

## Assessment of Biochemical Composition in “Yunxue No.1” Tobacco Leaf during the Air-Drying Process

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**ABSTRACT** The purpose of this study was to explore the change rule of tobacco leaf "Yunxue No. 1" during the drying process of cigar in Yunnan tobacco area. The tobacco leaves were collected at 3, 6, 9, 12, 15 and 21 days of drying to determine the chemical composition, and the changes of carbohydrate, pigment and polyphenol substances in cigar tobacco leaves during drying time were studied. The contents of various substances such as total sugar, reducing sugar, starch, total nitrogen, nicotine, chlorophyll a, chlorophyll b,  $\beta$ -carotene, lutein, chlorogenic acid, rutin, and scopolamine within the tobacco were meticulously examined. The experimental outcomes reveal that the moisture content present within the tobacco leaves has a considerable and substantial influence on the ultimate result of the air-drying process. This impact was mediated by changes in the levels of a diverse array of chemical constituents. The concentration of nicotine was closely and intrinsically linked with the levels of four plastidic pigments, namely chlorophyll a, chlorophyll b,  $\beta$ -carotene, and lutein. Furthermore, these four plastidic pigments mutually display a distinct and marked interdependence among themselves. In the future, subsequent research should primarily concentrate on optimizing the air-drying process of the Yunnan cigar variety "Yunxue No.1" in accordance with these firmly established rules, which will of great significance for the high-quality production in the later drying stage.

**Keywords:** Cigar; Air-drying; Carbohydrates; Plastidial; Pigments; polyphenols

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**INTRODUCTION** Cigar is a distinctive type of specialized tobacco product, renowned for its mellow aroma, a taste that delicately combines bitter and sweet nuances, and the remarkable characteristic of providing a strong sense of satisfaction (Dongchen et al., 2019). The drying stage of cigar tobacco is an absolutely crucial step in the entire tobacco production process. It represented a unified procedure involving both the dehydration and drying of tobacco leaves, as well as the transformation of their contents. Appropriate drying measures have the potential to effectively compensate for any deficiencies resulting from field growth conditions. The material metabolism of tobacco is a prolonged process of adaptation to the surrounding environment. This primarily encompasses primary metabolism and secondary metabolism. Primary metabolism during the field growth stage

plays a vital role in supplying energy and essential fundamental substances for tobacco. Its functioning is closely intertwined with the proportion and content of quality components. Secondary metabolism mainly consists of polyphenols, pigment metabolism, organic acid metabolism, alkaloid metabolism, and more. Furthermore, secondary metabolites not only enhance the self-protection ability and competitiveness of tobacco leaves within the environment but also serve as significant precursors for flavor in tobacco, exerting a substantial and influential impact on the overall quality of tobacco (Tiwari et al., 2016).

The quality of tobacco leaves is intimately associated with the chemical elements present within the tobacco, and these elements have a substantial influence on both the taste and the burning characteristics of the tobacco leaves. The research

conducted by Zhang Qing (Zhang et al., 2021) revealed that the carbon to nitrogen ratio of tobacco leaves demonstrated a secondary correlation with the total score of the tobacco smoking evaluation in each period. This indicates that it is essential for carbon and nitrogen metabolism to be maintained at an appropriate level in order to enhance the quality of the tobacco leaf smoking evaluation. As a result, the carbon nitrogen ratio is frequently employed as an indicator for measuring the aroma and taste of tobacco leaves. The precise balance of carbon and nitrogen in the metabolic processes of the tobacco leaves is a crucial factor that determines the overall quality and desirability of the tobacco for smoking. A well-regulated carbon nitrogen ratio ensures that the taste is harmonious and the burning process is smooth, thereby contributing to a more satisfactory smoking experience. This aspect of tobacco leaf composition and its correlation with smoking quality has gained significant attention in the field of tobacco research and production.

Moreover, the study carried out by Dong Shujun (Dong et al., 2015) indicated that polyphenols and plastids exert a significant role in the growth, modulation characteristics, appearance quality, and intrinsic quality of tobacco leaves. Not only that, their content and composition also have an impact on the evaluation results and the safety of tobacco products. At present, both domestic and foreign scholars have undertaken a considerable number of studies on the metabolic law of flue-cured tobacco (Yang et al., 2024). However, the research on the metabolic law of cigar tobacco is extremely limited. Especially when it comes to Yunnan cigar, it is basically in a state of blankness at the current stage. Based on this situation, the experiment selected the cigar variety "Yunxue No. 1" as the material and focused on studying the dynamic variations of various chemical contents in cigar tobacco leaves during the drying period.

