roasted wine onion fruity fusel alcoholic whiskey
20	2-Methyl-propanal dimer	78-84-2	129.858	1.284	Fresh aldehydic floral green
21	2-Methyl-propanal monomer	78-84-2	129.943	1.1036	Fresh aldehydic floral green
22	2-Octanol	123-96-6	554.477	1.4418	Fresh spicy green woody herbal earthy
23	2-Pentanone	107-87-9	170.77	1.3711	Sweet fruity ethereal wine banana woody
24	2-Pentylfuran	3777-69-3	574.13	1.254	Fruity green earthy beany vegetable metallic
25	2-Phenylethanol	1960/12/8	793.699	1.291	Floral rose dried rose flower rose water
26	3-Methylbutanal	590-86-3	168.208	1.4102	Ethereal aldehydic chocolate peach fatty
27	3-Methylvaleric acid dimer	105-43-1	498.284	1.6279	Animal sharp acidic cheesy green fruity sweaty
28	3-Methylvaleric acid monomer	105-43-1	499.201	1.283	Animal sharp acidic cheesy green fruity sweaty
29	5-Methylfurfuryl alcohol	3857-25-8	499.045	1.567	Sweet caramellic
30	6-Methyl-5-hepten-2-one	110-93-0	566.203	1.1776	Citrus green musty lemongrass apple
31	Acetoin	513-86-0	201.386	1.3308	Sweet buttery creamy dairy milky fatty
32	Acetone	67-64-1	115.973	1.1182	Solvent ethereal apple pear
33	Alpha-methylbenzenemethanol	98-85-1	691.94	1.1933	Fresh sweet acetophenone gardenia hyacinth
34	Alpha-terpieol	10482-56-1	959.064	1.2856	Lilac floral terpenic
35	Benzaldehyde dimer	100-52-7	498.052	1.4695	Strong sharp sweet bitter almond cherry
36	Benzaldehyde monomer	100-52-7	498.052	1.1491	Strong sharp sweet bitter almond cherry
37	Benzenemethanol	100-51-6	662.63	1.3237	Floral rose phenolic balsamic
38	Cyclohexanone	108-94-1	331.597	1.4537	Minty acetone
39	Dimethyl disulphide	624-92-0	238.664	1.1472	Sulfurous vegetable cabbage onion
40	E-2-hexen-1-ol	928-95-0	324.795	1.1757	Fresh green leafy fruity unripe banana
41	(E, Z)-2, 6-nonadienal	557-48-2	874.685	1.3705	Green fatty dry cucumber violet leaf
42	Ethyl-2-methylbutanoate	7452-79-1	295.452	1.2425	Sharp sweet green apple fruity
43	Ethyl acetate	141-78-6	148.578	1.3374	Ethereal fruity sweet weedy green
44	Ethyl butyrate	105-54-4	297.065	1.5575	Fruity juicy fruit pineapple cognac

Table 1: Continued.

