

## Changes of Major Chemical Compositions During Industrial Fermentation of Domestic Cigar Tobacco and Comparative Analysis with Imported Cigar Leaves

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### Abstract

Chemical substances of tobacco raw materials are closely related to product style and aroma quality. In this experiment, the alkaloid content, amino acid content, non-volatile organic acids and aroma-causing substances of domestic cigar tobacco were dynamically tested by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), and compared with those of Dominican cigar tobacco during the fermentation process. Under the proposed fermentation conditions, the alkaloid content of domestic cigar tobacco showed an overall decreasing trend, and the alkaloid content at the end of fermentation was lower than that of Dominican cigar tobacco, with a difference of 5.74%; the amino acid content at the end of fermentation was lower than that of the pre-fermentation by 90.60%, with a difference of 0.11% with that of the Dominican cigar tobacco; and the content of non-volatile organic acid was higher than that of the pre-fermentation by 20.74% at the end of fermentation, with a difference of 5.74% with that of the Dominican cigar tobacco. Dominican cigar leaf; carotenoid degradation products increased by 244.26% at the end of fermentation, which was not much different from Dominican cigar leaf; other aroma-causing products increased by 5.81% at the end of fermentation compared to pre-fermentation, which was 0.23% higher compared to Dominican cigar leaf.

### Keywords

Cigar Tobacco; Chemical Constituent; Aroma Substance; Artificial Fermentation.

### 1. Introduction

Cigar is a tobacco product with a long history, which is traditionally conceptualized as a tobacco roll rolled entirely with tobacco leaves, with a mellow aroma and a translucent sweet, concentrated eating flavor [1]. Unfermented tobacco has irritation and bad flavor, tobacco fermentation is a very important part of industrial processing, the fermentation process not only changes in the appearance of the quality of tobacco, but also further degradation of macromolecules, on the one hand, the decomposition of the aroma precursors and thus increase the amount of aroma and aroma of tobacco, on the other hand, reduce the birth of the miscellaneous gases, so that the tobacco smoking is more mellow and irritation of small [2-5]. There is a close relationship between the chemical base of the tobacco raw material and the style and aroma quality of the product, and the special style and aroma exhibited by cigar tobacco is also the result of the interaction of multiple chemical substances in cigar tobacco [6].

Tobacco alkaloids are a special class of nitrogen-containing organic alkaline substances, which are important quality elements of tobacco and have a greater impact on key sensory qualities such as aroma, amount of aroma, eating flavor, and irritation [7-9]. Amino acids are a class of important chemicals in tobacco, which play an important role in the formation of tobacco flavor substances, and the right amount of amino acids helps to improve the strength and increase the fullness of the smoke [10]. Non-volatile organic acids have no significant direct effect on the aroma in cigarette smoke, but they can combine with alkaloids to form salts, regulate the ratio of protonated and free nicotine, regulate the pH of tobacco, increase the concentration of smoke, improve the absorption of flavors, mellow the smoke, indirectly affect the smoke aroma of cigarettes, and act as a counterbalance in the smoke [11]. The content of carotenoids in tobacco is closely related to the quality of tobacco and is an important prerequisite substance for tobacco aroma components [12]. Degradation of carotenoids produces volatile isophorone,  $\beta$ -damascone, geranylacetone and other aroma substances, and the degradation produces relatively low thresholds of aroma substances, better aroma, and a large contribution to the aroma of tobacco [13-15]. In order to investigate the changes in chemical composition of cigar tobacco leaves during the fermentation process, this experiment used high performance liquid chromatography-tandem mass spectrometry (LC-MS) to dynamically test the changes in alkaloids, amino acids, non-volatile organic acids, and aroma-causing substances of domestic cigar tobacco leaves during the fermentation process.

