



Effects of coffee and cocoa as fermentation additives on sensory quality and chemical compositions of cigar tobacco leaves

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Abstract

Exogenous additives applied for cigar tobacco leaves fermentation played an important role in sensory quality of cigar. In this study, effects of additives including coffee and cocoa on the quality of cigar tobacco leaves were explored. The changes of sensory quality, conventional chemical components, nonvolatile organic acids, and aroma compositions in tobacco leaves were studied. Results showed that the richness, mellowness and maturity of aroma, as well as the fluency and sweetness of smoke in cigar were improved with the introduction of coffee or cocoa. According to chemical composition analysis, fermentation with coffee or cocoa increased the contents of alkaloids and sugar in tobacco leaves, which endowed the tobacco a relatively good performance on the sweetness and aroma richness. Besides, the decrease of citric acid, as well as the increase of saturated fatty acid and aroma components were found with additives, which was beneficial to improve the mellowness and aroma richness of cigar. It was suggested that coffee and cocoa exhibited positive effects on the chemical composition, especially increased the contents of sugar, saturated fatty acids and aromatic components, thus improving the sensory quality of tobacco leaves.

Keywords: cigar; tobacco fermentation; sensory quality; chemical composition.

Practical Application: Improving the sensory quality of tobacco leaves by fermented with additives and related mechanism research.

1 Introduction

Tobacco is an important economic crop that is widely sold around the world. As one of the largest producer and consumer of tobacco, China produced one third of the total global consumption of tobacco (Dai et al., 2020). Cigar is a kind of special tobacco products made by pure hand, which exhibited the characteristics of rich aroma, plump taste, and high smoke concentration. According to annual sales reports, the growth rate of China's cigar market exceeded 50% in 2019 and 80% in 2020, respectively. Unlike traditional cigarettes wrapped with papers, cigars are completely prepared by cigar tobacco leaves (CTLs). As a result, the quality of CTLs determined the unique flavor and aroma characteristic of cigar. Generally, CTLs of high quality are grown in the Dominican Republic, Nicaragua, Cuba and so on (Yu et al., 2021). Due to the influence of climate conditions, the aroma richness and sensory quality of CTLs harvested in China show a relatively low level compared with those in the above-mentioned places (Cai et al., 2019). Therefore, it is an important approach to improve the sensory quality of cigar by adjusting the preparation process.

The production of cigar involves several critical processes, including cultivation, drying, fermentation, and curing (Fan et al., 2016). The physical and chemical properties of CTLs would change obviously after fermentation, so as to improve the sensory quality of cigar. According previous reports, adopting fermentation additives to assist the fermentation of CTLs could effectively enhance the quality of cigar leaves (Li et al., 2022b;

Ye et al., 2022). Microbial agents, enzyme preparation, and plant extracts were widely researched for CTLs fermentation (Ye et al., 2022). Shorten fermentation time, harmonizing the chemical compositions, reducing irritation, and enrich aroma could be found. Mao et al. (2022) reported that tobacco fermented with *Bacillus subtilis* could improve the sugar content and enrich aroma of tobacco leaves. Zheng et al. (2022) found that phytase and pectinase could reduce the irritant of CTLs. For plant extracts, it is essential that they are rich in flavor ingredients and meet the requirement of food safety. Extracts of chrysanthemum and green tea, as well as rice wine were proven to improve the sensory quality of cigar (Li & Zhang, 2016). However, the research on exogenous additives in industrial fermentation of CTLs was still not in-depth. Generally, researchers only focus on the sensory changes caused by exogenous additives whose effects on the chemical composition of tobacco leaves have not been studied systematically. The mechanism of exogenous additives enhancing aroma and sensory quality of cigar remained unclear.

