



## Research article

# Analysis of differences in aroma and sensory characteristics of the mainstream smoke of six cigars

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## ABSTRACT

Cigars have unique aroma and style characteristics. In order to clarify the differences of aroma components between domestic and imported cigars and the material basis of the stylistic characteristics of different cigars, gas chromatography-mass spectrometry (GC-MS) and sensory evaluation were used to compare and analyze the aroma components in the mainstream smoke of four domestic cigars and two imported cigars. The GC-MS results showed that a total of 97 aroma components were measured in the smoke of the six cigars, and the types of aroma components were similar, but there were differences in their contents. In comparison with those of domestic cigars, imported cigars had suitable nicotine content, and higher contents of phytol, neophytadiene, 3-methylpentanoic acid, and (+)- $\delta$ -cadinene. To further explore the differences in the aroma components of the six cigars, GC-MS data combined with chemometrics were used to screen out 14 key aroma components based on  $P$ -value ( $P$ ) < 0.05, Variable Importance Projection (VIP) > 1, and Aroma Activity Values (OAV) > 1. The key aroma components of each cigar were obtained, Snow Dream No. 5: cedrol; Wangguan Guocui: 6-methyl-5-hepten-2-one, pyridine, 2-ethyl-6-methylpyrazine; General Achilles No. 3: p-cresol, 2-methylbutyraldehyde, methyl cyclopentenolone; Montecristo No. 4: cedrol, 2-methylbutyraldehyde, guaiaicol, 4-vinylguaiaicol, methyl cyclopentenolone; Romeo y Julieta Wide Churchills: cedrol, 2,6-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-heptanone, phenethyl alcohol; Great Wall No. 2: p-cresol, phenethyl alcohol, geranylacetone, methyl cyclopentenolone, dihydroactinidiolide. The odor descriptors of these compounds were consistent with the aroma profiles that were prominent in the senses of each cigar. This experiment initially explored the differences in aroma composition and style characteristics of cigars and provided data to support the quality improvement of domestic cigars.

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## 1. Introduction

The cigar is a type of tobacco product with unique aromas and cultural characteristics, and cigar consumption has steadily grown worldwide [1,2]. The market of domestic cigars has grown explosively in recent years and has good prospects for development. High-quality imported cigars, represented by Cuban cigars, are very popular in the domestic market due to their excellent aroma qualities and outstanding style characteristics [3]. Domestic cigars are still in the early stages of development, and there is still a large gap between the quality of domestic and imported cigars. Therefore, analyzing the quality differences between domestic and imported cigars and clarifying the development potential of domestic cigars will help improve the quality of domestic cigars and provide guidance for the cultivation, fermentation, and technology of domestic cigars.

Aroma components are one of the critical factors that influence the stylistic characteristics of the product and have an essential impact on the quality of the product. Differential analysis of aroma components provides a visual representation of the quality of different cigars and makes it easier to monitor cigar quality. At present, studies on the characteristic aroma components in cigars focus mainly on cigar tobacco leaves. Cigar tobacco leaves of different origins and varieties have important effects on the aroma components [4,5]. For example, Cuban cigar tobacco leaves have a strong spicy flavor; Indonesian and Chinese cigar tobacco leaves have leathery, peppery, and baked flavors [6]. Analysis of the aroma components of finished cigar tobacco leaves is helpful in determining style characteristics, clarifying aroma differences, and improving cigar quality [7]. Yu et al. [8] found that the content of degradation products of chlorophyll and cembranoids was higher in Cuban cigars, while the content of degradation products of chlorophyll was lower in Chinese cigars. In fact, smoke reflects the sensory quality and style characteristics more directly than tobacco leaves. Many researchers have used smoke to determine the style characteristics and key aroma components of cigarettes. It is helpful in the development of cigarette flavors and fragrances [9,10]. Hu et al. [11] discovered that the key components with burnt-sweet aroma in cigarette smoke were 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 3,4-dimethyl-1,2-cyclopentadione, methyl cyclopentenolone, and 3-ethyl-2-hydroxy-2-cyclopenten-1-one. Klupinski et al. [12] discovered the characteristic aroma component ambroxide in cigarillos by comparing their smoke with that of cigarettes. Nevertheless, there are currently no analyses of the differences of aroma components in different cigars in terms of cigar smoke.

This study aimed to explore the differences in the aroma components of domestic and imported cigars using instrumental and sensory methodologies, and to identify the key aroma components of different cigars to provide a basis for the development and product maintenance of domestic cigars.

## 2. Materials and methods

### 2.1. Chemicals

Dichloromethane ( $\geq 99.9\%$ ), C7–C30 n-alkanes (solvent: hexane), acetic acid (99%), isovaleric acid (98%), and 3-methylvaleric acid (97%) were obtained from Sigma-Aldrich Co., Ltd. (Shanghai, China). N,O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) ( $\geq 98\%$ ), 1-phenylethyl propionate (97%), and (E)-3-hexenoic acid (98%) were supplied by Aladdin (Shanghai, China). 2,3-Butanedione (98%) and isovaleraldehyde (98%) were obtained from J&K Scientific (Beijing, China). 2-Methylbutyraldehyde (98%), propionic acid (99%), octanoic acid (98%), benzoic acid (99%), myristic acid (99%), and palmitic acid (97%) were obtained from TCI (Shanghai, China).

### 2.2. Materials

Six cigars were typical samples of six cigar brands. The four domestic cigars, Snow Dream No. 5 (SD), General Achilles No. 3 (GA), Great Wall No. 2 (GW), and Wangguan Guocui (WG), were from the four major cigar manufacturers in China (China Tobacco Hubei Industrial Co., Ltd., China Tobacco Shandong Industrial Co., Ltd., China Tobacco Sichuan Industrial Co., Ltd., and China Tobacco Anhui Industrial Co., Ltd., respectively). The two imported cigars, Montecristo No. 4 (MT, Cuban Cigar Co., Ltd.) and Romeo y Julieta Wide Churchills (RJ, Cuban Cigar Co., Ltd.), were from two representative brands of Cuban cigars.

### 2.3. Sample preparation

The cigars were stored at a temperature of  $(22 \pm 2)^\circ\text{C}$  and a relative humidity of  $(60 \pm 5)\%$  for at least 72 h before smoking. According to CORESTA RECOMMENDED METHOD N°64: 2005, IDT, marked the length of the cigar butts, calculated the smoking capacity according to the diameter of the cigars, and prepared the holder. The puff frequency was 40 s, and the smoking duration was 1.5 s. Under these conditions, a Cambridge filter was used to capture the particulate matter in the mainstream smoke of a cigar.

Acidic components: After smoking, a Cambridge filter was placed in a 100 mL conical flask, 20 mL of dichloromethane was added, 200  $\mu\text{L}$  of (E)-3-hexenoic acid solution at 1.200 mg/mL was added as an internal standard, and the extraction time was 30 min. After the extract was filtered, 1 mL of the extract was added to the vial with 100  $\mu\text{L}$  of BSTFA and derivatized in a water bath at  $60^\circ\text{C}$  for 50 min, then the sample was injected after the vial cooled. Each sample had three parallel assays.

Other components: After smoking, a Cambridge filter was placed in a 100 mL conical flask, 20 mL of dichloromethane was added, 200  $\mu\text{L}$  of 1-phenylethyl propionate solution at 2.600 mg/mL was added as an internal standard, and the extraction time was 30 min. Nine cigars were smoked in each sample, and the extracts of every three cigars were combined and concentrated to 1 mL. Each sample had three parallel tests.

## 2.4. GC–MS analysis

GC-MS (7895A-5957C, Agilent, Santa Clara, CA) was used for the qualitative and semi-quantitative determination of the aroma components in cigars. In GC-MS analysis, carboxyl (-COOH) was difficult to detect due to its high polarity and low volatility [13]. Silylation combined with GC-MS could provide high sensitivity and selectivity [14], and BSTFA was a commonly used silylation reagent.

Acidic components: A DB-5MS capillary column (60 m × 0.25 mm × 0.25 μm) was used. The carrier gas was helium set at a constant flow rate of 1.00 mL/min. The oven temperature was initially set at 50 °C, then ramped to 280 °C at 4 °C/min, and kept at 280 °C for 5 min. The injection port was set to a split ratio of 10 at 270 °C and 5 min for the solvent delay time. The MS operated in the electron impact mode with an electron energy of 70 eV, with a mass scan range among 35–400 amu. The temperatures of the transfer line and ion source were set at 290 °C and 230 °C, respectively.

Other components: The oven temperature was initially set at 50 °C, then ramped to 250 °C at 2 °C/min, and finally, ramped to 280 °C at 5 °C/min and kept at this temperature for 5 min. Other conditions were similar to those for acidic compounds.

RI was calculated using n-alkanes (C7–C30). The components were identified by comparing their mass spectral data with the NIST 17 database (match greater than 85%) and by comparing the RI value with the literature value [NIST Chemistry Webbook (<https://webbook.nist.gov/chemistry/>)], the components for which the RI value could not be calculated were matched by retention time with standard injection. The aroma components were quantified using the semi-quantitation method based on the peak area and the known concentration of the internal standard (i.e., 1-phenylethyl propionate or (E)-3-hexenoic acid). The concentration of each compound was calculated using the following equation:

$$\omega_a = \frac{A_a}{A_{IS}} \times \frac{C_{IS} \times V_{IS}}{(M_t - M_b)} \quad (1)$$

where  $\omega_a$  is the content of the target compound in μg/g;  $A_a$  is the peak area of the target compound;  $A_{IS}$  is the peak area of the internal standard;  $C_{IS}$  is the known concentration of the internal standard;  $V_{IS}$  is the volume of the internal standard added;  $M_t$  is the weight of the cigar before burning;  $M_b$  is the weight of the cigar butt left after burning.