Compared with cigarettes, cigar quality is more strongly correlated with the quality of tobacco raw materials. Due to the short history of planting, the quality of domestic cigar tobacco leaves has limited the development of the cigar industry in China [7]. The areas in the world suitable for growing high-quality tobacco are very limited, and the foreign high-quality cigar tobacco producing areas include Cuba, Dominican Republic, Ecuador and so on [6]. In this experiment, the domestic cigar tobacco leaves and Dominican cigar tobacco leaves at the end of fermentation were compared and analyzed, in order to provide technical support for the application of domestic cigar tobacco leaves.

## 2. Materials and Methods

### 2.1. Test Materials and Instruments

#### 2.1.1. Experimental Materials

Cigar tobacco leaves from an agricultural fermentation followed by short-term mellowing in a production area in China, and cigar tobacco leaves from an agricultural fermentation followed by short-term mellowing in the Dominican Republic.

#### 2.1.2. Experimental Apparatus and Reagents

### 2.2. Tobacco Fermentation

#### 2.2.1. Tobacco Return

The central cigar tobacco was tied in accordance with the standard of 30~35 pieces/handle, and then placed in the intelligent artificial climate chamber at an interval of 10~15 cm between adjacent handles, under the conditions of relative humidity of 75%~85%, temperature of 25°C~27°C, and refluxing time of 8~10 h, and then atomized refluxing was carried out [2], and the handles were turned over during the period of refluxing to ensure that the tobacco refluxed uniformly, and ultimately, the moisture content of tobacco was controlled in the range of 25%~35% as the sample to be fermented. The final moisture content of the tobacco was controlled in the range of 25%~35%, which was used as the experimental sample to be fermented.

**Table 1.** Experimental instrument

Instrument	Model	Manufacturer
Intelligent Artificial Climate Chamber	RXM - 258B	Ningbo Jiangnan Instrument Factory
Ultra - high Performance Liquid Chromatograph	Thermo Vanquish UHPLC	Thermo Fisher Scientific
High - Resolution Mass Spectrometer	Q - Exactive HF	Thermo Fisher Scientific
Chromatographic Column	C18(1.8 $\mu$ m2.1mm 100mm)	Agilent technologies
Centrifuge	TGL - 16M	Changsha High - tech Industrial Development Zone Xiangyi Centrifuge Instrument Co., Ltd.
Automatic Rapid Sample Grinder	JXFSTPRP - 48	Shanghai Jingxin Industrial Development Co., Ltd.
CNC Ultrasonic Cleaner	KQ - 00DE	Kunshan Ultrasonic Instrument Co., Ltd.
Freeze Dryer	FD - IA - 50	Shanghai Bilanz Instrument Manufacturing Co., Ltd.
Balance	AUW120D	Shimadzu Corporation
Ultra - pure Water Machine	GWB - 2B	Beijing Purkinje General Instrument Co., Ltd.
Vortex Mixer	G560E	Scientific Industries, INC
Methanol	LC - MS	Merck KGaA
Acetonitrile	LC - MS	Merck KGaA
Formic acid	LC - MS	Xiya Reagent
2 - propanol	LC - MS	Merck KGaA

### 2.2.2. Tobacco Fermentation

The samples at the end of refluxing were placed in a constant temperature and humidity incubator for fermentation. The heap was turned once every 24h for a total of 5 times.

### 2.2.3. Sample

Five-point sampling method was used to take samples, and the sample volume was about 50 g. Each sample was sampled six times throughout the fermentation process, including unfermented samples and samples taken at 16:00 every day, and the collected samples were placed in a sterile sampling bag and preserved at -20°C to be analyzed and used.

## 2.3. Test Methods

### 2.3.1. Sample Preparation

Weigh 100 mg of the mixed samples in a 2 mL centrifuge tube, add 1 mL of 70% methanol and 3 mm steel beads, shake and break the samples with an automatic sample fast grinder for 3 min, cool down and low-temperature ultrasonic (40 KHZ) for 10 min, and centrifuge the samples at a low temperature of 12,000 rpm at 4°C for 10 min, and then take the supernatant and dilute it, and then pass through a 0.22  $\mu$ m PTFE filter head for on-line detection.