The aim of this was to regulate the substance metabolism of cigar tobacco leaves during the drying period, with the ultimate goal of providing a theoretical foundation for enhancing the quality of tobacco leaves. The exploration of these chemical content changes is of great significance in understanding the complex metabolic processes and finding effective ways to optimize the quality and characteristics of cigar tobacco. Such studies contribute to filling the knowledge gap and promoting further advancements in the field of cigar tobacco research and production.

## **MATERIALS AND METHODS**

### ***Test material***

The experimental study was conducted between February and June of 2022, within the dedicated research plots at Lujiang Point, Longyang District, Baoshan City, located in Yunnan Province (98°43'E, 24°46'N), characterized by an average field temperature of 20.1°C, monthly precipitation averaging 150 mm, and monthly solar irradiance averaging 150.3 hours. The experimental subjects was tobacco cultivars: the eggplant-coated "YUNXUE No.1". The experimental plot's soil was classified as colluvial sandy, characterized by its coarse particulate structure, significant intergranular space, rapid water percolation, limited water retention capacity, and high permeability. This soil profile facilitated ease of tillage, lay on level ground, and had been

previously planted with corn. The soil's pH was recorded at 5.8, with an organic matter content of 24.5g/kg, total nitrogen at 1.02g/kg, readily available potassium at 138mg/kg, and organic phosphorus at 89.5mg/kg, conditions that are conducive to the cultivation of cigar tobacco plants.

### ***Experimental design***

The tobacco air-drying process utilizes the traditional hanging method, where the leaves are methodically spaced out on the rods for uniform air-drying. Choose tobacco leaves that were consistent in length and display relatively uniform quality, handpicking 40 leaves per stick, culminating in a total of 720 leaves. Subsequently, the curated leaves were conveyed to the air-drying chamber, where they were fastidiously suspended upon tobacco rods to undergo a controlled air-drying process. The air-drying chamber was a sophisticated, climate-controlled environment, outfitted with an advanced automatic weighing mechanism. The chamber dimensions were meticulously specified as 30 meters in length, 10 meters in width, and 6 meters in height. Each suspension rod within the chamber, extending 300 centimeters in length and weighing 251 grams, was designed to accommodate 120 tobacco leaves. The rods were strategically spaced 20 centimeters apart to optimize airflow. The desiccation protocol employed was a traditional natural air-drying technique, inherent to the cigar production practices prevalent in Yunnan Province. On days 3, 6, 9, 12, 15, 18, and 21 of the air-drying regimen, 20 tobacco leaf samples were sequentially harvested and subjected to a thermal fixation process at 105°C for 15 minutes, followed by a air-drying phase at 60°C. Post-drying, the samples were shifted through a 60-mesh screen, a preparatory step for the subsequent analysis of chemical constituents and for quantifying the residual moisture content of the foliage.

### ***Fertilizer application***

Cigar tobacco is characterized by a substantial nitrogen assimilation capacity, necessitating the sustained provision of nitrogenous nutrients up to the point of harvest. Furthermore, augmenting the use of organic fertilizers, such as rapeseed cake, in lieu of inorganic variants, has been shown to be beneficial in enhancing the quality of cigar tobacco.

### ***Fertilization Strategy***

Dispense 150 kilograms of elemental nitrogen per hectare, amalgamate 1500 kilograms of organic fertilizer and 4500 kilograms of compound fertilizer (formulated in a ratio of N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O=10:10:25) per hectare with the base fertilizer, and establish intermediate strata of fertilization bands; At the 7-day post-transplantation of seedlings, apply 225 kilograms per hectare of a nitrogen-potassium blend and 3000 grams per hectare of water-soluble boron fertilizer (prepared at a 1.0% solution concentration), utilizing a deep placement applicator for thorough distribution; At the 20-day subsequent to the transplantation process, administer a mixture composed of 300 kilograms per hectare of compound fertilizer (N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O=10:10:25), 300 kilograms per hectare of potassium sulfate, and 150 kilograms per hectare of potassium nitrogen fertilizer (N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O=15:0:30) finely ground, followed by irrigation using a deep placement applicator to achieve a 2% nutrient solution concentration; Thirty days subsequent to applying the balanced fertilizer, blend 150

kilograms of compound fertilizer per hectare with water and administer it using a deep placement applicator.