## 2.5. Odor activity values (OAV)

The detection thresholds of the compounds (as shown in [Supplementary Material Table S1](#)) were quoted in this paper [15–17]. The OAV of each compound was calculated as  $OAV_a = \omega_a/T_a$ , where  $\omega_a$  was the content of compound a (μg/g) and  $T_a$  was the detection threshold of compound a (mg/kg). Compounds with  $OAV > 1$  contributed to the total system. The higher the OAV, the greater the contribution, and the contribution of compounds with  $OAV < 1$  can be ignored [11].

## 2.6. Sensory analysis

Six experts qualified in sensory quality evaluation of cigarettes were employed to perform the sensory evaluation according to the national standard “Cigars—Part 4: Technical Requirements for Sense Evaluation” (GB 15269.4–2011). The six cigars were evaluated for color, luster, flavor, offensive odor, irritancy, aftertaste, and ashing degree, while the aroma characteristics of the six cigars were assessed by reference to the Method for the Evaluation of the Sensory Quality and Style of Domestic Cigar Tobacco. The experts agreed that the aroma of the cigar samples could be described using fourteen attributes: nutty, bean, coffee, cocoa, woody, fruity, fresh-sweet, burnt-sweet, honey-sweet, floral, creamy, resinous, hay, and leathery. The intensities of the fourteen aroma attributes were described on a five-point scale ranging from 0 (none) to 5 (very strong) to assess acceptability.

## 2.7. Statistical analysis

Hierarchical cluster analysis (HCA) and one-way analysis of variance (ANOVA) were used to assess significance between groups using SPSS 23.0 (Chicago, IL, USA), and Duncan’s approach was used to assess statistical significance ( $P < 0.05$ ). Orthogonal partial least squares discriminant analysis (OPLS-DA) to calculate the variable importance projection (VIP) of the predictor variables was performed using Simca 14.1 (Umetrics, Umea, Sweden). The heat map was generated using TBtools (version 1.112; <https://github.com/CJ-Chen/TBtools/releases>). By using the R package ‘ggplot 2 [3.3.6]’, the Spearman correlation coefficient (r) was calculated and the correlation heat map was drawn.

## 3. Results and discussion

### 3.1. Sensory evaluation results of six cigars

[Table 1](#) shows the sensory evaluation scores for the six kinds of cigars. The six cigars had little difference in irritancy, luster, ashing degree, and offensive odor, but the main differences were in flavor and aftertaste. Flavor and aftertaste indexes were highly related to smoke constituents. RJ and SD had a softer, more harmonious aroma, so they had a higher flavor score. RJ, MT, and WG had a long, clean, and pleasant aftertaste, with the WG having the least irritating smoke and better balance. GW had a higher smoke concentration

and a slightly lower comfort level than the other cigars, so it scored lower on flavor and aftertaste.

Fig. 1 shows the aroma profiles of six kinds of cigars. Six cigars had some differences in the main aroma profiles. All six cigars had a bean aroma profile, mostly with nutty, woody aroma profiles. SD's woody and honey-sweet aroma profiles were relatively intense. At the same time, the RJ exhibited distinct floral and honey-sweet aroma profiles, and WG's hay and fresh-sweet aroma profiles were relatively intense. MT, GA, and GW had a pronounced nutty aroma profile, while the coffee and bean aroma profiles of MT were relatively intense. In contrast, GA had a pronounced woody aroma profile with slightly fruity and burnt-sweet aroma profiles, while GW presented distinct floral and woody aroma profiles.

### 3.2. Aroma components and characteristics of different cigars

#### 3.2.1. Composition of aroma substances in cigar smoke

In order to study the aroma characteristics of different cigars, the GC-MS method was used to analyze the aroma components and their relative contents in the smoke of six cigars. The qualitative results are shown in Table S1, and the quantitative results are shown in Table 2. A total of 97 aroma components were identified and classified into 10 categories (including 4 alcohols, 18 ketones, 4 aldehydes, 7 phenols, 10 acids, 3 esters, 6 alkaloids, 31 heterocycles, 6 alkenes, and 8 alkanes). In detail, 88, 88, 90, 91, 94, and 91 aroma compounds were found in SD, WG, GA, MT, RJ, and GW, respectively. The types of aroma components in the smoke of the six cigars were similar, but there were differences in the total concentration in each category. The content and percentage content of aroma components in the six cigars are shown in Fig. 2A and B, respectively. The total content of GW was the highest, reaching 1816.10  $\mu\text{g/g}$ . It was consistent with the results of the sensory evaluation, which found a higher concentration of GW in the smoke. The aroma components with the highest percentage content in cigars were alkaloids, followed by acids, heterocycles, alkenes, ketones, and phenols.

#### 3.2.2. Aroma characteristics of cigars

The composition and content of alkaloids affect the physiological strength, satisfaction, aroma and flavor, and other sensory qualities of cigars [18]. In the process of tobacco leaves fermentation, except for the decrease in alkaloid content, the content of most flavor components increased greatly. The nicotine content was the highest, accounting for more than 85% of the alkaloid content, and was the main source of satisfying smoking sensation. The nicotine content of GW was remarkably higher than that of the other cigars. An appropriate increase in nicotine content can improve the characteristic aroma of tobacco, but if the nicotine content is too high, it will produce a stimulating and pungent flavor that affects the aroma [19]. The lower flavor and total score of GW in the evaluation may be related to the high nicotine content of GW. Previous studies have shown that the total nicotine content and the total score of sensory evaluation are always in a negative correlation [20].

Heterocycles were the most abundant in cigar smoke, including pyridine, pyrrole, and pyrazine compounds, such as 2-methylpyrazine, 2,6-dimethylpyrazine, 2-ethyl-6-methylpyrazine, which are mainly derived from the Maillard reaction and have roasted and nutty aromas [21]. In the process of tobacco leaves modulation, aging, heating and burning, amino acids can react with reducing sugars to produce a variety of flavor components, which can not only remove the odor of tobacco, but also give it a unique flavor, which has an important impact on the flavor quality of tobacco leaves. 2,6-Dimethylpyrazine also presents coffee and cocoa aromas [22], and it can give the smoke coffee and cocoa aromas. Indoles are also heterocyclic compounds with mostly floral aromas. Indole was the most abundant compound among indoles in cigars, with bad odor at high content, its dilution had a jasmine aroma. And its content was remarkably higher in GW, MT, and RJ, reaching 24.60  $\mu\text{g/g}$ , 21.88  $\mu\text{g/g}$ , and 15.83  $\mu\text{g/g}$ , respectively.

Phenols in cigar smoke originate from the cleavage of polyphenolic compounds in the tobacco leaves, which are mainly produced during the combustion process, and phenols have an important influence on the quality of cigars. Phenol, p-cresol, guaiacol, and 4-vinylguaiacol detected in the six cigars have a smoky aroma [9]. The smoky aroma is a very strong aroma in tobacco products that is often used as a background and is not evaluated in the sensory evaluation of smoking, and a strong smoky aroma can usually cover the undesirable smell of the high concentration of indole and 3-methylindole [23]. Among the six cigars, the contents of phenol, p-cresol, and 4-ethylphenol were higher. The content of p-cresol in GA and GW was higher than in other cigars, and the content of guaiacol in MT was higher than in other cigars. The two substances, guaiacol, and p-cresol, have been considered to be the compounds that contribute more to the smoky aroma of cigarette smoke [9]. In terms of total phenols content, GW and MT were 60.29  $\mu\text{g/g}$  and 50.55  $\mu\text{g/g}$ , respectively. The total phenols contents of GW and MT were 1.5–2.5 times that of other cigars, and may bring a stronger smoky aroma.

**Table 1**  
Sensory evaluation results of six cigars.

Cigar	Color	Luster	Flavor	Offensive odor	Irritancy	Aftertaste	Ashing degree	Total
SD	5.00 $\pm$ 0.45a	4.00 $\pm$ 0.55a	33.00 $\pm$ 0.71a	10.50 $\pm$ 0.90 ab	12.00 $\pm$ 0.32 ab	19.50 $\pm$ 0.45cd	5.00 $\pm$ 0.45 ab	89.00 $\pm$ 0.84a
WG	5.00 $\pm$ 0.29a	4.00 $\pm$ 0.41a	31.00 $\pm$ 0.41b	10.50 $\pm$ 0.29 ab	12.50 $\pm$ 0.29a	20.50 $\pm$ 0.29 ab	5.50 $\pm$ 0.41a	89.00 $\pm$ 1.19a
GA	5.00 $\pm$ 0.32a	3.50 $\pm$ 0.45a	30.00 $\pm$ 0.71c	10.00 $\pm$ 0.32b	11.00 $\pm$ 0.63c	19.00 $\pm$ 0.63d	5.50 $\pm$ 0.45a	84.00 $\pm$ 1.48b
MT	4.50 $\pm$ 0.41a	3.50 $\pm$ 0.41 ab	30.50 $\pm$ 0.65bc	11.00 $\pm$ 0.29a	11.50 $\pm$ 0.29bc	20.00 $\pm$ 0.29bc	4.00 $\pm$ 0.29c	85.00 $\pm$ 0.82b
RJ	5.00 $\pm$ 0.41a	3.50 $\pm$ 0.29 ab	33.50 $\pm$ 0.29a	11.00 $\pm$ 0.29a	11.50 $\pm$ 0.29bc	21.00 $\pm$ 0.29a	4.50 $\pm$ 0.41bc	90.00 $\pm$ 1.08a
GW	4.50 $\pm$ 0.41a	3.00 $\pm$ 0.41b	29.00 $\pm$ 0.65d	10.00 $\pm$ 0.58b	11.50 $\pm$ 0.29bc	19.00 $\pm$ 0.58d	4.50 $\pm$ 0.29bc	81.50 $\pm$ 1.66c

The same letters within the same column were not significantly different at the 95% confidence level.