### 2.3.2. Chromatography-Mass Spectrometry Detection Conditions

For the samples, we used LC-MS technique with a C18 column. The conditions were as follows: the column temperature was 30°C; the flow rate was 0.3 mL/min; the mobile phase consisted of A: 0.1% formic acid aqueous solution, and B: pure acetonitrile; the injection volume was 2  $\mu$ L, and the temperature of the autosampler was 4°C. The elution procedure for the gradient of the mobile phase is shown in Table 2.

**Table 2.** Mobile phase conditions for liquid chromatography

Time (min)	Flow Rate ( $\mu\text{L}/\text{min}$ )	Gradient	B% Acetonitrile	A% Formic Acid
0 - 2	300	-	5	95
2-6	300	Linear Gradient	30	70
6-7	300	-	30	70
7-12	300	Linear Gradient	78	22
12-14	300	-	78	22
14-17	300	Linear Gradient	95	5
17-20	300	-	95	5
20-21	300	Linear Gradient	5	95
21-25	300	-	5	95

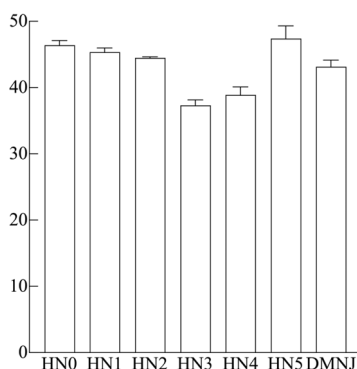
## 2.4. Data Processing and Display

Raw data from three sets of parallel experiments obtained after mass spectrometry analysis were used for substance identification using the Thermo mzCloud online database, the Thermo mzValut local database, and the ChemSpider database, and multivariate statistical analyses were carried out using the normalized data matrices.

## 3. Results and Discussion

### 3.1. Changes of Alkaloid Content During Fermentation of Domestic Cigar Tobacco in Comparison with Dominican Cigar Tobacco

The results of total alkaloid content changes during fermentation of domestic cigar tobacco leaves compared with Dominican cigar tobacco leaves are shown in Figure 1. The alkaloid content of domestic cigar tobacco showed an overall decreasing trend under the proposed fermentation conditions, and the alkaloid content at the end of fermentation was 19.39% lower than that before fermentation. The alkaloid content of domestic cigar tobacco was lower than that of Dominican cigar tobacco at the end of fermentation, and the difference in alkaloid content between the two was 5.74%.



**Fig 1.** Comparison of alkaloid content in Hainan cigar tobacco during fermentation with Dominican cigar tobacco

The results of the changes in the content of major alkaloids during fermentation of domestic cigar tobacco leaves compared with Dominican cigar tobacco are shown in Table 3. The changes in the content of nicotine, niacin and neotame alkaloids during fermentation of domestic cigar tobacco leaves were highly significant ( $p < 0.001$ ), but the contents in the post-fermentation samples were lower than those in the unfermented samples. Among them, the nicotine content

was higher in the domestic cigar tobacco samples, but the nicotine content was significantly reduced by 20.87% at the end of fermentation. After fermentation, the alkaloid contents of domestic cigar tobacco were significantly different from those of Dominican cigar tobacco except for neotobrassine. The contents of nicotine, hypocotinine and cotinine after fermentation of domestic cigar tobacco were lower than that of Dominican cigar tobacco, and the content of neotame was higher than that of Dominican cigar tobacco.

