Effect of Different Drying Temperatures on Biochemical Profile of Tobacco (*Nicotiana tabacum* L.)

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Abstract [**Objectives**] To elucidate the impact of different drying temperatures on the biochemical profile of tobacco (*Nicotiana tabacum* L.) leaves, the chemical constituents and ultrastructure of Yunyan 87 leaves subjected to different curing temperature settings. [**Methods**] Near-infrared spectroscopy techniques were utilized to analyze tobacco leaf samples, comparing the changes in chemical constituents at different curing temperatures. [**Results**] The CK treatment resulted in lower concentrations of nicotine, total nitrogen, chlorine, potassium, and starch, while simultaneously enhancing the levels of total sugar, reducing sugar, and protein. In comparison to the T treatment, the CK treatment appropriately altered the cell structure, reducing the content of cell wall substances. [**Conclusions**] These findings suggest that low-temperature curing at 44 ℃ during the color-fixing stage is beneficial for improving the quality of tobacco leaves.

Key words Chemical indicator, Ultrastructure, Nicotine content, Flue-cured

0 Introduction

Tobacco (*Nicotiana tabacum* L.) holds significant economic importance and serves as a vital model for investigating plant genetics, breeding, and biochemical processes[1-2]. The process of leaf curing is crucial for enhancing the quality of tobacco leaves for industrial applications and consists of several distinct stages: yellowing, flue-curing color fixing, and drying. These stages involve complex biochemical reactions in which carbohydrates and proteins continually degrade and convert, yielding molecular metabolites essential for tobacco quality[3-4]. The curing quality directly determines the final product's quality and effectiveness. However, the process faces several challenges, including the precise regulation of flue-curing temperature, duration, humidity, and variations in raw material traits[5-7]. Particularly, temperature control during the flue-curing process influences the drying rate and color of tobacco, and critically impacts the content and aromatic profiles of volatile components[8-9].

The flue-curing color fixing stage is crucial for the drying and enhancement of tobacco leaf quality. However, challenges such as brief periods of temperature stabilization, rapid heating rates, and inadequate calibration of parameters may result in undesirable outcomes, including stiffness, poor softness, insufficient aroma, excessive variegation, and uneven surfaces in the cured leaves[10-11]. Temperatures of 44 ℃ and 48 ℃ are critical for effective color fixing and aroma development. Stable curing at these temperatures facilitates the complete degradation of macromolecules, thereby achieving the desired yellowing and fragrance. Therefore, targeted research on stable temperature curing at these key points is essential[12-13].

The flue-curing process has been the subject of extensive research regarding its capacity to enhance the quality of tobacco leaves. Excessively high dry bulb temperatures or rapid heating rates during this process can hinder the formation of aromatic substances in tobacco leaves, thereby impairing their sensory quality[14]. Bortolini *et al.* [15] found that maintaining a maximum dry bulb temperature exceeding 68 ℃ during the drying stage can reduce energy use and costs, while enhancing the physical properties, chemical constituents, and sensory quality of upper flue-cured tobacco leaves. When the dry bulb temperature is 44 - 47 ℃ in the color fixing period, the water loss of tobacco leaves is less than 50%, the relative humidity of the air is high, and the enzyme activity is strong. After the curing process, the chemical constituents of the tobacco leaves are more coordinated, resulting in a complete transformation of their contents, which ultimately enhances the quality of flue-cured tobacco[9,16].

In the baking of tobacco leaves, the temperature of dry ball has a decisive effect on the quality of tobacco leaves. This study investigates a key temperature point, specifically the dry ball temperature, and its effects on the micro-structures of tobacco leaves. The effect of chemical constituent content clarifies the changes in the accumulation content inside the tobacco leaves on the biological process of quality control of tobacco leaves, identifies the key molecular mechanism, and determines the reasonable dry ball temperature. The ob-

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jective is to decrease the prevalence of smooth and stiff cigarettes. Additionally, enhancements are made to the quality of the baking process.