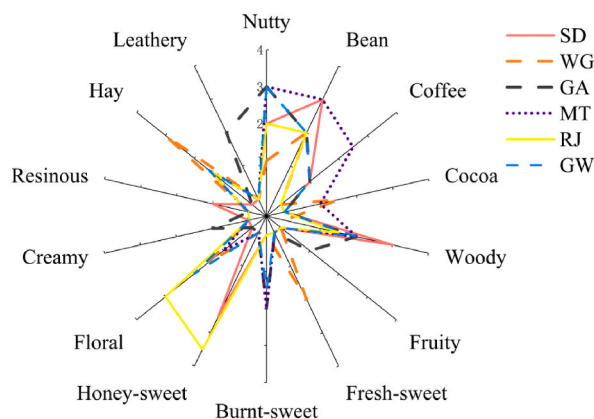


Fig. 1. Evaluation results of aroma profiles of six cigars.

Aldehydes and ketones are important aroma components of cigar smoke, including pyrolysis products from polysaccharides, pectin, and protein, as well as important aroma components formed by the non-enzymatic browning reaction of tobacco and transferred directly to the smoke [24]. The total content of aldehydes was the lowest of the six cigars, but still contributed to the aroma of cigars. Benzaldehyde has the nutty aroma of bitter almond [25], the content of benzaldehyde in GW and MT was higher than in other cigars. 2-Methylbutyraldehyde had nutty, coffee, and cocoa aromas [26], the content of 2-methylbutyraldehyde in MT was higher than in other cigars. A total of 18 ketones were detected in the six cigars. Of these, the components with the higher content were farnesylacetone (weak floral and sweet aromas), megastigmatrienone (herbal, green, and sweet aromas), and geranylacetone (floral, fruity, and sweet aromas). 2-Heptanone with a sweet aroma was detected only in RJ [22]. 6-Methyl-5-hepten-2-one has fresh-sweet and fruity aromas. It was detected in WG, GA, and RJ, which may be the reason why the fresh-sweet aroma of WG and the fruity aroma profile of GA were pronounced. 3-Ethyl-2-hydroxy-2-cyclopenten-1-one, with smoky and coffee aromas [27], was detected only in MT and GW, which enhanced their coffee aroma.

A total of four alcohols were detected in the six cigars, including furfuryl alcohol, phenethyl alcohol, cedrol, and phytol. Of these, the highest content was phytol with a weak floral aroma, followed by cedrol with a mild woody aroma [28]. The content of cedrol in SD was the highest (7.07  $\mu\text{g/g}$ ), it was more than three times that of other cigars, and this may be the reason why the woody aroma of SD was obvious. Phenylethanol is also an important aroma component in tobacco, giving the smoke floral and sweet aromas [29]. The content of phenylethanol in MT, RJ, and GW was higher than in other cigars.

The main sources of acidic components are in two forms: one is the direct transfer from the tobacco leaf into the smoke during smoking, and the other is through the transformation of macromolecular substances, such as the splitting and transformation of carbohydrates. 3-Methylpentanoic acid has an acidic herbal odor with a slight grassy aroma. The content of 3-methylpentanoic acid in MT was higher than in other cigars. It has been shown that 3-methylpentanoic acid contributes more to the sour aroma of the mainstream smoke of blend cigarettes [30]. Palmitic acid and myristic acid were detected in all six cigars. Although these two acids do not act directly on the smoke, they can reduce the irritation of the smoke, regulate the pH of the smoke, and indirectly affect the aroma of the smoke.

Neophytadiene had the highest content among the olefin compounds. In the process of tobacco leaves modulation and aging, neophytadiene is formed by the degradation of chlorophyll, and some of it continues to degrade into other aroma components. It has a clear aroma and can be transferred directly to the smoke during combustion, mellowing the smoke and reducing irritation [31]. Limonene has a lemony and sweet aroma and is common in natural essential oils. The contents in WG (14.60  $\mu\text{g/g}$ ) and RJ (11.68  $\mu\text{g/g}$ ) were higher than in other cigars. Ester compounds can reduce irritation and harmonize tobacco aroma. Sclareolide has a woody aroma. Its content in MT (7.11  $\mu\text{g/g}$ ) was higher than in other cigars. The content of alkane compounds was low, and only a few of them produced a unique aroma, which had a limited contribution to the overall aroma of the cigars.

Comparison of domestic and imported cigars showed that imported cigars had a higher content of alcohol compounds, mainly in the relatively high content of phytol. Phytol adds a faint floral aroma and makes the smoke full and delicate. The nicotine content of the imported cigars was suitable. Too low a nicotine content will make the cigar strength less powerful, and too high a content will be irritating. For example, the domestic cigar GW, with a significantly higher nicotine content, was more stimulating than imported cigars. Imported cigars had higher contents of neophytadiene and 3-methylpentanoic acid, which can soften the smoke and reduce irritation. (+)- $\delta$ -Cadinene has a dry woody aroma. It was only found in imported cigars. These differences in aroma compound content made domestic cigars slightly inferior to imported cigars in terms of cigar strength and harmony.

### 3.2.3. Comparison of aroma characteristics of cigars combined with OAV

The content of aroma components cannot be used completely as a basis for determining the aroma characteristics of the smoke, and usually aroma components with higher OAV can provide aroma characteristics of the smoke [32]. In previous studies, OAV was calculated to evaluate the contribution of an aroma component or groups of characteristic components to the overall aroma of the smoke, with the higher OAV, the higher the contribution [11]. A total of 17 aroma components with OAV >10 in the six cigars were