According to previous research of our group, coffee and cocoa were screened out for facilitating the fermentation process and enhancing the sensory quality of CTLs (Hu et al., 2022). In this study, mechanism of improved quality of CTLs by coffee and cocoa were analyzed. The changes of conventional chemical components, nonvolatile organic acids, and aroma compounds in CTLs were studied under three systems of water-fermentation, coffee-fermentation, and cocoa-fermentation, respectively. This

Received 12 Aug., 2022

Accepted 13 Oct., 2022

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study can not only explain the mechanism of flavor enhancement and quality improvement of cigar by fermentation additives, but also provide technical guidance for mining more fermentation additives.

2 Materials and methods

2.1 Sample collection and tobacco fermentation

The CTLs used in this study were provided by the Great Wall Cigar Factory (De-yang, China), which were harvested in 2020 and obtained after air-curing. For fermentation, 4 kg of CTLs were weighted, and 600 g of deionized water was sprayed evenly on the surface of tobacco leaves. Then, the leaves were fermented at 35 °C and 75% of relative humidity in a constant temperature and humidity incubator (Agilen, BINDER-KBF720). After 0, 7, 14, 21, 28 and 35 d, 400 g of CTLs were withdrawn at retention times. Besides, two other experiments with same procedure were carried out except that deionized water was replaced by 1% (w/w) of coffee solution or cocoa solution, respectively. Accordingly, the obtained CTL samples were named as T1, T2 and T3 based on the additives of water, coffee or cocoa, respectively. All samples were stored in a refrigerator at -20 °C for further analysis.

2.2 Sensory quality evaluation

Three CTL samples fermented with water, coffee, or cocoa for 35 d were adopted for sensory quality evaluation, which was performed by a five members-trained panel. Before the sensory evaluation, CTL samples were rolled by professional rollers to make cigars, and then maintained at 65% of relative humidity for 1 d. The scent components, as well as characteristics (aroma, smoke, aftertaste, and combustion) of cigar were clarified. Sensory characteristic scales were calculated by using a 9-point hedonic scale, while flavor components scales were calculated by using a 5-point hedonic scale. A higher score represented the better performance of the corresponding index. The scores were discussed and agreed by all panelists.

2.3 Determination of chemical compositions

The CTL samples were ground to 40-mesh size firstly. The contents of protein, total nitrogen (TN), alkaloid, total sugar (TS), reducing sugar (RS), potassium (K), and chlorine (Cl) in CTL samples were determined by a continuous flow analytical system according to the Tobacco Industry Standard (YC/T249-2008, YC/T161-2002, YC/T468-2013, YC/T159-2019, YC/T217-2007, and YC/T162-2011) (Liu et al., 2021). Nonvolatile organic acids (NOCs) in CTLs were analyzed through gas chromatograph (GC) equipped with hydrogen flame ionization detector (FID) (Liu et al., 2012). Gas Chromatography-Mass Spectrometry (GC-MS) was adopted to determine the contents of aroma components in CTLs (Yu et al., 2021).

3 Results and discussion

3.1 Sensory quality evaluation

CTL samples fermented with water (T1), coffee (T2), and cocoa (T3) were subjected to sensory evaluation. As shown

in Figure 1 and Table 1, CTL fermented with water showed weak bean and nut aromas, as well as mediocre performance on the sensory characteristics of aroma, smoke, aftertaste, and combustion. The results were consistent with the conclusion from a previous report, which further illustrated the necessity of exogenous additives (Li et al., 2019). It can be seen that the scores of aroma components and sensory characteristics showed great variations with the addition of coffee or cocoa. The CTL fermented with coffee possessed a relatively high score of coffee scent (4.0), followed by sample fermented with cocoa (3.0). Similarly, cocoa scent was introduced to T2 and T3 samples. Besides, the relatively flavorful nut scent, bean scent, and caramel scent were found. As shown in Table 1, with the introduction of coffee or cocoa, the mellowness and maturity of aroma, as well as the fluency and sweetness of smoke in cigar were improved obviously. It indicated that fermented additives contributed significantly to the perceived sensory characteristics of cigar, which was owing to the chemical profile of additives (Santoso et al., 2022; Seçuk & Seçim, 2022). Therefore, in addition to sensory quality, it is also significant to analyze the effects of coffee and cocoa on the chemical profile of tobacco leaves.