**Table 3.** Comparison of main alkaloid content changes between Hainan cigar tobacco and Dominican cigar tobacco during fermentation

Name	HN0	HN1	HN2	HN3	HN4	HN5	DMNJ
Nicotine	43.65%	42.95%	41.52%	32.06%	33.76%	34.54%	39.70%
Reduced Nicotine	0.98%	0.21%	0.42%	0.39%	0.50%	0.53%	0.81%
Neonicotinoids	0.92%	1.09%	1.29%	1.26%	1.08%	0.86%	0.83%
Cotinine	0.86%	1.09%	1.23%	3.60%	3.55%	1.48%	1.81%

The nicotine content of domestic cigar tobacco leaves was high, and nicotine, descending nicotine, and neotame alkaloids decreased at the end of fermentation, and the decrease of these alkaloids was conducive to the reduction of irritation and the improvement of the sensory quality of cigar tobacco leaves. Among them, nicotine content is one of the important chemical components that determine the quality of tobacco leaves, and the decrease of nicotine content during fermentation may be related to some nicotine-degrading bacteria and the volatilization of some free nicotine [16].

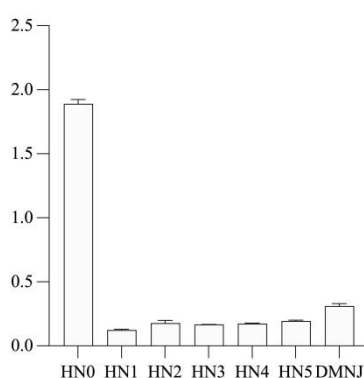
### 3.2. Changes of Amino Acid Content During Fermentation of Domestic Cigar Tobacco in Comparison with Dominican Cigar Tobacco

The results of the changes in the major amino acid contents of domestic cigar tobacco leaves during fermentation compared with those of Dominican cigar tobacco leaves are shown in Table 4. The amino acid content of domestic cigar tobacco showed an overall decreasing trend under the proposed fermentation conditions, and the amino acid content at the end of fermentation was 90.60% lower than that before fermentation. Although the amino acid content of domestic cigar tobacco was still lower than that of Dominican cigar tobacco at the end of fermentation, the amino acid content of the two cigar tobaccos was close to each other, with a difference of about 0.11%.

**Table 4.** Comparison of amino acid content of Hainan cigar tobacco during fermentation with Dominican cigar tobacco

Name	HN0	HN1	HN2	HN3	HN4	HN5	DMNJ
Phenylalanine	1.1058%	0.0519%	0.0519%	0.0311%	0.0238%	0.0464%	0.0540%
Arginine	0.0557%	0.0004%	0.0013%	0.0004%	0.0004%	0.0024%	0.0051%
Proline	0.1139%	0.0547%	0.0187%	0.0155%	0.0378%	0.0174%	0.1111%
Tryptophan	0.0121%	0.0253%	0.0269%	0.0651%	0.0479%	0.0228%	0.0136%
Tyrosine	0.0263%	0.0103%	0.0105%	0.0310%	0.0252%	0.0112%	0.0066%
Isoleucine	0.1649%	0.0009%	0.0324%	0.0295%	0.0450%	0.0388%	0.0448%
Aspartic acid	0.4281%	0.0116%	0.0276%	0.0102%	0.0045%	0.0401%	0.0544%

The results of amino acid content changes during fermentation of domestic cigar tobacco leaves compared with Dominican cigar tobacco leaves are shown in Figure 2. Under the proposed fermentation conditions, the changes in the content of various amino acids in domestic cigar tobacco during fermentation were highly significant ( $p < 0.001$ ), with phenylalanine being the most abundant in the domestic cigar tobacco samples and arginine being less abundant. Except for tryptophan, which showed an increase and then a decrease during the fermentation process, the contents of phenylalanine, arginine, proline, tyrosine, isoleucine and aspartic acid decreased significantly compared with those of the pre-fermentation samples, with decreases of 95.80%, 95.69%, 84.72%, 57.41%, 76.47% and 90.63%, respectively. Among them, the contents of phenylalanine, arginine, isoleucine and aspartic acid, which were the components of domestic cigar tobacco at the end of fermentation, were close to those of Dominican cigar tobacco, while the content of proline was significantly lower than that of Dominican cigar tobacco.