Field management involves the strict regulation of the transplanting process, including detailed field preparation, precise moisture control, and the use of hole-punching techniques. Mid-tillage management integrates agronomic practices such as careful moisture control, monitoring after planting, strategic mulching film disruption with soil cultivation, and timely plant topping and de-sprouting. Soil cultivation and weeding were conducted on March 30, March 31, and April 18, followed by debudding on April 27 and May 19. Pest management for Yunxue No. 1 focuses on controlling aphids, tobacco green worm, and whitefly using insecticides like thiamethoxam and imidacloprid, alongside yellow and blue sticky traps and insecticidal lamps. Disease management centers on preventing leaf curl, black shank, and green blight diseases, employing agents such as Chunrexin, Chlorobromoisocyanuric acid, and Manganese zinc methomyl, applied as a root-zone irrigation solution.

Measurement items and methods

#### ***Determination of water content***

Excise the apex and one-third of the basal portion of the entire tobacco leaf, insert it into an oven preset at a temperature of 100°C, and proceed to quantify its moisture content.

#### ***Measurement of temperature and humidity***

Commencing with the onset of the air-drying process, a Deli 9012 thermohygrometer (manufactured by Ningbo Deli E-commerce Co., Ltd.) was strategically positioned within each air-drying chamber. Systematic measurements were conducted to chronicle the ambient temperature and humidity levels, capturing data from 8:00 to 20:00 at sequential intervals on the 3rd, 6th, 9th, 12th, 15th, 18th, and 21st days.

#### ***Determination of chemical composition content***

The concentrations of total sugar, reducing sugar, total nitrogen, starch, and nicotine were meticulously quantified using flow analysis techniques (XiaohongZhang, 2018).

#### ***Determination of plastidic pigments content***

The assessment of plastidic pigments content was performed in conformity with the YC/T382-2010 standard for the determination of plastid pigments in tobacco and tobacco products utilizing high-performance liquid chromatography (HPLC) methodology.

#### ***Determination of polyphenol content***

The quantification of chlorogenic acid, scopolamine, and rutin in tobacco and tobacco products was accurately conducted in accordance with the protocol outlined in YC/T202-2006.

#### ***Data analysis***

Data visualization was achieved through Microsoft Excel 2010, while one-way ANOVA and correlation analyses were meticulously carried out using SPSS version 23.0.

## **RESULTS AND DISCUSSION**

### **Changes of temperature, humidity and moisture content of tobacco leaf during drying**

The graphical representation of temperature and humidity measurements throughout the air-drying process is depicted in Figure 1. The temperature within the air-drying

chamber exhibited an initial increase followed by a decrease over time, reaching a peak of 28.3°C on the 15th day before descending again. The air-drying chamber's humidity exhibited a cyclical pattern of increases and decreases over time, hitting the minimum on the 15th day, with the greatest fluctuations occurring between the 12th and 18th days. From the 12th to the 18th day of air-drying, the temperature within the air-drying room initially rose, subsequently fell, and finally stabilized, while the humidity decreased, then increased, followed by another decrease, culminating in a relative humidity at the end of the air-drying period that was lower than the initial levels. Table 1 delineates the temporal variations in the moisture content of tobacco leaves at designated intervals. A gradual decline in the moisture content of tobacco leaves was noted between day 3 and day 9, which then accelerated markedly from day 12 to day 21. The moisture content of tobacco leaves from 3 to 9 days did not significantly deviate; however, a highly significant disparity was observed when compared to the moisture levels from 12 to 15 days and from 18 to 21 days. The comparative analysis indicated that the moisture content of tobacco leaves from 12 to 15 days exhibited a significant variation from that observed in the interval of 18 to 21 days.

During the cigar tobacco air-drying process, the moisture content significantly influences the tobacco's appearance and color transitions (Gao et al., 2019). Excessively rapid dehydration can lead to a light and greenish hue in the dried cigar tobacco, whereas excessively slow dehydration can yield a dark and brownish hue, coupled with a reduction in the leaf's processing resilience (Zhang et al., 2017); Optimal water loss facilitates the biochemical transformation of leaf constituents, thereby harmonizing the internal chemical composition of the tobacco and augmenting the overall tobacco quality (Jiang et al., 2022). The current investigation reveals that the moisture level of the tobacco leaf diminished during the 9 to 12-day period. The reason for this is that, commencing on the ninth day of air-drying, the tobacco leaves underwent a air-drying process in an environment characterized by high temperatures and low humidity within the air-drying chamber. This observation was in concordance with the outcomes documented in the study conducted by Liu Boyuan (Liu et al., 2021).