3.2 Conventional chemical compositions analysis

A relative high content of nitrogen in cigar compared with other types of tobacco products were found, and the intensity and coordination of nitrogen metabolism play an important role in regulating the quality of tobacco leaves (Mao et al., 2022). Herein, the contents of protein, TN, and alkaloid in CTL samples were evaluated. As presented in Figure 2a, the protein content in CTL ranged from 22.56% to 26.69%, which was consistent with that of previous study (Kou et al., 2011). In T1 group (adding water), the protein content reached a highest value of 25.90% in 28 d, and then decreased with the fermentation proceed. In T2 and T3 groups, the addition of coffee and cocoa showed no significant effect on the protein content of CTL ($p > 0.05$) at the beginning of fermentation. Besides, there was no insignificant difference of protein content at the beginning (0 d) and the end (35 d) of fermentation. It can be concluded that the fermentation process and additives (coffee and cocoa) both exhibited little influence

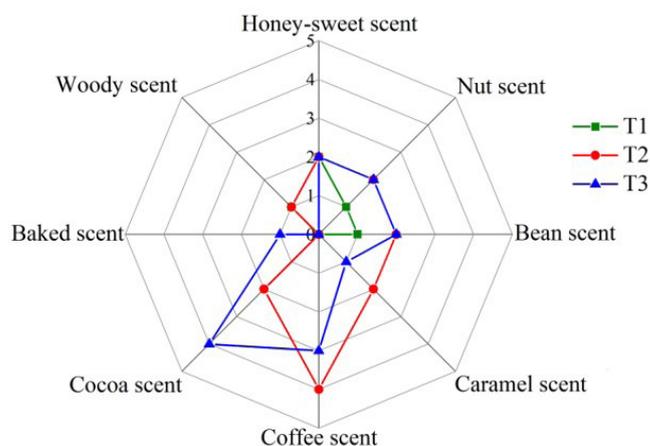


Figure 1. The flavor components radar chart of the three CTL samples.

Table 1. Sensory characteristics of the three CTL samples.

	Indicators	T1	T2	T3
		Score (0~9)		
Aroma characteristics	mellowness	3	6	6
	richness	3	4	5
	matureness	3	6	6
Smoke characteristics	plumpness	4	6	6
	fluentness	3	6	6
	smoothness	3	6	6
	sweetness	3	6	6
Aftertaste characteristics	cleanliness	3	4	5
	aftertaste	3	5	6
Combustion characteristics	combustibility	6	7	6
	grey	6	7	6
	ash coagulation	6	7	7