**Table 2**  
Contents of aroma components in the mainstream smoke of six cigars.

No.	Compound	Content ( $\mu\text{g/g}$ )					
		SD	WG	GA	MT	RJ	GW
1	Furfuryl alcohol	0.63 $\pm$ 0.17c	0.65 $\pm$ 0.15c	0.97 $\pm$ 0.42 abc	1.22 $\pm$ 0.29 ab	0.76 $\pm$ 0.21bc	1.45 $\pm$ 0.20a
2	Phenethyl alcohol	0.53 $\pm$ 0.05b	0.48 $\pm$ 0.05b	0.69 $\pm$ 0.13b	1.14 $\pm$ 0.10a	1.22 $\pm$ 0.19a	1.27 $\pm$ 0.15a
3	Cedrol	7.07 $\pm$ 0.63a	nd	nd	1.69 $\pm$ 0.32b	2.05 $\pm$ 0.45b	0.19 $\pm$ 0.02c
4	Phytol	9.34 $\pm$ 1.98 ab	10.33 $\pm$ 1.29 ab	8.39 $\pm$ 1.76b	11.48 $\pm$ 0.74 ab	12.06 $\pm$ 1.06a	9.96 $\pm$ 1.82 ab
Alcohols (4)		17.57 $\pm$ 2.17a	11.46 $\pm$ 1.22c	10.06 $\pm$ 1.54c	15.53 $\pm$ 0.59 ab	16.09 $\pm$ 1.65a	12.86 $\pm$ 1.94bc
5	2,3-Butanedione	1.38 $\pm$ 0.14bc	0.51 $\pm$ 0.16c	1.55 $\pm$ 0.39b	3.57 $\pm$ 0.76a	0.73 $\pm$ 0.03bc	3.23 $\pm$ 0.63a
6	2-Cyclopenten-1-one	2.75 $\pm$ 0.14c	2.16 $\pm$ 0.16e	2.46 $\pm$ 0.09d	3.56 $\pm$ 0.09b	2.46 $\pm$ 0.06d	4.17 $\pm$ 0.20a
7	2-Heptanone	nd	nd	nd	nd	0.22 $\pm$ 0.03a	nd
8	Cyclohexanone	0.45 $\pm$ 0.09b	0.55 $\pm$ 0.07 ab	0.42 $\pm$ 0.02b	0.52 $\pm$ 0.05 ab	0.55 $\pm$ 0.08 ab	0.65 $\pm$ 0.05a
9	2-Methyl-2-cyclopenten-1-one	5.23 $\pm$ 1.25d	8.72 $\pm$ 0.56a	4.72 $\pm$ 0.46d	5.85 $\pm$ 0.40cd	7.38 $\pm$ 0.92 ab	6.83 $\pm$ 0.33bc
10	3-Methyl-2-cyclopenten-1-one	1.29 $\pm$ 0.30d	2.06 $\pm$ 0.10bc	1.61 $\pm$ 0.24cd	2.12 $\pm$ 0.29b	1.67 $\pm$ 0.19bcd	3.00 $\pm$ 0.31a
11	6-Methyl-5-hepten-2-one	nd	0.76 $\pm$ 0.07a	0.65 $\pm$ 0.07b	nd	0.21 $\pm$ 0.04c	nd
12	3,4-Dimethylcyclopent-2-en-1-one	1.25 $\pm$ 0.27bc	1.99 $\pm$ 0.14 ab	0.71 $\pm$ 0.18c	2.37 $\pm$ 0.65a	2.00 $\pm$ 0.56 ab	2.11 $\pm$ 0.04a
13	Methyl cyclopentenolone	nd	nd	1.25 $\pm$ 0.10b	2.19 $\pm$ 0.17a	nd	1.40 $\pm$ 0.13b
14	2,3-Dimethyl-2-cyclopenten-1-one	2.21 $\pm$ 0.60b	2.46 $\pm$ 0.21b	2.80 $\pm$ 0.30b	3.73 $\pm$ 0.17a	2.56 $\pm$ 0.58b	4.20 $\pm$ 0.52a
15	Acetophenone	0.98 $\pm$ 0.13d	1.67 $\pm$ 0.15bc	1.44 $\pm$ 0.17c	1.88 $\pm$ 0.17 ab	2.03 $\pm$ 0.13a	1.85 $\pm$ 0.23 ab
16	3-Ethyl-2-hydroxy-2-cyclopenten-1-one	nd	nd	nd	2.17 $\pm$ 0.08a	nd	1.06 $\pm$ 0.16b
17	2'-Methylacetophenone	0.85 $\pm$ 0.08b	0.96 $\pm$ 0.15b	nd	nd	1.22 $\pm$ 0.14a	nd
18	Geranylacetone	1.53 $\pm$ 0.47d	2.44 $\pm$ 0.27c	5.24 $\pm$ 0.20b	5.18 $\pm$ 0.27b	2.59 $\pm$ 0.15c	6.24 $\pm$ 0.44a
19	Megastigmatrienone	1.02 $\pm$ 0.09bc	0.66 $\pm$ 0.04d	0.25 $\pm$ 0.03e	1.30 $\pm$ 0.08a	0.92 $\pm$ 0.12c	1.16 $\pm$ 0.08 ab
20	3-Hydroxy- $\beta$ -damascone	2.01 $\pm$ 0.24bc	1.63 $\pm$ 0.07c	1.44 $\pm$ 0.26c	2.75 $\pm$ 0.62a	1.94 $\pm$ 0.15bc	2.27 $\pm$ 0.12 ab
21	Phytone	12.54 $\pm$ 1.53 abc	8.69 $\pm$ 0.47d	9.88 $\pm$ 0.81cd	14.29 $\pm$ 2.83a	11.17 $\pm$ 0.98bcd	13.48 $\pm$ 1.32 ab
22	Farnesylacetone	7.18 $\pm$ 1.62b	5.45 $\pm$ 0.26b	6.58 $\pm$ 0.33b	6.86 $\pm$ 0.82b	5.53 $\pm$ 0.99b	9.07 $\pm$ 0.82a
Ketones (18)		40.70 $\pm$ 4.48b	40.70 $\pm$ 1.09b	41.01 $\pm$ 0.47b	58.34 $\pm$ 3.57a	43.16 $\pm$ 3.70b	60.71 $\pm$ 2.31a
23	Isovaleraldehyde	0.19 $\pm$ 0.00b	0.19 $\pm$ 0.03b	0.35 $\pm$ 0.02b	0.70 $\pm$ 0.11a	0.29 $\pm$ 0.03b	0.62 $\pm$ 0.20a
24	2-Methylbutyraldehyde	0.57 $\pm$ 0.05d	0.66 $\pm$ 0.04cd	0.89 $\pm$ 0.10b	1.17 $\pm$ 0.07a	0.68 $\pm$ 0.06cd	0.76 $\pm$ 0.07c
25	Furfural	0.17 $\pm$ 0.04d	0.14 $\pm$ 0.02d	0.23 $\pm$ 0.02c	0.32 $\pm$ 0.02b	0.12 $\pm$ 0.04d	0.38 $\pm$ 0.03a
26	Benzaldehyde	0.79 $\pm$ 0.15c	0.82 $\pm$ 0.06c	0.98 $\pm$ 0.13bc	1.57 $\pm$ 0.13a	1.15 $\pm$ 0.09b	1.78 $\pm$ 0.14a
Aldehydes (4)		1.72 $\pm$ 0.23c	1.80 $\pm$ 0.09c	2.45 $\pm$ 0.19b	3.76 $\pm$ 0.22a	2.24 $\pm$ 0.12b	3.53 $\pm$ 0.29a
27	Phenol	7.67 $\pm$ 2.78c	4.27 $\pm$ 0.20d	7.57 $\pm$ 0.74c	11.74 $\pm$ 1.06b	6.86 $\pm$ 0.94c	18.13 $\pm$ 1.00a
28	o-Cresol	3.85 $\pm$ 1.16b	4.96 $\pm$ 0.40b	4.48 $\pm$ 0.95b	8.27 $\pm$ 0.90a	5.16 $\pm$ 0.76b	9.43 $\pm$ 0.13a
29	p-Cresol	1.20 $\pm$ 0.12c	6.57 $\pm$ 0.13b	10.65 $\pm$ 2.44a	3.30 $\pm$ 0.23c	6.22 $\pm$ 0.13b	11.22 $\pm$ 1.11a
30	Guaiacol	1.05 $\pm$ 0.10c	nd	1.64 $\pm$ 0.15b	4.61 $\pm$ 0.15a	1.70 $\pm$ 0.12b	1.48 $\pm$ 0.03b
31	4-Ethylphenol	5.71 $\pm$ 1.5c	5.77 $\pm$ 0.38c	6.69 $\pm$ 1.05bc	13.22 $\pm$ 1.17a	7.96 $\pm$ 1.24b	11.96 $\pm$ 0.87a
32	2,4-Dimethylphenol	3.27 $\pm$ 0.53b	2.63 $\pm$ 0.20b	3.94 $\pm$ 0.59b	6.37 $\pm$ 0.92a	3.19 $\pm$ 0.78b	5.36 $\pm$ 0.97a
33	4-Vinylguaiacol	1.51 $\pm$ 0.13c	1.45 $\pm$ 0.11c	nd	3.03 $\pm$ 0.41a	2.10 $\pm$ 0.08b	2.70 $\pm$ 0.15a
Phenols (7)		24.04 $\pm$ 4.18d	25.65 $\pm$ 0.62d	34.97 $\pm$ 4.75c	50.55 $\pm$ 3.58b	33.17 $\pm$ 3.71c	60.29 $\pm$ 2.66a
34	Acetic acid	78.33 $\pm$ 9.05d	43.04 $\pm$ 9.95e	98.34 $\pm$ 7.13c	143.95 $\pm$ 8.11a	74.50 $\pm$ 5.33d	126.62 $\pm$ 7.71b
35	Propionic acid	5.32 $\pm$ 0.68c	5.33 $\pm$ 1.35c	5.93 $\pm$ 0.49c	13.61 $\pm$ 4.12a	8.85 $\pm$ 0.38bc	10.28 $\pm$ 0.73 ab
36	Isovaleric acid	1.36 $\pm$ 0.19cd	0.49 $\pm$ 0.08d	2.12 $\pm$ 0.46bc	3.12 $\pm$ 0.81a	0.99 $\pm$ 0.21d	2.45 $\pm$ 0.49 ab
37	3-Methylvaleric acid	4.61 $\pm$ 1.40d	6.31 $\pm$ 0.44cd	8.51 $\pm$ 1.19c	19.63 $\pm$ 3.24a	17.04 $\pm$ 1.88 ab	14.29 $\pm$ 2.03b
38	Lactic acid	1.74 $\pm$ 0.64d	1.47 $\pm$ 0.33d	4.39 $\pm$ 1.12c	7.82 $\pm$ 1.21b	1.49 $\pm$ 0.29d	10.80 $\pm$ 1.03a
39	Benzoic acid	3.74 $\pm$ 1.07bc	2.77 $\pm$ 0.17c	3.06 $\pm$ 0.86c	4.92 $\pm$ 0.51b	2.71 $\pm$ 0.72c	15.59 $\pm$ 1.56a
40	Octanoic acid	0.83 $\pm$ 0.09b	0.89 $\pm$ 0.14b	0.91 $\pm$ 0.21b	1.51 $\pm$ 0.32a	1.47 $\pm$ 0.28a	1.28 $\pm$ 0.22 ab
41	Phenylacetic acid	4.75 $\pm$ 0.68b	2.26 $\pm$ 0.44c	3.93 $\pm$ 0.35bc	8.38 $\pm$ 1.69a	2.70 $\pm$ 0.31bc	7.71 $\pm$ 1.67a
42	Myristic acid	3.00 $\pm$ 0.77 ab	1.78 $\pm$ 0.31b	1.97 $\pm$ 0.39b	3.82 $\pm$ 0.96a	1.93 $\pm$ 0.57b	3.82 $\pm$ 0.96a
43	Palmitic acid	25.10 $\pm$ 2.61b	8.74 $\pm$ 0.57c	24.17 $\pm$ 5.20b	45.95 $\pm$ 1.89a	12.27 $\pm$ 2.36c	42.26 $\pm$ 3.30a
Acids (10)		128.78 $\pm$ 8.25bc	73.06 $\pm$ 13.27d	153.35 $\pm$ 10.55b	252.70 $\pm$ 20.89a	123.96 $\pm$ 10.68c	235.10 $\pm$ 13.97a
44	Dihydroactinidiolide	2.42 $\pm$ 0.25b	2.15 $\pm$ 0.20b	2.22 $\pm$ 0.30b	2.24 $\pm$ 0.24b	2.14 $\pm$ 0.16b	3.40 $\pm$ 0.09a
45	Oxacycloheptadec-8-en-2-one	3.34 $\pm$ 0.08b	3.22 $\pm$ 0.12b	3.76 $\pm$ 0.07b	4.42 $\pm$ 0.52a	3.71 $\pm$ 0.47b	4.70 $\pm$ 0.36a
46	Sclareolide	1.65 $\pm$ 0.12d	3.52 $\pm$ 0.34c	4.15 $\pm$ 0.54bc	7.11 $\pm$ 0.39a	4.80 $\pm$ 0.77bc	5.52 $\pm$ 1.42b
Esters (3)		7.40 $\pm$ 0.44d	8.89 $\pm$ 0.54cd	10.13 $\pm$ 0.73bc	13.77 $\pm$ 0.14a	10.65 $\pm$ 1.08b	13.62 $\pm$ 1.23a
47	Quinoline	0.68 $\pm$ 0.16d	0.82 $\pm$ 0.07d	1.09 $\pm$ 0.05c	1.60 $\pm$ 0.07a	1.23 $\pm$ 0.17bc	1.33 $\pm$ 0.03b
48	Nicotine	538.27 $\pm$ 165.75c	507.52 $\pm$ 67.80c	381.55 $\pm$ 103.50c	768.77 $\pm$ 51.57b	567.81 $\pm$ 81.00bc	1048.32 $\pm$ 131.78a
49	Myosmine	37.28 $\pm$ 14.57a	12.33 $\pm$ 3.76c	19.72 $\pm$ 5.48bc	20.40 $\pm$ 6.64bc	11.24 $\pm$ 1.32c	28.21 $\pm$ 4.07 ab