1 Materials and methods

1.1 Experimental site and materials From 2022 to 2023, a field experiment was conducted at the Fuquan Experimental Base of the Guizhou Academy of Tobacco Science using the flue-cured tobacco variety Yunyan-87. A flat, well-drained plot with a row-to-plant spacing of 1.20 m × 0.50 m was selected, and local high-quality production practices were implemented. The top 4 – 6 tobacco leaves of the cigarette plant, exhibiting uniformity in phenotype characteristics such as maturity, color, and size during the mature period were used as experimental materials. The experimental curing barn, designed by the Guizhou Institute of Tobacco Science, consisted of 6 dense electric bulk curing barns measuring 3.5 m in length and 1.35 m in width. Three of these barns served as control (CK) and the remaining three as treatment (T), each replicated three times. Tobacco samples were placed on the second layer from the top of the curing barn. Four rods of tobacco leaves were loaded through the tobacco loading door, with one rod placed in each of the left and right paths.

1.2 Experimental design In this experiment, fresh tobacco leaves (F) were set up, and the dry bulb temperature at the initial stage of color fixing was 44 ℃ for control (CK) and 46 ℃ for treatment (T). In the mid-stage of color setting, the dry bulb temperature was adjusted to 44 ℃ (CK) and 46 ℃ (T) respectively, and kept for 87 h, while in other stages, it was carried out by three-stage baking[17-18].

1.3 Sampling method During the curing process, two key temperature points, referred to as the dry bulb temperatures, were 44 ℃ (CK) and 46 ℃ (T). After the fresh samples were collected in the field, they were promptly stored in a mixture of liquid nitrogen and formalin-aceto-alcohol (FAA) before being transported to the laboratory for testing. The samples used for the determination of chemical constituents consisted of flue-cured tobacco, with each sample being repeated three times. For ultrastructural observation, the samples included fresh tobacco leaves and flue-cured tobacco leaves subjected to 44 ℃ and 46 ℃.

1.4 Determination items and methods

1.4.1 Chemical index content analysis. Chemical indicators, including reducing sugars, total sugars, nicotine, total nitrogen, total potassium, and total chlorine, were quantified in dried and crushed tobacco leaf samples using a near-infrared spectrometer (NIR)[19-20]. Additionally, the protein and starch contents were also determined using established protocols[21-22]. The temperature was maintained at 25 ℃ with 68% relative humidity for conventional chemical constituent determination, while protein and starch determination were conducted at 15 ℃ with 40% relative humidity. A Bruker (Germany) MPA Fourier transform near-infrared spectrometer, equipped with a diffuse reflection integrating sphere and a sample rotator, served as the primary instrument for this study. Spectra were collected over the range of 12 000 to 4 000 cm^{-1} with a spectral resolution of 8 cm^{-1}. Each spectrum required 64 scans, approximately 30 sec per scan, and was obtained using the Windows XP platform. Methodically, samples were evenly distributed into quartz cups to a depth of 3 – 4 mm and subsequently flattened using a sample press. Initial background spectrum scans were conducted prior to the collection of the sample spectrum[23].

1.4.2 Ultrastructural analysis. A specimen of 1 mm × 2 mm was excised using a sharp double-edged blade from the central region of the main vein of flue-cured tobacco leaves. This specimen was subsequently fixed in a 4% glutaraldehyde solution, followed by washing with a phosphate buffer solution at pH 7.2, and finally fixed in a 1% osmium acid solution. Following fixation, standard ultrathin sectioning procedures were carried out, including dehydration, infiltration, embedding, polymerization, sectioning, and staining using uranyl acetate-lead citrate double staining[24]. Observations were made using a transmission electron microscope (JEM-1400, Japan) at 100 kV, focusing on 10 fields of view and key indicators like ultrastructures, cell walls, chloroplasts, and nuclei.

1.4.3 Statistical analysis. The Origin 2022 software was utilized for data processing and plotting. The IBM SPSS Statistics 27 software was employed to conduct variance and correlation analysis of the data, so as to analyze the significance of differences between different treatments at the 5% significance level.