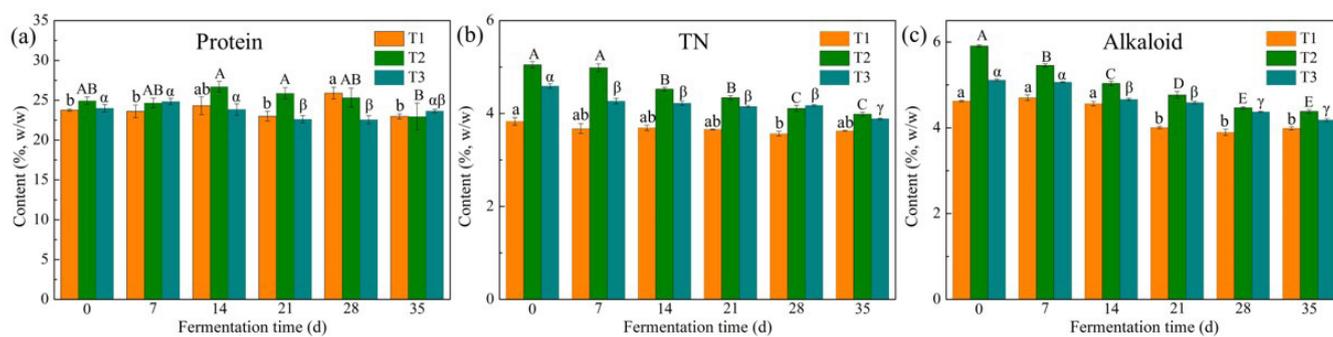


Figure 2. The contents of protein (a), TN (b), and alkaloid (c) in CTL samples. Note: Different lowercase letters indicate that the difference of samples in T1-group at different fermentation stages is statistically significant at $p < 0.05$ level. Different capital letters indicate that the difference of samples in T2-group at different fermentation stages is statistically significant at $p < 0.05$ level. Different Greek letters indicate that the difference of samples in T3-group at different fermentation stages is statistically significant at $p < 0.05$ level (same means for following figures and tables).

on the protein content of cigar tobacco leaves. In this study, a relative low temperature of 35 °C was adopted, thus the protein decomposition was mild.

Figure 2b showed the influences of fermentation time and additives on the TN contents in CTL samples. The content of TN in T1 group showed a similar value at the beginning and end of the fermentation process. However, the additives of coffee and cocoa solution affected the TN contents obviously. In T2 and T3 groups, the TN contents showed a significant decline as increasing fermentation time. At 35 d, the TN content decreased by 21.06% and 15.31% in T2 and T3 group, respectively. Similarly, increasing fermentation time reduced the alkaloid content remarkably (Figure 2c). After 35 d, 13.74%, 25.82%, and 18.22% of alkaloid degradation efficiencies in CTLs fermented with water, coffee, and cocoa were detected, respectively. Besides, at the initial stage of fermentation, TN and alkaloid contents in T2 and T3 groups were higher than these in T1 group. Considering nitrogen element in tobacco leaves mainly exists in the form of protein and alkaloid, the addition of coffee and cocoa introduced abundant alkaloid to CTLs, since protein content changed insignificantly. As shown in Figure 2b-2c, TN content increased from 3.83% (T1) to 5.05% (T2) and 4.59%

(T3), while alkaloid content increased from 4.62% (T1) to 5.90% (T2) and 5.11% (T3). According to previous reports, abundant caffeine, theophylline, and theobromine could be found in coffee and cocoa, which could be converted to various aroma components of furan and pyrazine (Mejía et al., 2021; Mostafa, 2022; Chavez et al., 2022). Therefore, the enrichment of alkaloid in CTLs fermented with coffee and cocoa would promote the improvement of aroma quantity. In addition, 13.74% of degradation efficiency of alkaloid was determined in T1 after 35 d, which was resulted by the degradation of nicotine into nitrosamine substance through nitrosation reaction (Sun et al., 2022). In conclusion, fermentation with coffee and cocoa increased the content of alkaloids and TN in tobacco leaves, which means that coffee and cocoa introduced rich N-containing aromatic substances into cigar.

Sugar, as the product of photosynthesis, is an important internal indicator of the quality of tobacco leaves. In detail, sugar could cover undesirable odors through generating acids which can neutralize or weaken the pungency of smoke (Yan et al., 2022). Besides, sugar has a positive influence on aroma quality since it could convert to flavor components by Maillard reaction and caramelization reaction (Banožić et al.,

2020; Li et al., 2022a). As shown in Figure 3, 0.5~0.6% of TS and 0.2~0.3% of RS were found in CTL raw materials (T1-0 d). With the extension of fermentation time, decreased trends on the contents of TS and RS were detected, indicating that more aroma components were formed by chemical reactions using sugar as substrate. By contrast, the contents of TS and RS in T2 and T3 groups were improved. It was reported that high concentration of carbohydrates was naturally present in coffee and cocoa (Lorenzo et al., 2022; Mostafa, 2022). Thus, CTLs fermented with coffee and cocoa would show a relatively good performance on the sweetness and aroma richness.