(continued on next page)

Table 2 (continued)

No.	Compound	Content ( $\mu\text{g/g}$ )					
		SD	WG	GA	MT	RJ	GW
50	$\beta$ -Nicotyrine	15.31 $\pm$ 7.34bc	12.69 $\pm$ 1.78bc	15.75 $\pm$ 4.39bc	19.08 $\pm$ 5.52 ab	9.55 $\pm$ 1.08c	26.16 $\pm$ 3.90a
51	2,3'-Bipyridine	19.02 $\pm$ 5.10bc	14.69 $\pm$ 1.82c	19.45 $\pm$ 3.21bc	26.54 $\pm$ 8.27b	17.43 $\pm$ 1.33bc	39.03 $\pm$ 5.63a
52	Cotinine	12.39 $\pm$ 3.26bc	7.36 $\pm$ 0.57c	11.49 $\pm$ 2.79bc	14.67 $\pm$ 4.73b	12.06 $\pm$ 0.62bc	22.69 $\pm$ 2.46a
Alkaloids (6)		622.94 $\pm$ 179.31c	555.43 $\pm$ 68.94c	449.05 $\pm$ 109.91c	851.06 $\pm$ 39.53b	619.31 $\pm$ 83.43c	1165.73 $\pm$ 118.10a
53	2,5-Dimethylfuran	nd	0.07 $\pm$ 0.00c	0.12 $\pm$ 0.02b	0.07 $\pm$ 0.01c	0.06 $\pm$ 0.01c	0.35 $\pm$ 0.06a
54	Pyridine	2.29 $\pm$ 0.47bc	3.76 $\pm$ 0.61a	2.10 $\pm$ 0.11c	2.25 $\pm$ 0.56bc	3.02 $\pm$ 0.42 ab	2.78 $\pm$ 0.32bc
55	Pyrrrole	8.23 $\pm$ 1.12b	15.94 $\pm$ 2.36a	8.77 $\pm$ 0.78b	9.53 $\pm$ 1.84b	16.22 $\pm$ 1.45a	7.22 $\pm$ 0.51b
56	2-Picoline	1.52 $\pm$ 0.47bc	3.45 $\pm$ 0.59a	1.83 $\pm$ 0.15bc	2.14 $\pm$ 0.52b	3.24 $\pm$ 0.51a	1.16 $\pm$ 0.10c
57	2-Methylpyrazine	0.36 $\pm$ 0.05c	2.67 $\pm$ 0.34a	0.83 $\pm$ 0.29bc	0.68 $\pm$ 0.26bc	2.17 $\pm$ 0.30a	0.89 $\pm$ 0.12b
58	3-Methylpyrrole	1.55 $\pm$ 0.28bc	2.72 $\pm$ 0.50a	1.30 $\pm$ 0.14c	2.05 $\pm$ 0.37b	3.00 $\pm$ 0.34a	2.06 $\pm$ 0.17b
59	3-Picoline	11.42 $\pm$ 2.89b	20.94 $\pm$ 2.98a	9.60 $\pm$ 0.45b	9.94 $\pm$ 1.19b	13.10 $\pm$ 1.41b	13.33 $\pm$ 1.09b
60	2,6-Lutidine	0.89 $\pm$ 0.35c	1.65 $\pm$ 0.39 ab	1.30 $\pm$ 0.27bc	1.58 $\pm$ 0.17 ab	1.96 $\pm$ 0.21a	0.98 $\pm$ 0.10c
61	2-Ethylpyridine	0.28 $\pm$ 0.04c	0.61 $\pm$ 0.10b	0.33 $\pm$ 0.02c	0.44 $\pm$ 0.08c	0.85 $\pm$ 0.12a	0.65 $\pm$ 0.07b
62	2-Acetylfuran	1.53 $\pm$ 0.19e	2.16 $\pm$ 0.14cd	2.66 $\pm$ 0.35bc	3.18 $\pm$ 0.35b	3.79 $\pm$ 0.13a	1.69 $\pm$ 0.31de
63	2,6-Dimethylpyrazine	0.29 $\pm$ 0.08c	1.78 $\pm$ 0.19b	1.61 $\pm$ 0.65b	1.22 $\pm$ 0.33b	2.94 $\pm$ 0.06a	1.68 $\pm$ 0.17b
64	Ethylpyrazine	0.69 $\pm$ 0.17bc	0.95 $\pm$ 0.11 ab	0.59 $\pm$ 0.16bc	0.81 $\pm$ 0.15 abc	1.13 $\pm$ 0.35a	0.56 $\pm$ 0.06c
65	2,5-Dimethylpyrrole	1.25 $\pm$ 0.13b	1.51 $\pm$ 0.06b	1.51 $\pm$ 0.15b	2.11 $\pm$ 0.22a	2.30 $\pm$ 0.43a	1.30 $\pm$ 0.14b
66	2,3-Dimethylpyrazine	0.22 $\pm$ 0.07c	0.84 $\pm$ 0.06a	0.42 $\pm$ 0.13bc	0.48 $\pm$ 0.19b	1.04 $\pm$ 0.09a	0.55 $\pm$ 0.04b
67	2-Ethylpyrrole	0.41 $\pm$ 0.06d	0.80 $\pm$ 0.17bc	0.59 $\pm$ 0.04cd	0.98 $\pm$ 0.15b	1.40 $\pm$ 0.17a	0.51 $\pm$ 0.10d
68	2,4-Lutidine	1.75 $\pm$ 0.82bc	2.36 $\pm$ 0.20 ab	1.29 $\pm$ 0.12c	1.83 $\pm$ 0.39bc	2.71 $\pm$ 0.45a	1.02 $\pm$ 0.09c
69	2,5-Dimethylpyridine	1.05 $\pm$ 0.15c	2.52 $\pm$ 0.13a	0.75 $\pm$ 0.06c	1.65 $\pm$ 0.11b	2.57 $\pm$ 0.14a	1.87 $\pm$ 0.32b
70	2-Vinylpyridine	nd	0.77 $\pm$ 0.07b	0.47 $\pm$ 0.08c	nd	0.95 $\pm$ 0.18a	nd
71	2,3-Lutidine	0.64 $\pm$ 0.06d	1.32 $\pm$ 0.08b	1.05 $\pm$ 0.11c	1.14 $\pm$ 0.13bc	1.35 $\pm$ 0.10b	2.31 $\pm$ 0.20a
72	3-Ethylpyridine	2.49 $\pm$ 0.78c	5.53 $\pm$ 0.61a	2.76 $\pm$ 0.14c	2.72 $\pm$ 0.15c	4.33 $\pm$ 0.29b	5.45 $\pm$ 0.84a
73	4-Vinylpyridine	6.67 $\pm$ 1.78c	15.58 $\pm$ 1.72a	7.16 $\pm$ 1.30c	6.76 $\pm$ 0.88c	11.43 $\pm$ 1.33b	12.10 $\pm$ 1.18b
74	2,4,6-Trimethylpyridine	0.54 $\pm$ 0.10d	1.46 $\pm$ 0.14b	0.66 $\pm$ 0.10cd	0.85 $\pm$ 0.11c	1.86 $\pm$ 0.11a	0.67 $\pm$ 0.05cd
75	2-Ethyl-6-methylpyrazine	0.94 $\pm$ 0.10d	2.69 $\pm$ 0.24b	1.23 $\pm$ 0.08d	1.70 $\pm$ 0.16c	3.99 $\pm$ 0.29a	1.83 $\pm$ 0.15c
76	3-Ethyl-2,4-dimethylpyrrole	nd	nd	nd	nd	0.29 $\pm$ 0.08a	nd
77	3-Hydroxypyridine	17.82 $\pm$ 6.41ab	18.61 $\pm$ 4.52ab	11.76 $\pm$ 3.72b	23.68 $\pm$ 4.12a	21.56 $\pm$ 1.95a	16.29 $\pm$ 1.06 ab
78	Indole	10.78 $\pm$ 0.87c	5.13 $\pm$ 1.11d	12.7 $\pm$ 0.70bc	21.88 $\pm$ 0.83a	15.83 $\pm$ 0.77b	24.60 $\pm$ 3.72a
79	3-Methylindole	8.15 $\pm$ 0.41c	7.71 $\pm$ 0.76c	7.08 $\pm$ 0.55c	12.87 $\pm$ 0.54b	7.53 $\pm$ 0.07c	20.29 $\pm$ 1.98a
80	2,5-Dimethylindole	1.65 $\pm$ 0.41ab	1.06 $\pm$ 0.08b	1.37 $\pm$ 0.29 b	2.08 $\pm$ 0.89a	1.14 $\pm$ 0.14 ab	1.29 $\pm$ 0.30 ab
81	2,3-Dimethylindole	4.80 $\pm$ 1.16b	4.40 $\pm$ 0.37b	5.78 $\pm$ 1.04ab	7.82 $\pm$ 2.17a	5.02 $\pm$ 0.88b	6.08 $\pm$ 0.37 ab
82	3-(1H-Pyrrol-2-yl)pyridine	7.60 $\pm$ 1.94a	4.59 $\pm$ 0.72b	3.80 $\pm$ 1.32b	4.41 $\pm$ 0.62b	2.65 $\pm$ 0.16b	7.66 $\pm$ 0.98a
83	9H-Pyrrodo [3,4-b]indole	2.31 $\pm$ 0.56c	2.68 $\pm$ 0.73bc	2.09 $\pm$ 0.22c	3.66 $\pm$ 1.03 ab	3.60 $\pm$ 0.51 ab	4.83 $\pm$ 0.42a
Heterocycles (31)		98.13 $\pm$ 15.93b	136.29 $\pm$ 12.07a	93.50 $\pm$ 7.92b	130.52 $\pm$ 11.52a	143.02 $\pm$ 6.61a	142.01 $\pm$ 11.28a
84	Styrene	0.65 $\pm$ 0.21bc	1.64 $\pm$ 0.37a	0.73 $\pm$ 0.16bc	1.05 $\pm$ 0.11b	2.06 $\pm$ 0.41a	0.43 $\pm$ 0.08c
85	Limonene	4.99 $\pm$ 1.41b	14.60 $\pm$ 3.72a	4.00 $\pm$ 0.82b	4.87 $\pm$ 0.99b	11.68 $\pm$ 4.43a	2.30 $\pm$ 0.33b
86	(4E,6Z)-2,6-dimethylocta-2,4,6-triene	1.00 $\pm$ 0.31c	1.68 $\pm$ 0.13 ab	1.05 $\pm$ 0.09bc	1.47 $\pm$ 0.06 abc	1.41 $\pm$ 0.69 abc	1.90 $\pm$ 0.12a
87	$\alpha$ -curcumene	1.61 $\pm$ 0.22a	1.40 $\pm$ 0.14a	1.43 $\pm$ 0.27a	1.70 $\pm$ 0.25a	1.34 $\pm$ 0.14a	1.35 $\pm$ 0.36a
88	(+)- $\delta$ -Cadinene	nd	nd	nd	9.78 $\pm$ 4.25a	3.44 $\pm$ 0.51b	nd
89	Neophytadiene	69.18 $\pm$ 11.74cd	58.15 $\pm$ 7.27cd	71.36 $\pm$ 7.16d	111.13 $\pm$ 12.38a	83.39 $\pm$ 12.09bc	93.88 $\pm$ 9.03 ab
Alkenes (6)		77.42 $\pm$ 12.02c	77.47 $\pm$ 10.79c	78.57 $\pm$ 7.47bc	130.00 $\pm$ 15.80a	103.31 $\pm$ 17.45b	99.85 $\pm$ 8.77bc
90	Dodecane	1.31 $\pm$ 0.37bc	2.57 $\pm$ 0.14a	1.10 $\pm$ 0.19c	2.05 $\pm$ 0.48a	2.61 $\pm$ 0.59a	2.02 $\pm$ 0.12 ab
91	Tridecane	1.81 $\pm$ 0.27d	3.30 $\pm$ 0.05bc	2.57 $\pm$ 0.11cd	4.05 $\pm$ 0.83ab	4.46 $\pm$ 0.37a	3.29 $\pm$ 0.19bc
92	Tetradecane	2.06 $\pm$ 0.36bc	3.19 $\pm$ 0.29 ab	2.27 $\pm$ 0.30bc	2.72 $\pm$ 1.40ab	3.71 $\pm$ 0.63a	1.34 $\pm$ 0.08c
93	Pentadecane	1.92 $\pm$ 0.55b	1.87 $\pm$ 0.09b	2.74 $\pm$ 0.45 ab	3.62 $\pm$ 1.69a	2.67 $\pm$ 0.35 ab	1.57 $\pm$ 0.11b
94	Hexadecane	1.38 $\pm$ 0.18b	1.19 $\pm$ 0.03b	1.60 $\pm$ 0.37b	2.89 $\pm$ 0.76a	1.61 $\pm$ 0.14b	2.75 $\pm$ 0.35a
95	Eicosane	3.64 $\pm$ 0.78a	2.66 $\pm$ 0.82a	3.00 $\pm$ 0.54a	3.18 $\pm$ 0.81a	2.89 $\pm$ 1.12a	3.58 $\pm$ 0.34a
96	Heneicosane	1.91 $\pm$ 0.67b	2.31 $\pm$ 0.17 ab	2.03 $\pm$ 0.54b	3.05 $\pm$ 0.64a	2.47 $\pm$ 0.24 ab	1.93 $\pm$ 0.32b
97	Docosane	2.43 $\pm$ 0.85c	3.07 $\pm$ 0.41bc	3.95 $\pm$ 1.16b	6.17 $\pm$ 0.37a	4.10 $\pm$ 0.80b	5.92 $\pm$ 0.25a
Alkanes (8)		16.47 $\pm$ 3.02c	20.15 $\pm$ 1.17bc	19.25 $\pm$ 2.48bc	27.74 $\pm$ 4.16a	24.52 $\pm$ 3.72 ab	22.40 $\pm$ 0.76 ab
Total (97)		1035.18 $\pm$ 215.17c	950.90 $\pm$ 87.50c	892.33 $\pm$ 124.25c	1533.97 $\pm$ 58.02b	1119.44 $\pm$ 125.40c	1816.10 $\pm$ 145.51a