#### ***Changes in carbohydrates of tobacco during air-drying process***

Table 2 presents the compositional dynamics of sugar and water compounds in "Yunxue No.1" tobacco leaves throughout the air-drying process. Each of the three carbohydrates peaked in concentration on the 9th day, subsequently underwent a decline, and exhibited a resurgence by the 18th day. During the air-drying process, the total sugar content in "Yunxue No.1" tobacco leaves manifested a downward trajectory from days 3 to 6, ascended from days 6 to 9 culminating in a peak at approximately 3.2%, then experienced a precipitous drop between days 9 and 18, bottoming out around 0.5%, followed by a recovery from days 18 to 21. In the tobacco leaves examined, the reducing sugar content and total sugar content exhibited synchronous fluctuations. Between days 3 and 6, the total sugar content demonstrated a decreasing trend, whereas from days 6 to 9, it manifested an increasing trajectory, culminating in a peak value of

approximately 1.72%. Subsequently, from days 9 to 18, there was a precipitous decline in total sugar content, reaching its lowest level of around 0.19% during this interval. A recovery in sugar content was observed from days 18 to 21. The data reveal that throughout the air-drying process, cigar tobacco leaves undergo considerable consumption of respiratory carbohydrates. The comparatively higher total and reducing sugar content in the leaf suggested that harvesting at an optimal maturity stage during field growth facilitates carbohydrate accumulation. Concurrently, maintaining appropriate temperature and humidity during the air-drying phase can mitigate the depletion of both total and reducing sugars, thereby favorably influencing the quality development of the leaf. Throughout the air-drying process, there was a notable decline in starch content from day 3 to day 6, which was then followed by an ascending trend from day 6 to day 9, peaking at approximately 1.27% on day 9—a value significantly divergent from measurements taken at other intervals. Subsequently, from day 9 to 18, the starch content experienced a decrement, descending to its minimum at around 0.69%, thereafter exhibiting an upturn from day 18 to 21.

The data elucidate that the total nitrogen content of cigar tobacco during the air-drying process undergoes a complex pattern, initially rising, subsequently declining, and then exhibiting a series of ascents and descents. Notably, the nitrogen content peaked at an approximate value of 5.3% on day six of the air-drying process. The nicotine content in cigar tobacco leaves throughout the air-drying process exhibited an undulatory pattern, characterized by phases of increase, followed by decrease, and subsequent cycles of ascent and descent. Notably, the nicotine content with no significant fluctuations detected between the periods of 3 to 6 days and 9 to 15 days. The peak of the nicotine concentration was observed on the 18th day of the air-drying process, where it reached approximately 5.5%.

Sun Yuqing study proved that tobacco characterized by an elevated saccharide content exhibits an oily sheen and glossiness, which enhance its visual quality (Sun et al., 2023c); showed that the levels of reducing sugar and total sugar present in tobacco exhibit a highly significant positive correlation with the equilibrium moisture content (Deng et al., 2019); Consequently, a diminution in starch content coupled with an augmentation in saccharide levels may be indicative of enhanced tobacco quality. The current investigation revealed that the concentrations of the three analytes—total sugar, reducing sugar, and starch—commenced a decline subsequent to attaining their apex at nine days and exhibited a recovery at eighteen days. The underlying cause of this alteration could be attributed to the dehydration of tobacco leaves during the initial air-drying phase, accelerated decomposition of macromolecular carbohydrates at this early juncture, and the resultant production of water-soluble total sugar, reducing sugar, and starch surpassing their respective rates of utilization (Liu et al., 2021). Consequently, there ensued a marked augmentation in the levels of total sugar, reducing sugar, and starch within a brief temporal frame. This conclusion was not consistent with the findings of and that Throughout the Air-drying process, the contents of total sugar and reducing sugar in the tobacco leaves manifested a discernible downtrend. The etiology

of this phenomenon could be ascribed to the employment of abbreviated intervals in the experimental design of the air-drying study, with a more pronounced manifestation of deficiencies observed during the 3-day interval as opposed to the 5-day period. Secondly, variability in the growth environment and cultivar types may also precipitate divergences in the trajectories of change observed between the two parameters (Deng et al., 2021).

The elevated levels of total nitrogen and nicotine constitute a salient characteristic that differentiates cigars from other cigarette varieties, with the concentration and composition of nicotine exerting a significant influence on the organoleptic quality, stylistic intensity, and safety profile of cigars. In the course of the experiment, the total nitrogen content exhibited an initial increase followed by a subsequent decline (Deng et al., 2021). The augmentation of total nitrogen content observed in the pre-air-drying phase and extending into the mid-to-late air-drying stages can potentially be attributed to the inherent abundance of nitrogenous compounds in cigar tobacco. The catabolism of macromolecular nitrogenous substances into smaller nitrogenous entities throughout the air-drying process contributed to the elevation of the overall nitrogen content. This catabolic activity predominantly transpired during the senescent phase, aligning with the findings presented by Li Yifan (Li et al., 2019).