**Fig 2.** Comparison of amino acid content in domestic cigar tobacco during fermentation with Dominican cigar tobacco

The decrease of amino acids is favorable to reduce the strength and irritation of cigar tobacco and improve the quality of cigar. In this study, the decrease of amino acid content in domestically produced cigar tobacco was mainly due to the decrease of phenylalanine, which may be due to the fact that phenylalanine, as an aromatic amino acid, is directly decomposed into flavor compounds (e.g., benzyl alcohol, phenylethanol, etc.) on its own, and they are also the more abundant flavor constituents in tobacco [2]. Domestic cigar tobacco leaves, through fermentation, contained lower levels of phenylalanine, arginine, proline, isoleucine and aspartic acid than Dominican cigar tobacco leaves, except for tyrosine and tryptophan. Among them, the content of phenylalanine, arginine, isoleucine and aspartic acid in domestic cigar tobacco at the end of fermentation did not differ much from that of Dominican cigar tobacco, while the content of proline was significantly lower than that of Dominican cigar tobacco.

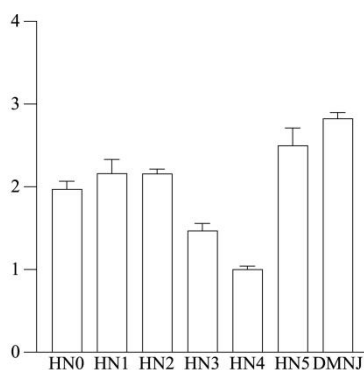
### 3.3. Comparative Analysis of Changes in Nonvolatile Organic Acid Content of Domestic Cigar Tobacco during Fermentation with Dominican Cigar Tobacco

The results of the changes in the content of major non-volatile organic acids during the fermentation of domestic cigar tobacco leaves compared with that of Dominican cigar tobacco leaves are shown in Table 5. Under the proposed fermentation conditions, the non-volatile organic acids of domestic cigar tobacco showed a decreasing and then increasing trend, and the content of non-volatile organic acids at the end of fermentation was 20.74% higher than that before fermentation. Although the non-volatile organic acid content of domestic cigar tobacco after fermentation is still lower than that of Dominican cigar tobacco, the non-volatile organic acid content of the two is very close to that of Dominican cigar tobacco, with a difference of only 0.03315%.

**Table 5.** Comparison of non-volatile organic acid content in Hainan cigar tobacco during fermentation with Dominican cigar tobacco

Name	HN0	HN1	HN2	HN3	HN4	HN5	DMNJ
Palmitic acid	0.105585%	0.130077%	0.089490%	0.188305%	0.170786%	0.181194%	0.114700%
Linoleic acid	0.142187%	0.241758%	0.160246%	0.248099%	0.293153%	0.280385%	0.002655%
Citric acid	0.008511%	0.001886%	0.005917%	0.005420%	0.001627%	0.008449%	0.008449%
Linolenic acid	0.296106%	0.078226%	0.062581%	0.047194%	0.059752%	0.129467%	0.100102%
Gluconic acid	1.705838%	1.786041%	1.902343%	1.021303%	0.536193%	2.127028%	2.533769%

The results of non-volatile organic acid content changes in domestic cigar tobacco during fermentation compared with Dominican cigar tobacco are shown in Figure 3. Under the proposed fermentation conditions, the content of various non-volatile organic acids in domestic cigar tobacco changed significantly during the fermentation process ( $p < 0.001$ ), of which the higher content of non-volatile organic acids was gluconic acid, and gluconic acid had a significant increase at the end of the fermentation, with an increase of 19.80%. Palmitic acid and linoleic acid showed a general trend of increasing content during fermentation, and citric acid content did not change much before and after fermentation. Linolenic acid showed a decreasing trend with the change of fermentation time, and the content decreased by 128.71% at the end of fermentation. After fermentation, the contents of palmitic acid, linoleic acid, citric acid and linolenic acid were higher than those of Dominican cigar tobacco, except gluconic acid. Although the content of gluconic acid in domestic cigar tobacco increased significantly after fermentation, it was still significantly lower than that of Dominican cigar tobacco.