The same letters within the same row were not significantly different at the 95% confidence level.  
nd: not detected.

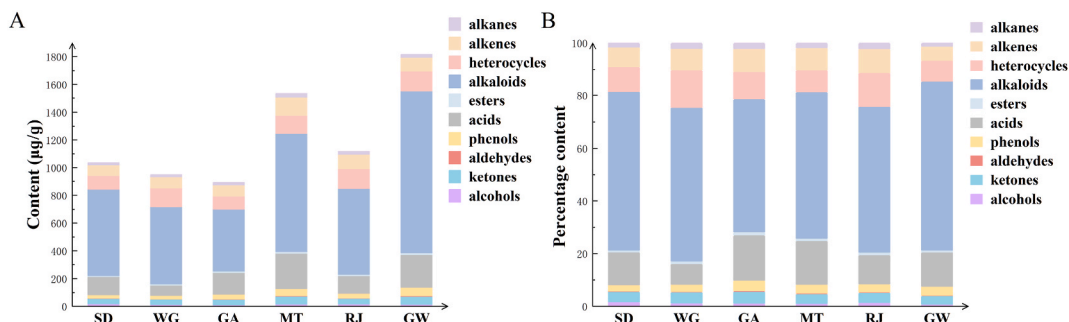


Fig. 2. Content (A) and percentage content (B) of aroma components in the six cigars.

considered to be important aroma components in cigar smoke, including 2,3-butanedione, isovaleraldehyde, 2-methylbutanal, styrene, 6-methyl-5-hepten-2-one, 2-ethyl-6-methylpyrazine, limonene, acetophenone, p-cresol, guaiacol, 4-ethylphenol, 2,4-dimethylphenol, geranial acetone, cedar alcohol, phytol, 3-methylpentanoic acid, and phenylacetic acid.

By grouping the OAV of the compounds exhibiting similar odor descriptors into an aromatic series, establishing aroma profiles of cigars containing several aromatic series [33]. Accordingly, to further understand the differences of the aroma compounds of six cigars, the figure on aroma properties of cigars was obtained and the coordinates were sum of OAV of the aroma compounds in the same odor types with natural logarithm computation. Eight odor types were confirmed based on their odor descriptors including nutty, burnt-sweet, smoky, woody, floral, fruity, creamy, and sour. Some compounds with nutty aroma also have coffee or cocoa aroma, these compounds are classified in the nutty category. Honey-sweet aroma is difficult to classify. Most of the compounds with sweet aroma also have floral or fruity aroma, these compounds are classified in the floral or fruity category. As can be seen in Fig. 3, nutty, smoky, and woody were the primary characteristic odors of cigars. The strongest woody attribute was found in the SD, consistent with the sensory evaluation outlined above, might be from the contribution of cedrol with high OAV. The strongest nutty attribute was found in MT, consistent with the stronger nutty coffee aromas of MT in the sensory evaluation. This could be due to the contribution of 2-methylbutyraldehyde at high OAV. GA, GW, and MT had a stronger burnt-sweet aroma than that in SD, WG, and RJ, in agreement with the sensory evaluation described above, might be from the contribution of methyl cyclopentenolone.

### 3.3. Aroma difference analysis of six cigars

#### 3.3.1. Multivariate statistical analysis

Data analysis mainly includes supervised analysis and unsupervised analysis. Unsupervised analysis can cluster samples in the absence of sample information, such as by HCA. Supervised analysis methods, such as OPLS-DA, filter out information irrelevant to classification through orthogonal signal correction technology. HCA and OPLS-DA will be combined to discuss the aroma difference of cigars here.

The HCA based on 97 aroma components detected via GC-MS is shown in Fig. 4. It shows that it can be divided into three groups at

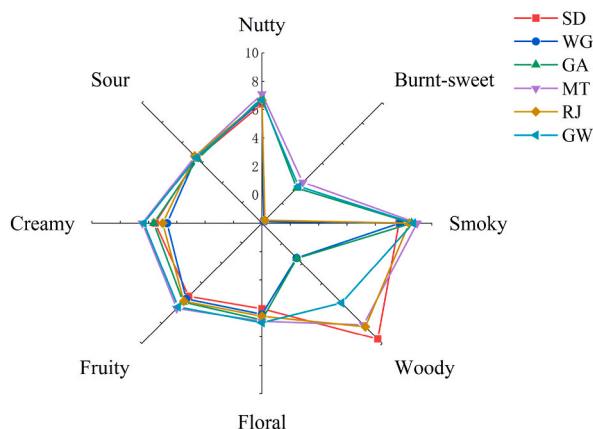


Fig. 3. Aromatic series of six cigars. The coordinates in the figure were OAV with natural logarithmic computation.



the Euclidean distance of 16, WG and RJ can be regarded as a group, GA and SD can be regarded as a group, and GW and MT can be regarded as a group. The samples were not categorized according to domestic and imported cigars because the clustering was based on compounds that to some extent reflect the aroma characteristics of the cigars. From the sensory evaluation, the two imported cigars MT and RJ had completely different aroma characteristics, while the domestic cigar GW and the imported cigar MT both had an obvious nutty aroma, which were grouped together by clustering. Therefore, the HCA cluster analysis reflected the similarity of the aroma characteristics of the six cigars. WG and RJ had similar aromas, GA and SD had similar aromas, and GW and MT had similar aromas.