2 Results and analysis

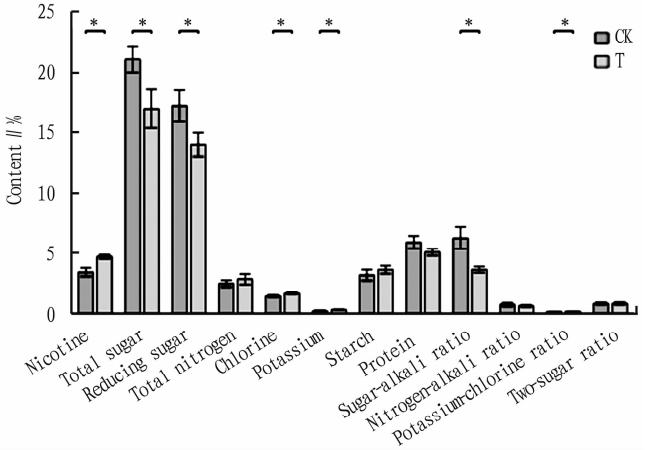
2.1 Changes in the chemical constituent of tobacco leaves The nicotine content of high-quality grilled cigarettes in China is 1.5% – 3.5%. The total nitrogen content is 1.5% – 3.5%. The starch content is 4% – 5%, while the total sugar content varies from 18% to 22%. The reducing sugar content is reported to be 16% – 18%. The potassium content exceeds 2%, and the chlorine content is below 1%. The two-sugar ratio is greater than 0.85, the sugar-alkali ratio ranges from 4 to 10, the nitrogen-alkali ratio is less than 1, and the potassium-chlorine ratio is equal to or greater than 4. The nicotine content of tobacco cigarettes under T treatment was about 4.67%, while the total nitrogen content was around 2.8%. Additionally, the starch content was about 3.70%, and the two-sugar ratio was approximately 0.9%. These values were more in line with the standards of high-quality cigarettes. As can be seen from Fig. 1, with the advancement of the curing process, the changing trend of chemical constituents of tobacco leaves was as follows.

The nicotine content in tobacco leaves increased. The nicotine content in tobacco leaves under CK treatment was about 3.76%, and that under T treatment was about 4.67%. This represents a significant increase of 0.91%.

The content of total sugar and reducing sugar decreased. The contents of total sugar and reducing sugar in tobacco leaves under CK treatment were about 21.04% and 17.19%, respectively,

while those under T treatment were about 16.93% and 13.96%, respectively. The reductions in total sugar and reducing sugar are statistically significant, amounting to 4.11% and 3.23%, respectively.

The total nitrogen content increased. The total nitrogen content in tobacco leaves under CK treatment was about 2.46%, and that under T treatment was about 2.83%. This represents an increase of 0.37%. The chlorine content increased. The chlorine content in tobacco leaves under CK treatment was about 1.43%, and that under T treatment was about 1.65%. This represents an increase of 0.22%. The content of potassium increased. The potassium content in tobacco leaves under CK treatment was about 0.21%, and that under T treatment was about 0.32%. This represents an increase of 0.11%. The starch content increased. The starch content in tobacco leaves under CK treatment was about 3.17%, and that under T treatment was about 3.61%. This represents an increase of 0.44%. The content of protein decreased. The protein content in tobacco leaves under CK treatment was about 5.88%, and that under T treatment was about 5.04%. This represents a decrease of 0.44%.

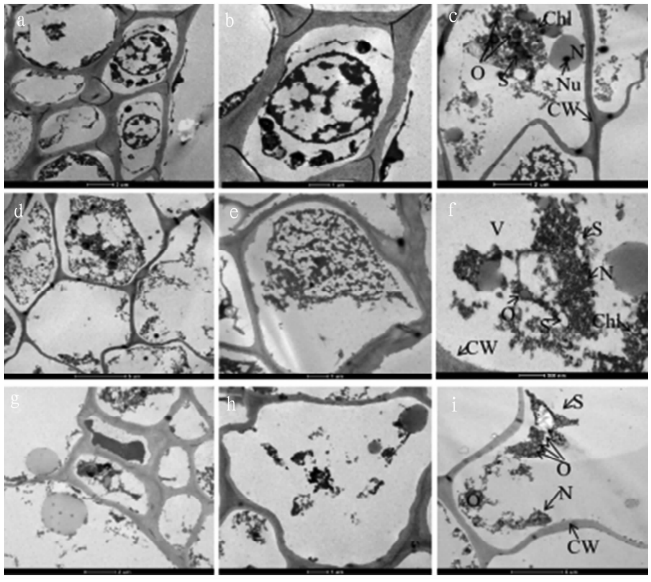


NOTE Error bars depict standard deviation. * means that there is a significant difference between the two groups when $P < 0.01$.