#### **Changes in the pigmentation of tobacco during air-drying**

Table 3 delineates the quantified concentrations of each pigment class throughout the air-drying process of "Yunxue No.1" tobacco. The chart reveals a discernible declining trend in the chlorophyll a content within "Yunxue No.1" tobacco leaves. There was a rapid degradation of chlorophyll a observed from day 3 to day 9, followed by a plateau in the rate of decline. The chlorophyll a content experienced a marked reduction, with a decrement of 93.91% over the course of the air-drying process (Xie et al., 2022). Analogous to chlorophyll a, the chlorophyll b concentration exhibited a similar downtrend. Between days 3 and 9, there was an accelerated decrease in chlorophyll b content, culminating in near-complete degradation by day 9 of the air-drying process. Subsequently, the chlorophyll b levels tended to stabilize, with the air-drying process resulting in a total reduction of 98.83%. In contrast to chlorophyll a and b, the  $\beta$ -carotene levels initially exhibited an upward trend, followed by a subsequent decline. Between days 3 and 6, there was a marked surge in  $\beta$ -carotene content, which was then swiftly followed by a pronounced degradation phase from day 6 to day 9. Post this phase, the changes in  $\beta$ -carotene content became more gradual, with no significant differences observed between the measurements on days 12, 15, 18, and 21. Overall, there was a decrease of 63.97% in  $\beta$ -carotene content from day 3 to day 21 (Xiong et al., 2024).

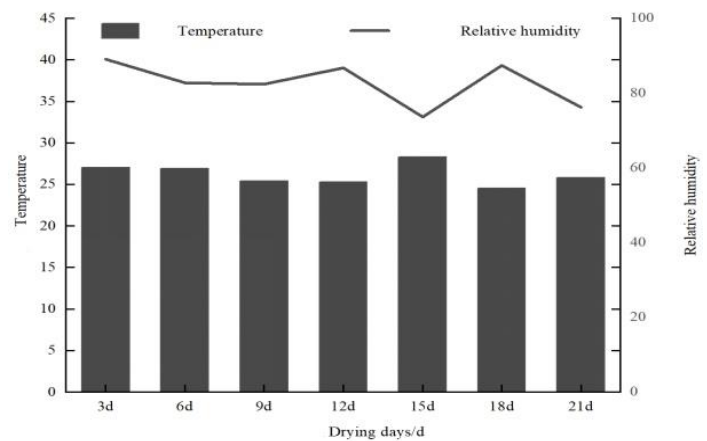
Lutein levels in the leaves similarly manifested an initial increase followed by a decrease throughout the air-drying period. The concentration of lutein peaked at 450.02  $\mu\text{g/g}$  on the sixth day. There was a significant reduction in lutein content, amounting to a 57.00% decrease from day 3 to day 21. Plastidial pigments within tobacco, encompassing chlorophyll and carotenoids, play a pivotal role in dictating the hue of the leaves air-drying. The degradation byproducts of these pigments exert a

significant influence on both the intensity and profile of the tobacco's aroma. During the initial phase of the air-drying process, a substantial degradation of carotenoids in the tobacco leaf was observed, whereas their concentration reached a state of equilibrium in the latter stages of the modulation period (Li et al., 2021, Muhammad Tahir, 2024, Li et al., 2024, Khan et al., 2024). The present investigation revealed that the concentrations of plastidial pigments in cigar tobacco foliage underwent a marked diminution throughout the air-drying phase, relative to their initial levels. However, an ascendant trend was observed for the two carotenoids during the pre-drying stage, culminating in a peak at six days. The data gleaned from the experiments indicated that the catabolism of chlorophyll a outpaced that of chlorophyll b. Similarly, the breakdown of  $\beta$ -carotene exceeded that of lutein. Notably, the diminution of carotenoids was significantly less pronounced than the depletion of chlorophylls, implying that the latter's degradation during the air-drying phase could be instrumental in the alteration of color in the cured tobacco leaves (Yang et al., 2020). This is the same as the results obtained by Lu Shaohao (Lu et al., 2019) in terms of chlorophyll, but slightly different in terms of carrots. The reason for this phenomenon may be that this experiment adopted a shorter interval time in the experimental design of the drying experiment. Different curing conditions, different types of tobacco leaves and different geographical and ecological environment also lead to the differences.