To further analyze the differences in aroma components among the six cigars, we conducted OPLS-DA on the volatile and semi-volatile components. Quality parameters of the generated OPLS-DA model indicated the fit index of the independent variable ( $R^2X$ ) in this analysis was 0.903, the fit index of the dependent variable ( $R^2Y$ ) was 0.988, and the model prediction index ( $Q^2$ ) was 0.939.  $R^2$  and  $Q^2$  exceed 0.5, indicating that the model fit results were acceptable [34]. After 200 permutation tests, as shown in Fig. 5B, the intersection of the  $Q^2$  regression line with the vertical axis was less than zero, indicating that there was no overfitting of the model and the model was reliable.

As shown in Fig. 5A, the six cigar samples were well separated. As shown in Fig. 6, 39 characteristic aroma components in cigars were screened based on  $P < 0.05$  and  $VIP > 1$ , including 2 alcohols, 11 ketones, 1 aldehyde, 1 acid, 3 phenols, 1 ester, 1 alkene, and 19 heterocycles. The heat map of the characteristic aroma components is shown in Fig. 7. Compared to the other cigars, the three aroma components with high content were cedrol, myosmine, and 3-(1H-pyrrol-2-yl)pyridine in SD. The contents of 7 aroma components were higher in WG, such as 6-methyl-5-hepten-2-one, pyridine, 3-picoline, 2-methylpyrazine. The contents of 6-methyl-5-hepten-2-one and p-cresol were relatively high in GA. The contents of 9 aroma components were higher in MT, such as guaiacol, 3-ethyl-2-hydroxy-2-cyclopenten-1-one, methyl cyclopentenolone, 2-methylbutyraldehyde. The contents of 11 aroma components were higher in RJ, such as 2-heptanone, 2'-methylacetophenone, 2,6-dimethylpyrazine. There were 7 aroma components with higher content in GW, including benzoic acid, dihydroactinidiolide, 3-methyl-2-cyclopenten-1-one, p-cresol, geranylacetone, 2,3-lutidine, 2,5-dimethylfuran. The differences in the content of aroma compounds are highly dependent on the composition of the tobacco leaves and the production process [5,35].

### 3.3.2. Aroma difference analysis combined with OAV

The 39 characteristic aroma components were screened according to  $P < 0.05$  and  $VIP > 1$ . Combined with the OAV for further analysis, there were 14 aroma components with  $OAV > 1$  (Table 3). These key aroma components may play an important role in determining the aroma characteristics of the six cigars.

A comparison of the OAV of the 14 key aroma components in the six cigars. Cedrol (14140.41) was more prominent in SD, the OAV of cedrol was more than three times that of other cigars, highlighting the woody aroma of SD. 6-methyl-5-hepten-2-one (11.23), pyridine (1.88), and 2-ethyl-6-methylpyrazine (67.28) were more prominent in WG. 6-methyl-5-hepten-2-one has fruity and fresh-sweet aromas, making the sweet aroma more refreshing and clean. The more prominent OAV were p-cresol (2731.20), 2-methylbutyraldehyde (887.33), and methyl cyclopentenolone (4.17) in GA, having smoky, nutty, coffee and burnt-sweet aromas. Cedrol (3393.07), 2-methylbutyraldehyde (1168.67), guaiacol (5491.27), 4-vinylguaiacol (252.41), and methyl cyclopentenolone (7.30) were more prominent in MT, with woody, nutty, coffee, smoky, and burnt-sweet aromas. The more prominent OAV were cedrol (4095.91), 2-ethyl-6-methylpyrazine (99.78), 2,6-dimethylpyrazine (4.10), phenethyl alcohol (2.16), and 2-heptanone (1.59) in RJ, having woody, nutty, floral, and sweet aromas. p-Cresol (2875.64), phenethyl alcohol (2.25), geranylacetone (103.96), methyl cyclopentenolone (4.67), and dihydroactinidiolide (6.80) were more prominent in GW, with smoky, floral, sweet, burnt-sweet, and woody aromas. The results show that the aroma profiles of the more prominent key aroma components of each cigar are consistent with their

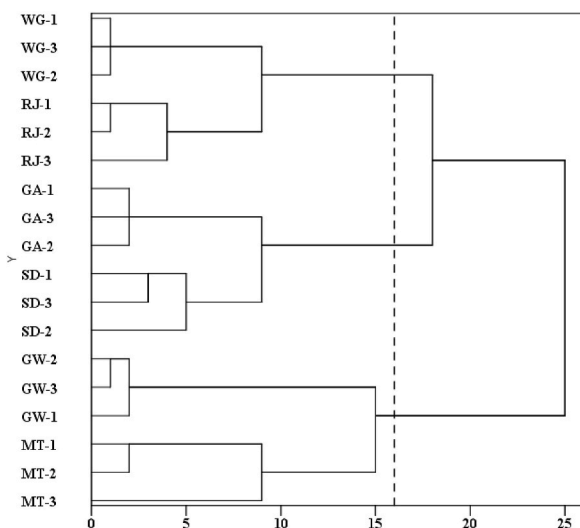


Fig. 4. Cluster analysis results based on aroma compounds of cigars.

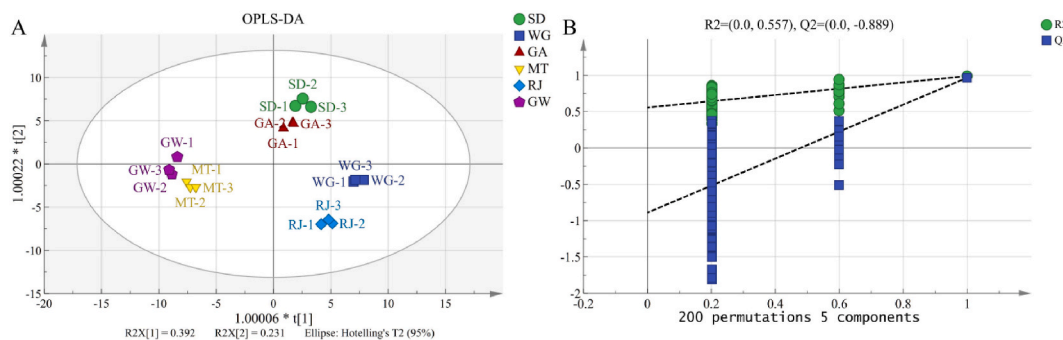


Fig. 5. Orthogonal partial least squares discriminant analysis (A), permutation test (B) of six cigars.

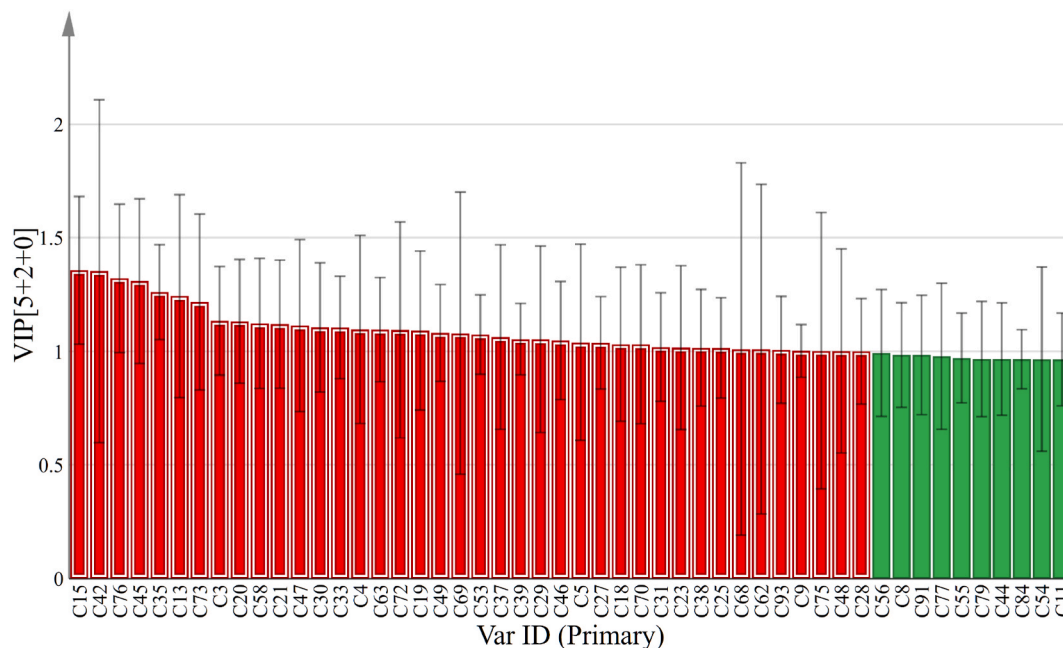


Fig. 6. Compounds satisfying VIP > 1 of six cigars (the aroma compounds represented by the numbers are shown in Table S1).

main aroma profiles in the sensory evaluation. These key aroma compounds may have contributed to the different stylistic profiles of the six cigars.