Count	Compound	CAS#	Rt (sec)	Dt (RIPrel)	Odor
45	Ethyl pentanoate	539-82-2	393.1	1.6844	Sweet fruity apple pineapple green tropical
46	Ethyl propanoate	105-37-3	194.587	1.1507	Sweet fruity rum juicy fruit grape pineapple
47	Furfural dimer	1998/1/1	305.714	1.3334	Sweet woody almond fragrant baked bread
48	Furfural monomer	1998/1/1	307.159	1.0829	Sweet woody almond fragrant baked bread
49	Gamma-butyrolactone dimer	96-48-0	423.483	1.3024	Creamy oily fatty caramel
50	Gamma-butyrolactone monomer	96-48-0	428.134	1.0823	Creamy oily fatty caramel
51	Heptanal dimer	111-71-7	399.962	1.6938	Fresh aldehydic fatty green herbal wine-lee ozone
52	Heptanal monomer	111-71-7	398.964	1.3305	Fresh aldehydic fatty green herbal wine-lee ozone
53	Hexanal dimer	66-25-1	270.35	1.5626	Fresh green fatty aldehydic grass leafy fruity sweaty
54	Hexanal monomer	66-25-1	270.613	1.2518	Fresh green fatty aldehydic grass leafy fruity sweaty
55	Isoamyl acetate dimer	123-92-2	364.495	1.7505	Sweet fruity banana solvent
56	Isoamyl acetate monomer	123-92-2	365.85	1.3075	Sweet fruity banana solvent
57	Isopentanol	123-51-3	239.511	1.1195	Fusel oil alcoholic whiskey fruity banana
58	Linalool	78-70-6	776.337	1.2204	Citrus floral sweet bois de rose woody green blueberry
59	Methional	3268-49-3	372.522	1.0868	Musty potato tomato earthy vegetable creamy
60	Acetic acid, methyl ester	79-20-9	122.656	1.1938	Ether sweet fruity
61	Methyl-5-furfural	620-02-0	512.507	1.1275	Spice caramel maple
62	*n*-Nonanal dimer	124-19-6	782.302	1.9402	Waxy aldehydic rose fresh orris orange peel fatty peely
63	*n*-Nonanal monomer	124-19-6	783.105	1.472	Waxy aldehydic rose fresh orris orange peel fatty peely
64	Octanal	124-13-0	600.438	1.4018	Aldehydic waxy citrus orange peel green fatty
65	Pentanal dimer	110-62-3	190.197	1.4247	Fermented bready fruity nutty berry
66	Pentanal monomer	110-62-3	190.413	1.1796	Fermented bready fruity nutty berry
67	Pentanoic acid	109-52-4	404.26	1.2271	Sickening putrid acidic sweaty rancid
68	Phenylacetaldehyde dimer	122-78-1	670.411	1.5386	Green sweet floral hyacinth clover honey cocoa
69	Phenylacetaldehyde monomer	122-78-1	668.193	1.2532	Green sweet floral hyacinth clover honey cocoa
70	Phenylacetic acid	103-82-2	1010.29	1.3311	Sweet honey floral honeysuckle sour waxy civet
71	Propanal	123-38-6	109.401	1.0436	Earthy alcohol wine whiskey cocoa nutty
72	Propanoic acid	1979/9/4	232.834	1.1038	Pungent acidic cheesy vinegar
73	Propylsulfide	111-47-7	376.951	1.1582	Garlic onion
74	Toluene	108-88-3	228.112	1.018	Sweet
75	Z-3-hexen-1-ol	928-96-1	340.959	1.5085	Fresh green cut grass foliage vegetable herbal oily

The retention time of a certain compound in the sample was determined by using the standard curves, the detected compounds in this work were referred to the IMS database. The odor data of these compounds are referred to the standard database of the Perflavory information system.

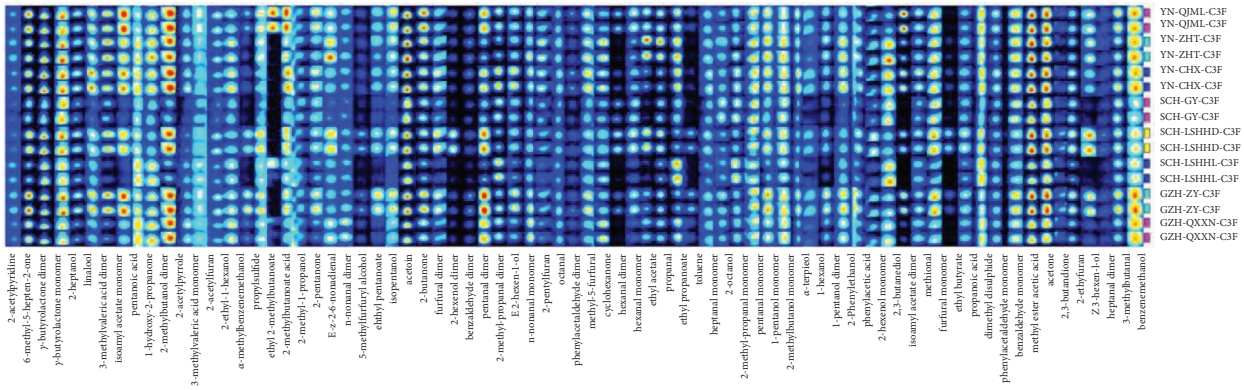

Figure 3: The fingerprint spectrum of aroma compounds in the tobacco samples from eight producing areas.