The existent of K could facilitate the complete burning of tobacco, while Cl tends to slow down the burning, thus the ratio of K and Cl (K/Cl) was an important indicator of combustibility for cigar (Yin et al., 2019). Figure 4 illustrated the content variation of K, Cl and K/Cl in CTLs during fermentation, the K content remained at a stable level (~3.5%), while the trends of Cl content decreased gradually. The loss of Cl was resulted by the volatilization of chlorine contained compounds during the fermentation. As a result, the value of K/Cl increased from 3.20 (0 d) to 3.61 (35 d) in T1-group. Besides, compared with that of T1, K/Cl value of T2 and T3 showed a higher level. Therefore, it could be concluded that the fermentation process and additives of coffee and cocoa solutions were beneficial to improve the combustibility of cigar.

3.3 Nonvolatile organic acids analysis

Nonvolatile organic acid (NOC) is an important component of tobacco leaves, which accounted for 70-80% (w/w) of organic

acids (Kong et al., 2018). The composition and content of nonvolatile organic acids were of great significance in optimizing the sensory quality of tobacco leaves by regulating the pH and reducing the irritation of smoke (Lu et al., 2021). In this study, a total of fourteen nonvolatile organic acids were detected in CTL samples, including arachidic acid, stearic acid, oleic acid, linoleic acid, palmitic acid, myristic acid, vanillic acid, citric acid, malic acid, succinic acid, levulinic acid, malonic acid, oxalic acid and lactic acid.

As shown in Figure 5, different variation trends of the total amount of NOCs in the three groups were found. In the initial stage (0 d), the total content of NOCs was 11543.22, 12861.25, and 9630.78 $\mu\text{g g}^{-1}$ in T1, T2 and T3 group, respectively. In T1 group, the content of NOC decreased first and then increased as fermentation proceeded, and a lowest value of 7697.13 $\mu\text{g g}^{-1}$ was determined in 21 d. Besides, there are no significant difference in NOC content at the beginning and end of fermentation. It can also be seen that a decreased trend of NOCs content was detected, and a lowest level of 7546.03 $\mu\text{g g}^{-1}$ was found at 35 d in T2 group. In contrast, T3 group owned a relatively steady NOC content (ranging from 9269.35 to 10532.22 $\mu\text{g g}^{-1}$), demonstrating that the addition of cocoa is beneficial to maintain the acid and alkaline balance of cigar smoke, thus to improve the sensory of cigar.

Additionally, it can be seen that citric acid, malic acid, and malonic acid were the three main acids, which accounted for about 90% of all NOCs and were important indicators of cigar quality. As shown in Figure 5, the citric acid content in

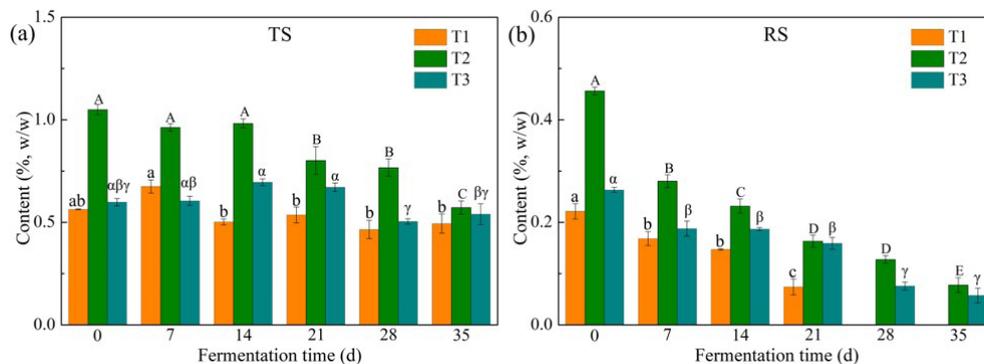


Figure 3. The contents of TS (a) and RS (b) in CTL samples.