### 3.3.3. Relationship between key aroma components and sensory attributes

Spearman correlation analysis was performed on the OAV results of key aroma components and sensory data, and the correlation heat map was plotted (Fig. 8). The Spearman correlation coefficients revealed that sensory qualities and the key aroma components had a strong correlation. 2-Methylbutyraldehyde (nutty, coffee, cocoa), geranylacetone (floral, sweet), methyl cyclopentenolone (burnt-sweet), and guaiacol (smoky, fermented) correlated positively ( $r > 0.5$ ,  $p < 0.05$ ) with nutty and burnt-sweet aromas. Corresponding to the results of Table 2, these aroma compounds involved above showed the higher contents and OAV in the GA, MT, and GW, they contributed to the prominent nutty and burnt-sweet attributes of GA, MT, and GW. 2-Heptanone (sweet) positively correlated with honey-sweet and floral aromas, and was detected only in RJ, it significantly affected the honey-sweet aroma of RJ. Cedrol (woody) positively correlated with honey-sweet, bean, and woody aromas. 2-Ethyl-6-methylpyrazine (nutty) was negatively correlated with woody aroma. Cedrol showed the highest content and OAV in SD, 2-ethyl-6-methylpyrazine showed the lowest content and OAV in SD, they contributed to the prominent woody aroma of SD. 6-Methyl-5-hepten-2-one (fruity, fresh-sweet) and pyridine (pungent) were positively correlated with fresh-sweet aroma, especially 6-methyl-5-hepten-2-one showed the stronger Spearman correlation coefficients. 6-Methyl-5-hepten-2-one showed the highest content and OAV in WG, which was consistent with the sensory results that WG had stronger fresh-sweet aroma. Furthermore, p-cresol (smoky) was negatively correlated with bean aroma. p-Cresol showed the higher content and OAV in GA and GW, which was consistent with the sensory results that GA and GW had weaker bean aroma. According to Spearman correlation coefficients ( $|r| > 0.5$ ,  $p < 0.05$ ), these key aroma components were generally considered

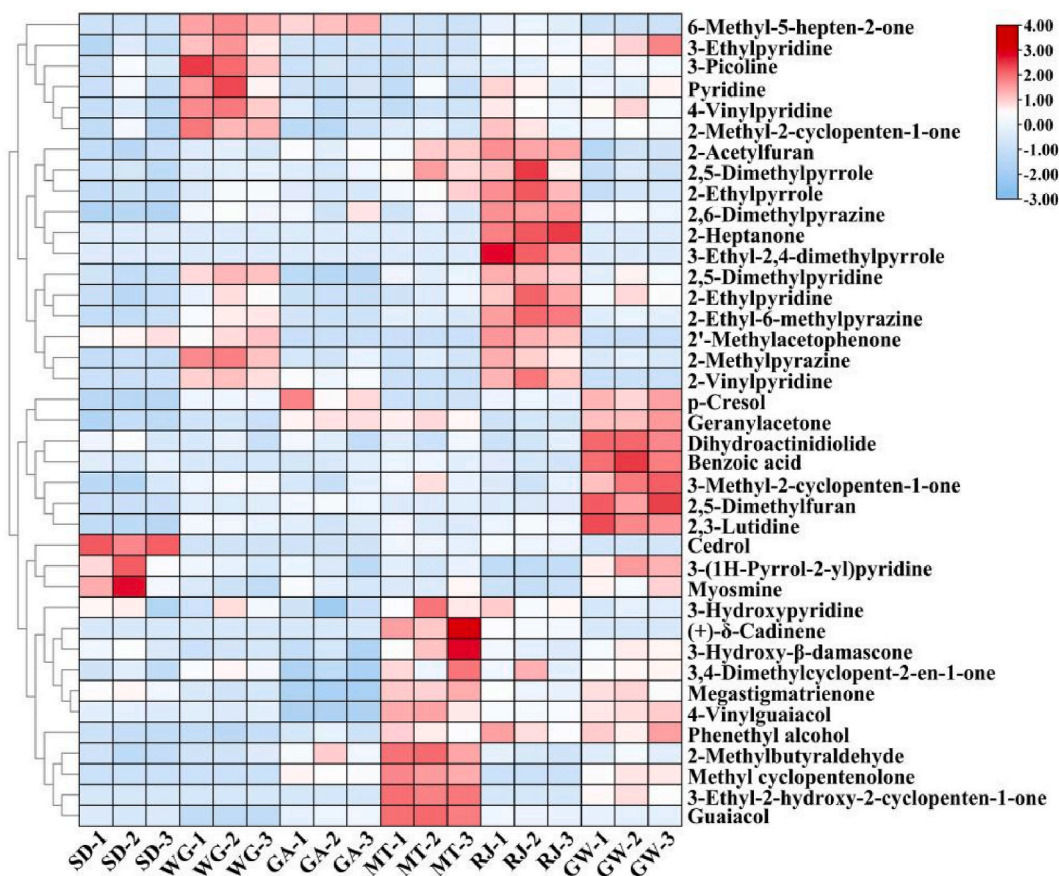


Fig. 7. Heat map of differential components satisfying  $VIP > 1, P < 0.05$  of six cigars.

Table 3

OAV of key aroma components in the six cigars.

Compound	OAV						Odor description
	SD	WG	GA	MT	RJ	GW	
2-Heptanone	0.00	0.00	0.00	0.00	1.59	0.00	sweet
Cedrol	14140.41	4.31	4.44	3393.07	4095.91	384.14	woody
p-Cresol	308.89	1684.02	2731.20	846.58	1593.93	2875.64	smoky
6-Methyl-5-hepten-2-one	0.00	11.23	9.56	0.00	3.07	0.00	fruity, fresh-sweet
2-Methylbutyraldehyde	569.33	655.00	887.33	1168.67	683.00	756.47	nutty, coffee, cocoa
4-Vinylguaiaicol	125.24	120.30	0.00	252.41	175.10	225.01	smoky, fermented
2,6-Dimethylpyrazine	0.41	2.48	2.24	1.70	4.10	2.34	nutty, coffee
Phenethyl alcohol	0.94	0.84	1.23	2.02	2.16	2.25	floral, sweet
Geranylacetone	25.58	40.70	87.36	86.40	43.09	103.96	floral, sweet
Methyl cyclopentenolone	0.00	0.00	4.16	7.30	0.00	4.67	burnt-sweet
Guaiacol	1250.79	0.00	1952.38	5491.27	2020.24	1765.57	smoky, fermented
Pyridine	1.14	1.88	1.05	1.13	1.51	1.39	pungent
Dihydroactinidiolide	4.83	4.31	4.44	4.47	4.28	6.80	woody
2-Ethyl-6-methylpyrazine	23.54	67.28	30.68	42.45	99.78	45.87	nutty

significant and were mostly responsible for the different stylistic profiles of the six cigars.

#### 4. Conclusions

In this study, GC-MS and sensory evaluation were used to analyze and compare the aroma components of four types of domestic cigars and two types of Cuban cigars. Sensory evaluation revealed that the six cigars were apparent differences in their main aroma characteristics. SD had more obvious woody and honey-sweet aroma profiles; RJ had pronounced floral and honey-sweet aroma profiles; the hay and fresh-sweet aromas of WG were relatively strong; MT had prominent nutty, coffee, and bean aroma profiles; GA

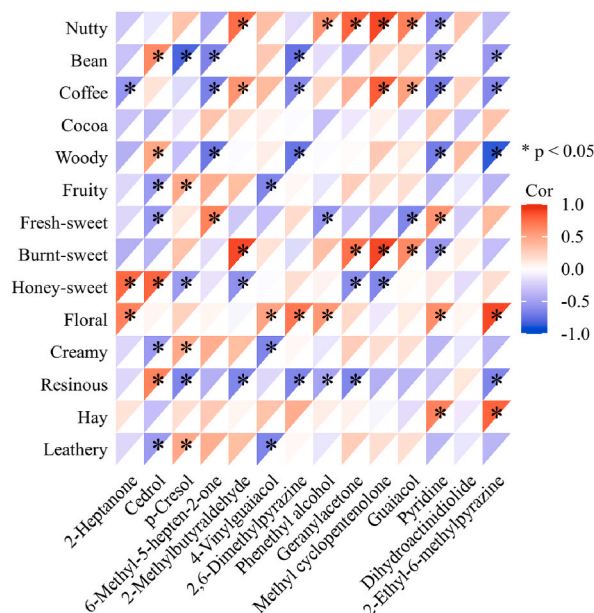


Fig. 8. Correlation heat map between the key aroma components and sensory attributes.

had pronounced nutty and woody aroma profiles; GW had more obvious nutty, woody, and floral aroma profiles. A total of 97 aroma components were detected in six cigars. Compared with domestic cigars, imported cigars had suitable nicotine content, and higher contents of phytol, neophytadiene, 3-methylpentanoic acid, and (+)- $\delta$ -cadinene. To further explore the differences in aroma components of the six cigars, 14 key aroma components were screened on the basis of  $P < 0.05$ ,  $VIP > 1$ , and  $OAV > 1$ . The key aroma components of each cigar were obtained by comparing the OAV of the 14 key aroma components in six cigars, SD: cedrol; WG: 6-methyl-5-hepten-2-one, pyridine, 2-ethyl-6-methylpyrazine; GA: p-cresol, 2-methylbutyraldehyde, methyl cyclopentenolone; MT: cedrol, 2-methylbutyraldehyde, guaiacol, 4-vinylguaiacol, methyl cyclopentenolone; RJ: cedrol, 2,6-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-heptanone, phenethyl alcohol; GW: p-cresol, phenethyl alcohol, geranylacetone, methyl cyclopentenolone, dihydroactinidiolide. In addition, correlation analysis showed that key aroma components in six cigars were significantly correlated with sensory attributes. This study initially explores the differences in aroma composition and stylistic characteristics of cigars. To some extent, these results provide data support and lay a foundation for improving the quality of domestic cigars.

#### Data availability statement

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

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#### CRediT authorship contribution statement

**Lin Yang:** Writing – original draft. **Lulu Liu:** Methodology, Data curation. **Lingbo Ji:** Writing – review & editing. **Chenxi Jiang:** Methodology, Data curation. **Zhongrong Jiang:** Methodology, Data curation. **Dongliang Li:** Writing – review & editing. **Zhen Yang:** Writing – review & editing. **Wen Cai:** Writing – review & editing. **Quanwei Zhou:** Writing – review & editing. **Jinshan Lei:** Methodology, Data curation. **Pinhe Li:** Writing – review & editing. **Yuhong Jia:** Writing – review & editing. **Jie Liu:** Methodology, Data curation. **Heng Xu:** Resources. **Jun Hu:** Supervision, Resources.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e26630>.

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