**Changes in tobacco polyphenols during air-drying process**

Table 4 elucidates the dynamic alterations in polyphenol concentrations observed during the air-drying process of "Yunxue No.1" tobacco. Throughout the air-drying process, the chlorogenic acid levels demonstrated an upward trajectory followed by a decline, culminating in a peak concentration at day 9. At this zenith, the chlorogenic acid concentration was measured at 0.4840 mg/g, subsequent to which it exhibited a gradual decrement. Between days 12 and 21, the chlorogenic acid levels stabilized, as evidenced by the lack of significant variance, indicating a plateau in the content variation during the latter stages of the air-drying process. The rutin content was characterized by a recurrent fluctuating trend, exhibiting three principal peaks occurring on days 9, 15, and 21, respectively. At these three temporal markers, the rutin concentrations were comparably high and did not differ significantly from one another, each surpassing the levels noted during the initial air-drying phase (Xie et al., 2022). The apex of rutin concentration was recorded on day 9, reaching 2.1471 mg/g. In contrast to the levels of chlorogenic acid and rutin, the scopoletin content within the tobacco leaf was comparatively low, yet it displayed an overarching trend of increment. It was characterized by an initial increase, followed by a decrease, and a subsequent rise. The concentration of scopoletin peaked on day 9, registering a maximum of 0.0126 mg/g. Polyphenolic compounds and plastidic pigments constitute critical constituents within tobacco, exerting a direct influence on the hue and the overall visual quality of the cured tobacco leaf (Zhou et al., 2019). Chlorogenic acid, scopoletin, and rutinoides represent the predominant polyphenolic constituents in tobacco. Chen Qingqing found that the aggregate phenolic concentration

in the white ribbed tobacco TN86 exhibited a decrement concomitant with the air-drying process, reaching a nadir at 3 days (Chen et al., 2021). There was a modest resurgence in phenolic content at 6 days post-drying, albeit the levels remained subpar in comparison to those measured at the juncture of harvesting, followed by a subsequent gradual diminution. In this study, chlorogenic acid and rutinoides demonstrated analogous trajectories; however, their concentrations at 9 days were observed to surpass the levels recorded at 3 days. Furthermore, scopoletin exhibited a pattern of recurrent oscillation throughout the experimental timeline, with its concentration peaking thrice, specifically at 9, 15, and 21 days. Notably, the levels of brassinolide at these intervals surpassed those measured at the air-drying onset. The causative factors underlying this phenomenon could be attributed primarily to the significant dissimilarities between cigar tobacco leaves and white rib tobacco leaves, which manifest in distinct polyphenolic compound profiles. Furthermore, the air-drying processes for white rib tobacco diverge markedly from those for cigar tobacco. Lastly, the disparities in geographic locales and ecological milieus contribute to the observed differences (Zhang et al., 2024).



**Figure 1:** Temperature and humidity changes in the air-drying room during the air-drying process

**Table 1:** Moisture content of cigar tobacco leaves at different times

Air-drying time/days	Moisture content
3	83.95±0.62 <sup>a</sup>
6	81.82±1.48 <sup>a</sup>
9	72.88±3.46 <sup>a</sup>
12	40.03±25.3 <sup>b</sup>
15	42.93±2.21 <sup>b</sup>
18	25.09±6.29 <sup>c</sup>
21	28.6±6.02 <sup>c</sup>

Note: Distinct lowercase letters assigned within the same column denote statistically significant differences among treatments at the  $P < 0.05$  level.

**Correlation analysis of different chemicals in the air-drying process**

As delineated in Table 5, an in-depth correlation analysis was conducted on the content variation of diverse chemical constituents within "Yunxue 1" tobacco throughout the air-drying process. The findings indicated that the association between relative humidity and the concentration of individual chemical constituents was not markedly evident (Zhao et al., 2023, Zeng et al., 2024, Saeed et al., 2024). The moisture level of the tobacco leaves exhibited a substantial correlation with the concentrations of a myriad of chemical compounds. Specifically, the moisture level was found to be significantly positively correlated with the total sugar, chlorophyll a,  $\beta$ -carotene, and lutein concentrations. Additionally, a pronounced positive correlation was observed with the levels of reducing sugar, total nitrogen, and chlorophyll b, while a significantly negative correlation was discerned with nicotine content. This finding was in confirmation with (Sun et al., 2023b). The concentration of total sugars was found to be significantly and positively correlated with the levels of reducing sugars, and exhibited a substantial and negative correlation with nicotine concentrations. The total nitrogen concentration exhibited a notably positive correlation with  $\beta$ -carotene levels. Moreover, the concentration of nicotine was found to be inversely correlated with the concentrations of the four pigments—