characteristic aroma compounds. The same phenomenon was observed between samples GZH-ZY-C3F and GZH-QXXN-C3F from Guizhou province. Other compounds, such as 2-methylbutanoate acid, (E, Z)-2, 6-nonadienal, pentanal dimer, ethyl propanoate, 2, 3-butandiol, and isoamyl acetate dimer are various much among the samples from different producing areas. These results demonstrated the big influences of producing areas on aroma compound compositions in tobacco samples. The difference in the concentrations of different aroma compounds between producing areas are significant. Apart from the difference in the content of a certain compound in the tested samples, there are some compounds, such as γ-butyrolactone dimer, γ-butyrolactone monomer, acetoin, 2-methylbutanol monomer, methyl ester acetic acid, and acetone, are all rich in all tobacco samples. It means that the content of these compounds will not be affected by the producing areas, the intrinsic factor decides their content. As discussed above, the fingerprint analysis could help us to find out which tobacco sample has low or high concentrations of a certain aroma compound easily. Moreover, the fingerprint analysis can also be used to compare the concentrations of different aroma compounds in the same sample. Thus, the fingerprint spectrum and its related analysis is a very powerful tool to correlate the content of aroma compounds with the producing area.

3.4. Principal Component and Similarity Analysis. To further analyze the relationship among samples, principal component analysis (PCA) was conducted based on the information acquired for all volatile aroma compounds in tobacco samples. The results are shown in Figure 4, in which the contribution rate of the first principal component was 54%. The contribution rate of the second principal component was 16%, and the cumulative contribution rate of the two principal components reached 70%. Judging from Figure 4, each sample from a different province had its characteristics, and the determination of the first and second principal components rate could be used to clearly distinguish eight tobacco samples from each other. The dispersion degree of tobacco samples was high in samples SCH-GY-C3F, SCH-LSHHD-C3F,

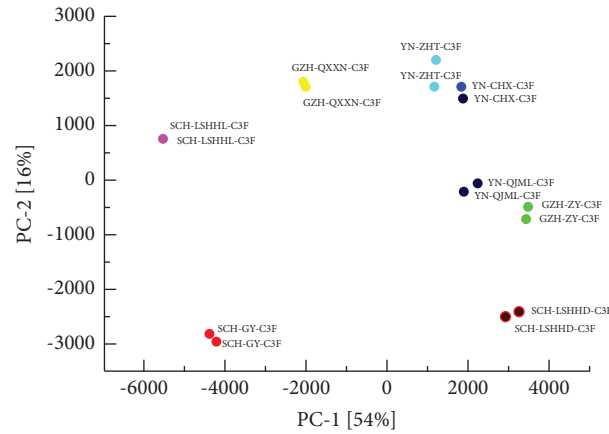

Figure 4: The principal component analysis of each sample.

SCH-LSHHL-C3F, GZH-ZY-C3F, and GZH-QXXN-C3F, but low in YN-QJML-C3F, YN-CHX-C3F, and YN-ZHT-C3F. The similarity between the two parallel samples in SCH-LSHHL-C3F was very high. Conversely, the two parallel samples in other cities had a certain difference, which could be caused by the tiny difference in soil constituents and temperature, and the variation of environmental humidity because of the long distance between the two cities.

To improve the accuracy of the analysis of tobacco samples from different producing areas, all volatile material information were used to calculate the similarity of each sample. Two parallel samples from the same producing area were also selected for the comparison. The results are shown in Table 2. Reasonably, the similarity between the two parallel tobacco samples is high, which achieved 96% at the lowest and 99% at the highest coinciding with the fingerprint spectrum analysis. For the tobacco samples YN-CHX-C3F and YN-ZHT-C3F, YN-CHX-C3F, and YN-QJML-C3F from the same province, their similarity was located between 90 and 92%. However, the tobacco samples YN-ZHT-C3F and YN-QJML-C3F were relatively low (87–88%), even though they are produced in the same province. To our surprise, most of the tobacco samples' similarity is low (72–89%) in Sichuan province. The highest similarity value is only (88–89%), which is obtained from samples SCH-LSHHL-C3F and SCH-GY-C3F. The same phenomenon

Table 2: The similarity between samples.