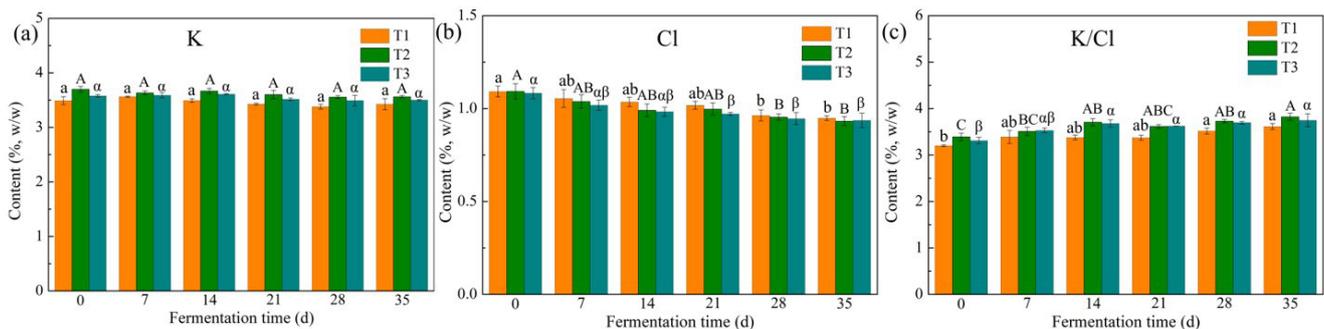


Figure 4. The contents of K (a), Cl (b), and K/Cl (c) in CTL samples.

T1 underwent a generally decreased trend, while that of T2 and T3 increased at the end of fermentation. As for malic acid, its content in T1 and T3 groups decreased firstly and then increased, while that of T2 decreased as time increased. Besides, after fermentation, the contents of malic acid in CTLs fermented with coffee and cocoa were significantly lower than that in T1 group ($3101.29 \mu\text{g g}^{-1}$). In terms of citric acid, its content in T2 group decreased during the fermentation process, while that in T1 and T3 groups exhibited little changes. Compared with that in T1 group ($4602.67 \mu\text{g g}^{-1}$), the citric acid contents in T2 and T3 groups at the end of fermentation (35 d) was significantly lower, which were $1944.86 \mu\text{g g}^{-1}$ and $3727.45 \mu\text{g g}^{-1}$, respectively. According to relevant research report, content of citric acid is negatively correlated with the quality of tobacco leaves (Li et al., 2020). Therefore, CTLs fermentation with coffee or cocoa was beneficial to improve the quality of cigar.

Besides, several saturated fatty acids including arachidic, stearic, palmitic, and myristic acids, as well as unsaturated fatty acids such as oleic and linoleic acids were detected in cigar (Figure 5). Liu et al. (2020) reported that high fatty acids showed important influence on the aroma and taste quality of CTLs. As shown in Figure 5, the total contents of high fatty acids in T2 and T3 groups at the same fermentation time were higher than that in T1 group. At the end of fermentation process, 559.43 , 597.92 , and $395.97 \mu\text{g g}^{-1}$ of high fatty acids were found in cigar leaves fermented with water, coffee, and cocoa, respectively. It is worth noting that the effects of high saturated fatty acids and high unsaturated fatty acids on tobacco quality are opposite. Generally, saturated high fatty acids can enrich the aroma of smoke, as well as improve the mellowness of cigar, while superfluous unsaturated fatty acids would increase the irritation and rough feeling of smoke (Li et al., 2020). Compared with that in T1, the total content of saturated fatty acids in T2 and T3 at 21 d were increased by 154.34% and 224.95%, respectively. According to a previous report, high levels of saturated fatty acids, mainly palmitic and stearic acids, were predominant in coffee and cocoa (Dong et al., 2022). As a result, the addition of coffee or cocoa lead to the increasement of these acids, thus improving the mellowness and fluentness of cigar, which was consistent with results of sensory evaluation (Table 1).