chlorophyll a, chlorophyll b,  $\beta$ -carotene, and lutein—in a statistically profound manner. The contents of chlorophyll a and chlorophyll b, as well as  $\beta$ -carotene and lutein, demonstrated a statistically significant positive correlation (Chen et al., 2023, Rashid et al., 2024, Mushtaq et al., 2024, Murtaza et al., 2024). The concentration of chlorophyll b exhibited a significantly positive correlation with the levels of  $\beta$ -carotene, while its correlation with lutein concentrations was of an even higher statistical significance. Additionally, the relationship between  $\beta$ -carotene and lutein concentrations was characterized by a profoundly significant positive correlation. This finding suggested that the moisture levels within the tobacco leaves markedly influence the ultimate quality of the dried tobacco, mediated through alterations in the concentrations of diverse chemical compounds (Sun et al., 2023a). The concentrations of the four plastidic pigments—namely, nicotine, chlorophyll a, chlorophyll b,  $\beta$ -carotene, and lutein—were intricately interconnected, exhibiting strong interdependencies amongst each other. These experimental results were consistent with the conclusions of Wang Huifang's previous experiments (Wang et al., 2024).

**Table 2:** Changes in carbohydrate content (%) during air-drying of cigar tobacco

Traits	Air-drying days/d						
	3d	6d	9d	12d	15d	18d	21d
Total sugar	2.6931±0.0703 <sup>b</sup>	1.9889±0.075 <sup>c</sup>	3.2009±0.1736 <sup>a</sup>	0.8821±0.0941 <sup>e</sup>	0.8695±0.2755 <sup>e</sup>	0.5147±0.122 <sup>f</sup>	1.305±0.0974 <sup>d</sup>
Reducing sugar	1.4253±0.0701 <sup>b</sup>	1.4874±0.075 <sup>ab</sup>	1.7215±0.1738 <sup>a</sup>	0.4328±0.0947 <sup>d</sup>	0.4182±0.2755 <sup>d</sup>	0.1919±0.1224 <sup>d</sup>	0.8638±0.0972 <sup>c</sup>
Starch	0.8541±0.0702 <sup>b</sup>	0.785±0.0751 <sup>b</sup>	1.2722±0.1739 <sup>a</sup>	0.8817±0.0938 <sup>b</sup>	0.7741±0.2753 <sup>b</sup>	0.6861±0.1218 <sup>b</sup>	0.9603±0.0966 <sup>b</sup>
Total nitrogen	4.9142±0.0702 <sup>b</sup>	5.308±0.075 <sup>a</sup>	4.5225±0.1739 <sup>c</sup>	4.0936±0.1002 <sup>e</sup>	4.3439±0.2753 <sup>cde</sup>	4.4505±0.1227 <sup>cd</sup>	4.1733±0.0971 <sup>de</sup>
Nicotine	4.0895±0.1337 <sup>c</sup>	4.4251±0.1966 <sup>c</sup>	4.9587±0.2545 <sup>b</sup>	5.028±0.2776 <sup>b</sup>	5.0707±0.1368 <sup>ab</sup>	5.5031±0.2795 <sup>a</sup>	5.1579±0.1852 <sup>ab</sup>

Note: Distinct lowercase letters assigned within the same column denote statistically significant differences among treatments at the  $P < 0.05$  level.

**Table 3:** Variation of total nitrogen and nicotine content in different parts of cigar tobacco leaves during air-drying process (ug/g)

Traits	Air-drying days/d						
	3d	6d	9d	12d	15d	18d	21d
Chlorophyll a	62.4292±3.5616 <sup>a</sup>	45.9173±3.7937 <sup>b</sup>	6.899±2.4746 <sup>cd</sup>	8.2367±3.3061 <sup>cd</sup>	9.1542±1.2751 <sup>c</sup>	3.2352±0.37 <sup>d</sup>	3.7981±0.31 <sup>d</sup>
Chlorophyll b	82.8159±3.5616 <sup>a</sup>	26.9545±3.7937 <sup>b</sup>	3.0715±2.4746 <sup>c</sup>	3.4113±3.3061 <sup>c</sup>	2.6605±1.2751 <sup>c</sup>	1.1957±0.37 <sup>c</sup>	0.9691±0.31 <sup>c</sup>
Beta carotene	2431.9777±153.4866 <sup>b</sup>	3226.8859±203.7267 <sup>a</sup>	1482.3972±276.2978 <sup>c</sup>	1183.4284±189.7359 <sup>cd</sup>	1239.6560±196.9036 <sup>cd</sup>	1013.3740±105.4835 <sup>d</sup>	876.3596±137.9171 <sup>d</sup>
Lutein	288.3439±13.4937 <sup>b</sup>	450.0196±38.7855 <sup>a</sup>	196.2840±17.6467 <sup>c</sup>	182.2751±13.1502 <sup>c</sup>	173.8013±13.8488 <sup>c</sup>	166.0975±18.9208 <sup>c</sup>	123.9880±10.2955 <sup>d</sup>