Matching (%)	YN-QJML-C3F	YN-QJML-C3F	YN-CHX-C3F	YN-CHX-C3F	YN-ZHT-C3F	YN-ZHT-C3F	SCH-LSHHD-C3F	SCH-LSHHD-C3F	SCH-GY-C3F	SCH-GY-C3F	SCH-LSHHL-C3F	SCH-LSHHL-C3F	GZH-QXXN-C3F	GZH-QXXN-C3F	GZH-ZY-C3F	GZH-ZY-C3F
YN-QJML-C3F	100	97	90	90	87	88	89	89	77	77	74	74	83	83	88	88
YN-QJML-C3F	97	100	90	90	87	88	89	89	79	79	77	76	85	85	89	88
YN-CHX-C3F	90	90	100	99	91	92	87	87	78	78	76	77	85	85	90	90
YN-CHX-C3F	90	90	99	100	91	92	87	87	78	78	76	77	85	86	90	89
YN-ZHT-C3F	87	87	91	91	100	96	85	85	80	80	78	78	85	85	86	85
YN-ZHT-C3F	88	88	92	92	96	100	86	86	80	79	78	78	86	86	87	86
SCH-LSHHD-C3F	89	89	87	87	85	86	100	98	77	76	72	73	81	81	90	89
SCH-LSHHD-C3F	89	89	87	87	85	86	98	100	79	79	74	75	82	82	90	89
SCH-GY-C3F	77	79	78	78	80	80	77	79	100	99	88	88	86	86	77	77
SCH-GY-C3F	77	79	78	78	80	79	76	79	99	100	89	89	86	86	77	77
SCH-LSHHL-C3F	74	77	76	76	78	78	72	74	88	89	100	98	86	86	73	72
SCH-LSHHL-C3F	74	76	77	77	78	78	73	75	88	89	98	100	86	86	73	73
GZH-QXXN-C3F	83	85	85	85	85	86	81	82	86	86	86	86	100	98	82	82
GZH-QXXN-C3F	83	85	85	86	85	86	81	82	86	86	86	86	98	100	82	82
GZH-ZY-C3F	88	89	90	90	86	87	90	90	77	77	73	73	82	82	100	98
GZH-ZY-C3F	88	88	90	89	85	86	89	89	77	77	72	73	82	82	98	100

happened to samples GZH-QXXN-C3F and GZH-ZY-C3F from Guizhou province, resulting in a similarity of 82%. By comparing and analyzing the similarity between tobacco samples from different regions. Interestingly, the similarity between tobacco samples GZH-ZY-C3F and YN-CHX-C3F, GZH-ZY-C3F, and SCH-LSHHD-C3F reached 90%. This is not normal because the similarity between two tobacco samples from other regions was lower than 90%. The high similarity between the two samples from different provinces may originate from the similar planting climate, soil condition, moisture, sunshine condition. All of the above discussion indicates that the aroma compounds in tobacco samples were greatly affected by the producing area.

4. Conclusion

In this study, GC-IMS technology was used for the qualitative analysis of aroma compounds in tobacco samples from eight different producing areas. Moreover, owing to the two-dimensional analytic ability of this technology, the relationship between the composition/content of tobacco leaves and their producing areas was well-built. In tobacco leaves of this work, as high as 197 volatile aroma compounds were detected and 75 of them were well identified, including 23 alcohols, 20 aldehydes, 6 acids, 8 ketones, 9 lipids, and 3 furans. In addition to the qualitative analysis of the aroma compounds in a certain tobacco sample, the ion mobility spectrum and its corresponding fingerprint spectrum were successfully applied to directly reveal the content of the 75 identified compounds in the tobacco samples varying with producing areas. With these two advanced spectrums, the content of aroma compounds in the tobacco leaves is compared and discussed in detail in the light of different producing areas. This kind of comparison facilitates us to understand the impact of producing areas at the molecular level, bridging the gap between the macro tobacco-producing areas and the micro aroma compounds composition/content. Furthermore, PCA and similarity analysis were used to distinguish samples accurately and clearly from different production regions, as well as to identify the origin of unknown samples. This work provides an example of the analysis of the composition/content of aroma compounds in tobacco leaves from different producing areas by GC-IMS for tobacco flavor control and tobacco leaves grade.

Data Availability

The data used to support the findings of this study are included in Supplementary Materials.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

The peak position and area of identified compounds in the fingerprint spectrum of tobacco samples from eight producing areas (Table S1) are included in Supplementary Materials. (*Supplementary Materials*)

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