**Fig 3.** Comparison of non-volatile organic acid content in domestic cigar tobacco during fermentation with Dominican cigar tobacco

Non-volatile organic acids have no significant direct effect on the aroma of cigar smoke, but they can combine with alkaloids to form salts, regulate the ratio of protonated and free nicotine, regulate the pH of cigarettes, increase the concentration of smoke, improve the taste of smoke, mellow the smoke and indirectly affect the aroma of cigarette smoke, and act as a counterbalance in the smoke [11]. Palmitic acid can increase the fatty and waxy flavor of cigarette smoke, give the smoke a soft suction flavor, and make the smoke mellow, and its content is positively correlated with the burning quality of tobacco; the content of unsaturated fatty acids, such as linoleic acid and linolenic acid, is negatively correlated with the aroma and eating flavor of the tobacco, and they increase the roughness, irritation, and greenish gas of the smoke, and produce astringent flavor [20]. Therefore, the increase of palmitic acid content and the decrease of linolenic acid content are favorable to improve the sensory quality of domestic cigar tobacco leaves. The content of palmitic acid, linoleic acid and citric acid of domestic cigar

tobacco leaves through fermentation is still lower than that of Dominican cigar tobacco leaves, so how to increase the content of these organic acids can be a further development direction to improve domestic cigar tobacco leaves.

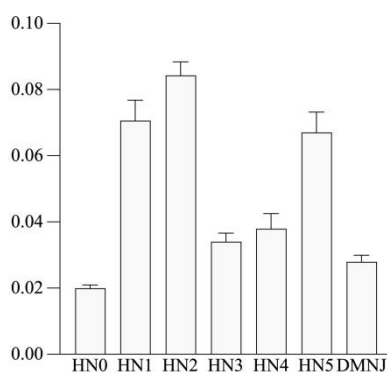
### 3.4. Comparative Analysis of the Changes of Aromatizing Substances in The Fermentation Process of Domestic Cigar Tobacco and Dominican Cigar Tobacco

#### 3.4.1. Comparative Analysis of Changes in Carotenoid Degradation Product Content with Dominican Cigar Leaf

**Table 6.** Comparison of carotenoid degradation products in Hainan cigar tobacco during fermentation with dominican cigar tobacco

Name	HN0	HN1	HN2	HN3	HN4	HN5	DMNJ
Isophorone	0.00314%	0.00764%	0.00671%	0.00680%	0.00757%	0.00495%	0.00449%
$\beta$ -Cyclocitral	0.00134%	0.00177%	0.00110%	0.00139%	0.00139%	0.00149%	0.00149%
$\beta$ -Damascenone	0.01078%	0.04825%	0.06584%	0.01573%	0.01983%	0.04759%	0.01646%
Geranylacetone	0.00435%	0.01302%	0.01075%	0.01041%	0.00909%	0.01348%	0.00556%

The changes in the content of the main carotenoid degradation products during the fermentation of domestic cigar tobacco were compared with those of Dominican cigar tobacco as shown in Table 6. The carotenoid degradation content of domestic cigar tobacco showed a trend of increasing, then decreasing and then increasing under the proposed fermentation conditions, and the content of carotenoid degradation products at the end of fermentation was 244.26% higher than that before fermentation. The content of carotenoid degradation products of domestic cigar tobacco was higher than that of Dominican cigar tobacco at the end of fermentation, and the difference in the content of carotenoid degradation products between the two was 0.03951%.