Fig. 1 Contents of the main chemical constituents in Yunyan-87 tobacco leaves

2.2 Observation of the cell ultrastructure of tobacco leaves

Cell ultrastructural analysis of flue-cured tobacco leaves revealed predominantly elliptical cells with some square or irregular shapes (Fig. 2). The cell membranes were observed to be continuous and clear. In fresh tobacco leaves, chloroplasts and other organelles were found to be clustered near the cell wall within the palisade tissue. A large central vacuole contained numerous bubbles. Oval-shaped chloroplasts displayed prominent starch and osmiophilic granules. The cytoplasm contained irregular osmiophilic substances, while the mitochondria were sparse and difficult to observe. The cell nucleus exhibited a clear double-membrane structure, distinct nucleolar boundaries, uniform chromatin, and a dense nucleolus.



NOTE a – c. Supermicrostructural forms of fresh – like blades; d – f. Supermicrostructural forms after CK treatment and baking; g – i. Supermicrostructural forms of the leaves after baking. Cw. Cell wall; Chl. Chloroplast; O. Osmiophilic granule; S. Starch granule; V. Vacuole; N. Nucleus; Nm. Nuclear membrane; Nu. Nucleolus; Ch. Chromatin.

Fig. 2 Changes in the cell ultrastructure of Yunyan-87 tobacco leaves

Exposure to the temperature of CK treatment resulted in a slight curling of tobacco leaves. Cell organelles such as chloroplasts were scattered around the nucleus. Furthermore, there was a notable decrease in the number of chloroplasts, with many exhibiting deformation, cracking, and aggregation, and the nucleus was condensed and deformed. The area of osmiophilic particles within chloroplasts increased, showing cavitation, while mitochondria disappeared. The central vacuole filled the central region of the cell, and the number of large starch particles was small, but small particles remained clearly observable.

Under T treatment, it was found by scanning electron microscope that there were few identifiable substances in the cells. The distribution of starch granules decreased, with osmiophilic granules being the most prominent among them. Cell nuclear shrinkage and deformation were observed. The cell wall exhibited shrinkage and twisting, resulting in a reduction in thickness, while its coloration became lighter and more diffuse. Furthermore, the phenomena of rupture and degradation were more obvious.

3 Discussion

3.1 Changes in the chemical constituent of tobacco leaves during the tobacco curing process The chemical constituent of tobacco leaves is crucial in determining their quality and characteristics. During the curing process, various chemical constituents undergo significant changes that impact the aroma, taste, and overall quality of tobacco[25]. Nicotine and tar, which are essential to tobacco's sensory attributes, undergo degradation and transformation during the curing process, altering their distribution and

content in the final product[26]. The key indicators of flue-cured tobacco quality include total sugar, reducing sugars, nicotine, total nitrogen, and protein levels[27-28].

The optimal protein content in high-quality tobacco leaves typically ranges from 7% to 9%, as excessive protein levels can negatively affect aroma and taste. This study corroborates previous findings that protein degradation during the curing process is gradual and stable[29]. Additionally, the study found no significant differences in potassium or protein content among different curing conditions, indicating consistent effects. With increasing dry bulb temperature, the total and reducing sugar contents in the CK treatment exceeded those in the T treatment, indicating an upward trend[30]. Although the contents of both sugars were significantly higher in the CK treatment than in the T treatment, the low sugar content in the T treatment may lead to a sharp and irritating smoke, negatively affecting the sensory quality of the tobacco leaves. The sugar contents in the CK treatment fall within the range associated with high-quality tobacco leaves. Additionally, the nicotine, total sugar, reducing sugar, and chlorine contents in tobacco leaves under the CK and T treatments exhibited differences, indicating that the different treatment methods have a differential impact on these constituents. The environmental and soil conditions during tobacco cultivation also influence the chemical constituent, affecting the levels of total nitrogen, sugar, chlorine, and protein. Sugars and lipids are primary precursors for tobacco aroma, and their transformation during curing is crucial for aroma development[31]. During the color-fixation stage of tobacco leaves, the transformation of aromatic compounds is relatively active. However, as the dry bulb temperature becomes excessively high, the chemical reactions in the tobacco leaves essentially cease, leading to the easy volatilization and loss of aromatic substances[32].