3.4 Aroma components analysis

As shown in Figure 6, the total contents of aroma components in the three groups possessed the similar change trend of increased

firstly and then decreased with fermentation proceeded, which exhibited the highest level at 21 d. Hence, the fermentation period of 21 d would be appropriate, which was consistent with the conclusion from Guo et al. (2021). Compared with tobacco leaves fermented with water (T1), CTLs fermented with coffee (T2) and cocoa (T3) possessed significantly higher total contents of aroma components. It can be seen that the highest content of 2.5458 mg g^{-1} in T1 was detected, while that in T2 and T3 were 3.0872 and 2.9291 mg g^{-1} , respectively, meaning that 21.27% and 15.06% of improved efficiencies were found. At the end of fermentation, the total content of aroma components was enhanced from 1.9716 mg g^{-1} (T1) to 2.7204 mg g^{-1} (T2) and 2.5458 mg g^{-1} (T3), respectively, demonstrating that the addition of coffee and cocoa could enrich the flavor of CTLs, which was agree with the results of sensory evaluation (Table 1).

In terms of the types of aroma substances, ketones, alkenes and alcohols were main components in cigar, which accounted for more than 80% of the total content. As the fermentation proceeded, contents of the above three compounds increased first and then decreased, showing a similar trend of total content of aroma components. Neophytadiene is the most abundant aroma component in cigar, accounting for about 30% of the total aroma component. Neophytadiene is formed by the degradation of chlorophyll in tobacco leaves during ripening and air-curing. Since neophytadiene can directly mix with smoke to optimize the aroma and taste, it can mellow the CTLs and reduce irritation, showing an important impact on the quality of cigar (Wang et al., 2021). It can be seen that the variation trend of neophytadiene is consistent with the change of the total content of aroma components. As fermentation proceeded, the content of neophytadiene shows a unimodal trend, reaching the highest level at 21 d. In addition, as fermentation continued, the content of neophytadiene decreased, because the content of chlorophyll decreased at the later stage of fermentation, and neophytadiene continued to be consumed as a precursor of furans. The change of neophytadiene may be the main factor leading to the variation of aroma components during the fermentation of CTLs. Although coffee and cocoa had no significant effect on the content of neophytadiene, they affected the contents of ketones and acids obviously. With the addition of coffee and cocoa, the content of saturated fatty acids such as stearic acid and palmitic acid in tobacco leaves increased significantly, and abundant caffeine and theobromine were also introduced. Caffeine and theobromine can introduce coffee and cocoa aromas to tobacco leaves. Although saturated fatty acids have no direct effect on

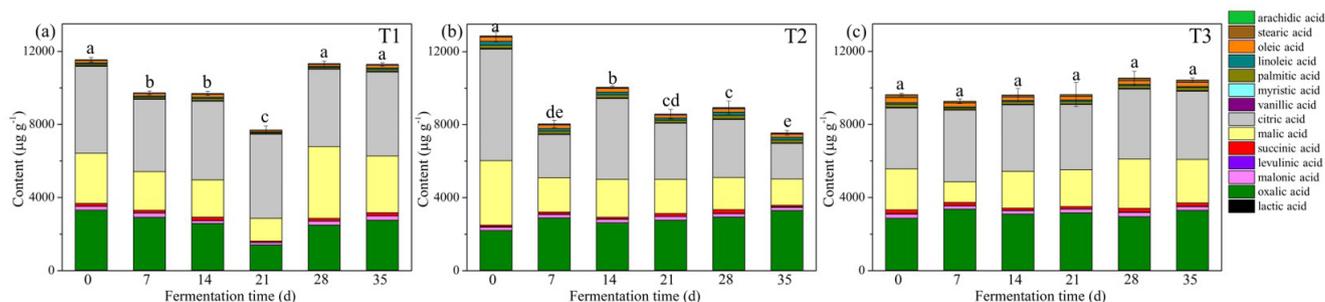


Figure 5. The contents of NOCs in T1 (a), T2 (b), and T3 (c) CTLs.