**Fig 4.** Comparison of carotenoid degradation products content in Hainan cigar tobacco leaves and Dominican cigar tobacco leaves during fermentation

The results of carotenoid degradation product content changes during fermentation of domestic cigar tobacco leaves compared with Dominican cigar tobacco leaves are shown in Figure 4. Under the proposed fermentation conditions, the content changes of isophorone,  $\beta$ -damascone and geranylacetone substances during fermentation were highly significant ( $p < 0.001$ ), and the content increased at the end of fermentation, by 57.64%, 341.47% and 209.89%, respectively. Among them,  $\beta$ -damascenone and geranylacetone increased more, while  $\beta$ -cyclocitral did not change significantly and only increased by 11.19%. The content of carotenoid degradation products of domestic cigar tobacco through fermentation were higher than that of Dominican cigar tobacco, in which the content of isophorone and  $\beta$ -cyclocitral was

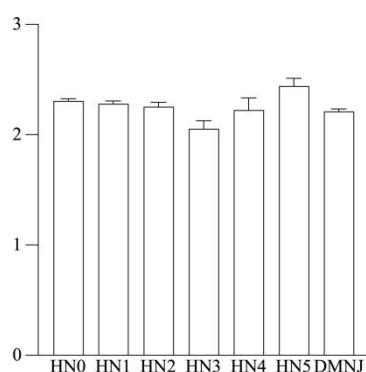
closer to that of Dominican cigar tobacco, while the content of  $\beta$ -damascenone and geranylgeranylacetone was significantly higher than that of Dominican cigar tobacco.

Aroma substances such as isophorone,  $\beta$ -damascenone, and geranylacetone, the degradation of which produces a relatively low threshold of aroma substances, less irritation, better aroma, and a large contribution to the aroma of tobacco [13-15]. After domestic cigar tobacco leaves were fermented, the content of all carotenoid degradation products was higher than that of Dominican cigar tobacco, of which the content of  $\beta$ -damascenone and geranylacetone was significantly higher than that of Dominican cigar tobacco.

### 3.4.2. Comparative Analysis of Changes in the Content of Other Aroma-Producing Products with Dominican Cigar Leaf

**Table 7.** Comparison of content changes of other odorants in Hainan cigar tobacco during fermentation with Dominican cigar tobacco

Name	HN0	HN1	HN2	HN3	HN4	HN5	DMNJ
Quinoline	2.20827%	2.11451%	2.08645%	1.86286%	2.00423%	2.27359%	2.06391%
Indole	0.03294%	0.04005%	0.04662%	0.03001%	0.03031%	0.04458%	0.04054%
Acetophenone	0.01606%	0.01493%	0.01723%	0.00876%	0.00460%	0.01144%	0.01056%
Methylheptenone	0.00066%	0.00255%	0.00203%	0.00423%	0.00528%	0.00211%	0.00098%
Methyl linolenate	0.00364%	0.00924%	0.00694%	0.00553%	0.00576%	0.00482%	0.00654%
Dibutyl phthalate	0.00566%	0.00673%	0.00691%	0.00910%	0.00964%	0.00918%	0.00805%
p-Methoxyacetophenone	0.00159%	0.00302%	0.00258%	0.00298%	0.00215%	0.00220%	0.00192%
L-Carvone	0.00423%	0.00530%	0.00576%	0.00438%	0.00220%	0.00503%	0.00519%
Cinnamaldehyde	0.00385%	0.00423%	0.00559%	0.00476%	0.00317%	0.00584%	0.00263%
Cinnamyl alcohol	0.00202%	0.00195%	0.00151%	0.00374%	0.00204%	0.00179%	0.00386%
2,4-Dimethylbenzaldehyde	0.00202%	0.00255%	0.00239%	0.00285%	0.00335%	0.00226%	0.00668%
Coumarin	0.00066%	0.00032%	0.00028%	0.00034%	0.00032%	0.00039%	0.00503%
Clove aldehyde	0.00194%	0.00147%	0.00137%	0.00304%	0.00339%	0.00239%	0.00156%
3-Acetylpyridine	0.02362%	0.07496%	0.06710%	0.11153%	0.14620%	0.07548%	0.05521%