Under the treatment condition of 46 ℃, the dehydration of tobacco leaves occurs rapidly, with a higher degree of membrane lipid peroxidation and an accelerated decline in physiological function, ultimately resulting in a higher total nitrogen content[33]. Studies by Su *et al.* [34] have shown that within a certain range, the higher the total nitrogen content in tobacco leaves, the more likely it is for the cured leaves to become stiff. The technique of reducing the curing temperature has a positive impact on decreasing the proportion of stiff tobacco leaves, which may be related to the degree of degradation of macromolecular substances within the leaves. Appropriate moisture and temperature are necessary to ensure the activity of cell wall enzymes and amylase, allowing for the thorough degradation of the corresponding macromolecular substances[35].

3.2 Observation of the ultrastructure of tobacco leaves Curing is a critical stage in flue-cured tobacco production, significantly influencing the quality and flavor of the final product. This process induces significant structural changes in tobacco leaves, affecting their physiological and biochemical characteristics[36]. Electron microscopy of fresh tobacco leaves revealed closely arranged organelles near the cell wall, oval chloroplasts, intact cell membranes, sparse mitochondria, clear nuclear structures with uniform chromatin and dense nucleoli, and thick, dark cell walls. As flue-curing progresses and temperature increases, the distortion and rupture of cell organelles and walls become pronounced. The starch granule content decreases, and low electron density osmiophilic substances accumulate within the cells. Electron microscopy revealed that before 44 ℃, tobacco leaf cells exhibit significant structural and compositional changes, triggering intense cellular responses and partial damage. At 46 ℃, severe cell membrane damage occurs, impairing normal functions. The pre-46 ℃ stage is thus critical for the physiology and ultrastructure of tobacco leaves, influencing tissue quality.

Research shows that osmiophilic particles in palisade tissue are crucial for determining tobacco leaf aroma and oil content, accumulating lipid substances likely derived from chloroplast inner membrane system degradation[37]. As the curing process advances, tobacco leaf cells gradually lose vitality, halting substance transformation. Consequently, this significantly changes the cell structure, shape, and internal constituents, along with a decrease in cellular water content. The cell walls undergo hardening, while the leaf color transitions to a brown hue. Physiological and biochemical processes cease, leading to a reduction in identifiable substances. Additionally, there is a decrease in starch granules, shrinkage of nuclei, and contraction and twisting of cell walls. This leads to thinner, lighter-colored cells with increased degradation and rupture[38].

4 Conclusions

This study showed that lowering the dry bulb temperature led to a timely softening of the leaf texture, improved tissue structure, and reduced proportion of stiffness in the cured leaves. The curing treatment at 44 ℃ was observed to induce a more appropriate level of disruption to the cellular structures and a reduction in cell wall substance content when compared to the 46 ℃ curing treatment. These findings offer further insights into the physiological and biochemical changes associated with tobacco leaf curing.

References

[1] WU SJ, GAO CC, PAN H, *et al.* Advancements in tobacco (*Nicotiana tabacum* L.) seed oils for biodiesel production[J]. Frontiers in Chemistry, 2021, 9: 834936.

[2] KINAY A, YILMAZ G, KANDEMIR N. Yield and quality properties of some oriental tobacco (*Nicotiana tabacum* L.) hybrids[J]. Genetika, 2020, 52: 735-750.

[3] BANOZIC M, JOKIC S, ACKAR D, *et al.* Carbohydrates key players in tobacco aroma formation and quality determination[J]. Molecules, 2020, 25: 1734.