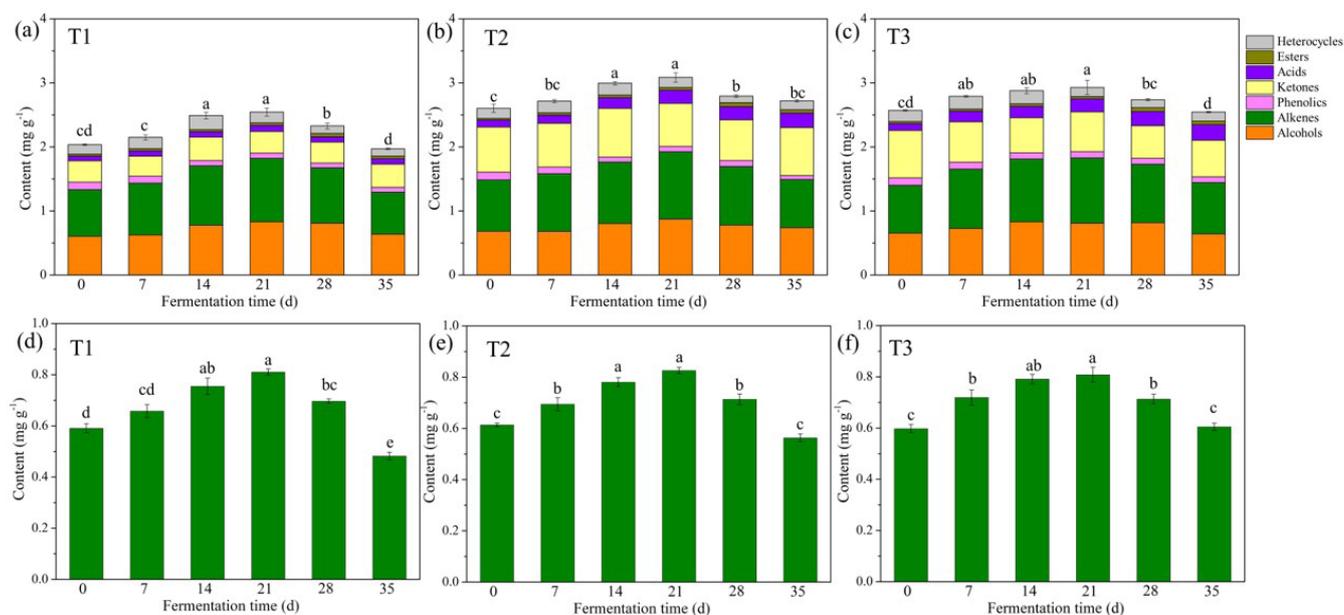


Figure 6. The contents of aroma components in T1 (a), T2 (b), and T3 (c). The contents of neophytadiene in T1 (d), T2 (e), and T3 (f).

flavor, they can adjust the pH of tobacco, improve the mellowness and smoke concentration, thereby indirectly affecting flavor characteristics of cigar.

In conclusion, coffee and cocoa can directly introduce rich aroma components into tobacco leaves, and increase the content of aroma components by affecting the metabolic pathways of chemical compositions, thus improving the sensory quality of cigar.

4 Conclusion

In summary, the mechanism of improved quality of CTLs fermented with coffee or cocoa was revealed. According to the evaluation of sensory quality, it was verified that characteristics of aroma, smoke, aftertaste, and combustion of CTLs were improved with the addition of coffee or cocoa. As proved by chemical compositions analysis, contents of total nitrogen, alkaloid, and sugar were enhanced with additives. Besides, higher levels of saturated fatty acid and aroma compositions were found in coffee- and cocoa-groups than that in water-group. The optimization of chemical compositions promoted the improvement of sensory quality of tobacco leaves. This study not only fill the blank of mechanism research on flavor enhancement and quality improvement of cigar by fermentation additives, but also provide technical guidance for mining more fermentation additives.

Acknowledgements

Financial support from the Major Science and Technology Program of China National Tobacco Corporation [110202101014(XJ-06) and 110202101066(XJ-15)] are gratefully acknowledged.

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