**Fig 5.** Comparison of the content changes of other fragrance-causing products in Hainan cigar tobacco leaves and Dominican cigar tobacco leaves during fermentation

The results of changes in the content of other aromatizing products during the fermentation of domestic cigar tobacco compared with Dominican cigar tobacco are shown in Table 7. Under the proposed fermentation conditions, the total amount of other aroma-causing products of domestic cigar tobacco showed a trend of decreasing and then increasing, and the total amount of other aroma-causing products at the end of fermentation was 5.81% higher than that before fermentation. The content of other aroma-causing products of domestic cigar tobacco was

higher than that of Dominican cigar tobacco at the end of fermentation, and the total amount of other aroma-causing products of the two categories differed by 0.22844%.

The results of the changes in the total amount of other aroma products during the fermentation of domestic cigar tobacco compared with Dominican cigar tobacco are shown in Figure 5. Under the proposed fermentation conditions, changes in the content of most of the aroma-causing products in domestic cigar tobacco were highly significant ( $p < 0.001$ ) except for 2,4-dimethylbenzaldehyde, coumarin, butyraldehyde and p-methoxyacetophenone, which were significant ( $p < 0.05$ ). The contents of quinoline, indole, methylheptenone, methyl linolenate, dibutyl phthalate, p-methoxyacetophenone, L-caryophyllene, cinnamaldehyde and butyraldehyde increased at the end of fermentation; among them, the content of quinoline was higher, and the content of quinoline increased at the end of fermentation, but the change was not significant compared with that of the pre-fermentation period; whereas the contents of cinnamyl alcohol and coumarin both decreased at the end of fermentation. The contents of methylheptenone, p-methoxyacetophenone, cinnamyl alcohol, 2,4-dimethylbenzaldehyde, coumarin and butyraldehyde were still lower than those of Dominican cigar tobacco after fermentation, among which the contents of cinnamyl alcohol and 2,4-dimethylbenzaldehyde were closer to those of Dominican cigar tobacco. The contents of quinoline, indole, acetophenone, methyl linolenate, dibutyl phthalate, L-caryophyllene, cinnamaldehyde and 3-acetylpyridine in the domestic cigar tobacco at the end of fermentation were significantly higher than those in the Dominican cigar tobacco.

Among the aroma-causing products with elevated content, methylheptenone has a lemongrass and isobutyl acetate-like aroma; p-methoxyacetophenone has a strong and long-lasting hay aroma with floral and hawthorn scent[6]; L-caryophyllene ketone has an orangy odor; and cinnamaldehyde has a special cinnamon aromatic odor. The increase of these aroma substances is conducive to increasing the aroma of domestic cigar tobacco and improving the sensory quality of cigar tobacco. The contents of quinoline, indole, acetophenone, methyl linolenate, dibutyl phthalate, L-caryophyllene, cinnamaldehyde and 3-acetylpyridine in the domestic cigar tobacco leaves after the end of fermentation were significantly higher than those in the Dominican cigar tobacco leaves. The contents of methylheptenone, p-methoxyacetophenone, cinnamyl alcohol, 2,4-dimethylbenzaldehyde, coumarin and butyraldehyde were still lower than those of Dominican cigar tobacco after fermentation. Among them, cinnamyl alcohol has a balsamic aroma with sweet floral and hyacinth notes; 2,4-dimethylbenzaldehyde has a mild, sweet, bitter almond odor [16]; and coumarin has a hay aroma similar to black coumarin. Therefore, how to further improve the content of these aroma substances in domestic cigar tobacco can be a further development direction to improve domestic cigar tobacco.

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