**Table 4:** Changes of polyphenol content in different parts of cigar tobacco leaves during air-drying process (mg/g)

Traits	Air-drying days/d						
	3d	6d	9d	12d	15d	18d	21d
Chlorogenic acid	0.2316±0.0803 <sup>b</sup>	0.2828±0.09 <sup>b</sup>	0.4840±0.1051 <sup>a</sup>	0.2886±0.0803 <sup>b</sup>	0.2879±0.0609 <sup>b</sup>	0.2423±0.0373 <sup>b</sup>	0.1592±0.0308 <sup>b</sup>

Rutin	1.6275±0.2326 <sup>b</sup>	2.2731±0.19 <sup>a</sup>	2.5057±0.2001 <sup>a</sup>	1.614±0.2326 <sup>b</sup>	2.4272±0.1604 <sup>a</sup>	0.9086±0.13 <sup>d</sup>	2.1471±0.2427 <sup>a</sup>
Scopoletin	0.0072±0.0006 <sup>c</sup>	0.0089±0.0024 <sup>bc</sup>	0.0126±0.0021 <sup>a</sup>	0.0111±0.0013 <sup>ab</sup>	0.0095±0.0005 <sup>bc</sup>	0.0068±0.001 <sup>c</sup>	0.0097±0.0019 <sup>abc</sup>

**Table 5:** Correlation analysis of different chemicals in the air-drying process

Traits	Relative Humidity	Moisture content	Total sugar	Reducing sugar	Starch	Total nitrogen	Nicotine	Chlorophyll a	Chlorophyll b	Beta carotene	Lutein	Chlorogenic acid	Rutin
Moisture content	0.29												
Total sugar	0.19	0.85*											
Reducing sugar	0.11	0.874	0.95**										
Starch	-0.11	0.30	0.70	0.62									
Total nitrogen	0.29	0.81*	0.51	0.64	-0.16								
Nicotine	-0.32	-0.96**	-0.78*	-0.75	-0.28	-0.64							
Chlorophyll a	0.17	0.89**	0.42	0.39	-0.07	0.5	-0.85*						
Chlorophyll b	0.53	0.86*	0.53	0.5	0.03	0.57	-0.93**	0.85*					
Beta carotene	0.21	0.93**	0.60	0.64	-0.03	0.82*	-0.89**	0.82*	0.85*				
Lutein	0.35	0.89**	0.64	0.67	0.07	0.75	-0.92**	0.78*	0.92**	0.96**			
Chlorogenic acid	-0.21	0.14	0.14	0.21	0.21	-0.07	-0.17	0.03	0.21	0.28	0.32		
Rutin	-0.70	0.39	0.46	0.54	0.55	0.13	-0.32	0.25	0.03	0.42	0.28	0.42	
Scopoletin	-0.32	0.07	0.31	0.29	0.80	-0.36	-0.07	-0.14	-0.10	-0.10	-0.03	0.60	0.53

Note: A single asterisk (\*) denotes a statistically significant correlation at the  $P < 0.05$  level, while a double asterisk (\*\*) signifies a highly significant correlation at the  $P < 0.01$  level.

**CONCLUSION**

In this experiment, an in-depth study was carried out to explore the alterations in the chemical composition of cigar over the course of the drying period. The contents of various substances such as total sugar, reducing sugar, starch, total nitrogen, nicotine, chlorophyll a, chlorophyll b, β-carotene, lutein, chlorogenic acid, rutin, and scopolamine within the tobacco were meticulously examined. The experimental outcomes revealed that the moisture content present within the tobacco leaves had a considerable and substantial influence on the ultimate result of the air-drying process. This impact was mediated by changes in the levels of a diverse array of chemical constituents. The concentration of nicotine was closely and intrinsically linked with the levels of four plastidic pigments, namely chlorophyll a, chlorophyll b, β-carotene, and lutein. Furthermore, these four plastidic pigments mutually displays a distinct and marked interdependence among themselves. In the future, subsequent research should primarily concentrate on optimizing the air-drying process of the Yunnan cigar variety "Yunxue No.1" in accordance with these firmly established rules. This undertaking holds significant academic worth and merit as it has the potential to contribute towards the advancement and refinement of the air-drying techniques for cigar and the overall quality of the final product.

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