[4] CHEN YJ, REN K, HE X, *et al.* Dynamic changes in physiological and biochemical properties of flue-cured tobacco of different leaf ages during flue-curing and their effects on yield and quality[J]. BMC Plant Biology, 2019, 19: 555.

[5] LIU HP, DUAN SM, LUO HL. Design and temperature modeling simulation of the full closed hot air circulation tobacco bulk curing barn[J]. Symmetry, 2022, 14: 1300.

[6] ZONG JJ, HE X, LIN ZL, *et al.* Effect of two drying methods on chemical transformations in flue-cured tobacco[J]. Drying Technology, 2022, 40: 188-196.

[7] JIA JX, ZHANG MJ, ZHAO JC, *et al.* The effects of increasing the dry-

bulb temperature during the stem-drying stage on the quality of upper leaves of flue-cured tobacco[J]. Processes, 2023, 11: 726.
[8] ZOU CM, HU XD, HUANG W, *et al.* Different yellowing degrees and the industrial utilization of flue-cured tobacco leaves[J]. Scientia Agricola, 2019, 76: 1 - 9.
[9] CHEN J, LI Y, HE X, *et al.* Influences of different curing methods on chemical compositions in different types of tobaccos[J]. Industrial Crops and Products, 2021, 167: 113534.
[10] MENG Y, WANG YH, GUO WM, *et al.* Analysis of the relationship between color and natural pigments of tobacco leaves during curing[J]. Scientific Reports, 2024, 14: 166.
[11] CONDORI M, ALBESA F, ALTBELLI F, *et al.* Image processing for monitoring of the cured tobacco process in a bulk-curing stove[J]. Computers and Electronics in Agriculture, 2020, 168: 105113.
[12] ABUBAKAR Y, YOUNG JH, JOHNSON WH, *et al.* Modeling moisture and chemical changes during bulk curing of flue-cured tobacco[J]. Transactions of the ASAE, 2003, 46(4): 1123 - 1134.
[13] HU BB, GU KY, GONG JSQ, *et al.* The effect of flue-curing procedure on the dynamic change of microbial diversity of tobaccos[J]. Scientific Reports, 2021, 11: 5354.
[14] CAO GS, BAO YF, WU C, *et al.* Analysis on efficiency optimization of tobacco leaf flue-curing process[J]. Procedia Engineering, 2017, 205: 540 - 547.
[15] BORTOLINI M, GAMBERI M, MORA C, *et al.* Greening the tobacco flue-curing process using biomass energy: A feasibility study for the flue-cured Virginia type in Italy[J]. International Journal of Green Energy, 2019, 16: 1220 - 1229.
[16] MENG Y, WANG YH, GUO WM, *et al.* Analysis of the relationship between color and natural pigments of tobacco leaves during curing[J]. Scientific Reports, 2024, 14: 166.
[17] SUN CL, ZHANG HL, ZHOU DB, *et al.* Based on metabolomics, the optimum wind speed process parameters of flue-cured tobacco in heat pump bulk curing barn were explored[J]. Scientific Reports, 2023, 13: 21558.
[18] CHEN J, Li Y, HE X, *et al.* Influences of different curing methods on chemical compositions in different types of tobaccos[J]. Industrial Crops and Products, 2021, 167: 113534.
[19] BORGES-MIRANDA A, PEREZ-MARTINEZ C, JIMENEZ-CHACON J, *et al.* Near infrared spectroscopic analysis of total alkaloids as nicotine, total nitrogen and total ash in *Cuban cigar* tobacco[J]. Journal of Near Infrared Spectroscopy, 2019, 27: 123 - 133.
[20] WEI KS, BIN J, WANG F, *et al.* On-line monitoring of the tobacco leaf composition during flue-curing by near-infrared spectroscopy and deep transfer learning[J]. Analytical Letters, 2022, 55: 2089 - 2107.
[21] HU WR, ZHOU QW, CAI W, *et al.* Effects of coffee and cocoa as fermentation additives on sensory quality and chemical compositions of cigar tobacco leaves[J]. Food Science and Technology, 2022, 43: e96122.
[22] CAI X, GAO TY, ZHENG SY, *et al.* Effects of enclosure succession on the morphological characteristics and nutrient content of a bamboo whip system in a moso bamboo (*Phyllostachys edulis*) forest on Wuyi mountain, China[J]. Forests, 2023, 14: 2193.
[23] JIANG DY, HU G, QI GQ, *et al.* A fully convolutional neural network-based regression approach for effective chemical composition analysis using near-infrared spectroscopy in cloud[J]. Journal of Artificial Intelligence and Technology, 2021, 1: 74 - 82.
[24] BUKHARI SA, ZHENG WT, XIE LP, *et al.* Cr-induced changes in leaf protein profile, ultrastructure and photosynthetic traits in the two contrasting tobacco genotypes[J]. Plant Growth Regulation, 2016, 79: 147 - 156.
[25] FORSTER M, LIU C, Duke, M G, *et al.* An experimental method to study emissions from heated tobacco between 100 - 200 ℃[J]. Chemistry Central Journal, 2015, 9: 1 - 10.
[26] CAMLICA M, YALDIZ G. Analyses and evaluation of the main chemical components in different tobacco (*Nicotiana tabacum* L.) genotypes[J]. Grasas Aceites, 2021, 72: e389.
[27] LI XF, BIN J, YAN XF, *et al.* Application of chromatographic technology to determine aromatic substances in tobacco during natural fermentation: A review[J]. Separations, 2022, 9: 187.
[28] DUAN J, HHUANG Y, LI ZH, *et al.* Determination of 27 chemical constituents in Chinese southwest tobacco by FT-NIR spectroscopy[J]. Industrial Crops and Products, 2012, 40: 21 - 26.
[29] WEI KS, BIN J, WANG F, *et al.* On-line monitoring of the tobacco leaf composition during flue-curing by near-infrared spectroscopy and deep transfer learning[J]. Analytical Letters, 2022, 55: 2089 - 2107.
[30] BORGES-MIRANDA A, SILVA-MATA FJ, TALAVERA-BUSTAMANTE I, *et al.* The role of chemosensory relationships to improve raw materials' selection for *Premium cigar* manufacture[J]. Chemical Papers, 2021, 75: 4075 - 4091.
[31] CECILIO-FILHO AB, FELTRIM AL, MENDOZA-CORTEZ JW, *et al.* Nitrogen and potassium application by fertigation at different watermelon planting densities[J]. Journal of Soil Science and Plant Nutrition, 2015, 15: 928 - 937.
[32] KROUMOVA AB, ZAITLIN D, WAGNER GJ. Natural variability in acyl moieties of sugar esters produced by certain tobacco and other Solanaceae species[J]. Phytochemistry, 2016, 130: 218 - 227.
[33] ZHAO JY, LI L, ZHAO YN, *et al.* Metabolic changes in primary, secondary, and lipid metabolism in tobacco leaf in response to topping[J]. Analytical and Bioanalytical Chemistry, 2018, 410: 839 - 851.
[34] SU J, CHEN Y, ZHU Y, *et al.* The response of Hongda, a flue-cured tobacco cultivar, to nitrogen fertilizer rate[J]. Archives of Agronomy and Soil Science, 2020, 67: 536 - 550.
[35] ZHAO SC, WU ZY, LAI M, *et al.* Determination of optimum humidity for air-curing of cigar tobacco leaves during the browning period[J]. Industrial Crops and Products, 2022, 183: 114939.
[36] CHEN YJ, REN K, HE X, *et al.* Dynamic changes in physiological and biochemical properties of flue-cured tobacco of different leaf ages during flue-curing and their effects on yield and quality[J]. BMC Plant Biology, 2019, 19: 1 - 21.
[37] FAHN A, EVERT RF. Ultrastructure of the secretory ducts of *Rhus glabra* L.[J]. American Journal of Botany, 1974, 61: 1 - 14.
[38] XIA SS, LIU H, CUI YJ, *et al.* UDP-N-acetylglucosamine pyrophosphorylase enhances rice survival at high temperature[J]. New Phytologist, 2022, 233: 344 - 359.