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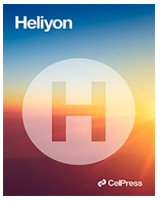

Research article

Application of PLSR in correlating sensory and chemical properties of middle flue-cured tobacco leaves with honey-sweet and burnt flavour

Jing Li [a], Zhiyuan Ma [b], Huawei Dai [b], Hu Li [b], Jun Qiu [a,**], Xueli Pang [a,*]

[a] Tobacco Research Institute of Chinese Academy of Agricultural Sciences, Qingdao, 266101, China
[b] Shandong Zibo Tobacco Co., Ltd, Zibo City, Shandong Province, 255035, China

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ABSTRACT

Among the eight types of aroma and flavour characteristics of Chinese flue-cured tobacco (FCT), tobacco grown in Shandong is classified as having a honey-sweet and burnt aroma. To elucidate the key chemical components that determine the characteristics of the honey-sweet and burnt aroma styles of Shandong FCT, we qualitatively and quantitatively evaluated the smoke flavour quality and five categories of flavour-related chemical components (routine components, water-soluble sugars, free amino acids, Amadori compounds and key aroma-active compounds) in Shandong middle FCT leaves using sensory analysis and modern instrumental analysis techniques. The association between the chemical components and sensory quality was analysed. Our results showed that the total sugars, reducing sugars (fructose, glucose, and psicose), total sugar-nicotine ratio, proline-total amino acid ratio, sulphur-containing amino acid-total amino acid ratio and fructosyl-proline (Fru-Pro) were high in premium FCT leaves. The aroma-active compounds associated with the honey-sweet burnt flavour style of the Shandong Middle FCT included sweet-scented 2,3-pentanedione, 2,3-butanedione, butyrolactone, 2-furanmethanol, roasted-like 2-pentylfura, and green-like 1-penten-3-one. Partial least squares regression (PLSR) analysis revealed that 29 aroma precursors were positively correlated with the sensory quality characteristics of Shandong FCT. The results of our study can provide guidance for the targeted improvement and precise regulation of the flavour-style characteristics of Shandong FCT.

1. Introduction

Tobacco is a special cash crop and its value is expressed through smoking [1]. The evaluation of the quality of flue-cured tobacco (FCT) is mainly based on the sensory evaluation of quality and flavour style. The evaluation of sensory quality includes gas diffusivity, agglomeration, aroma quality, aroma quantity, sweetness, softness, dryness, irritation, miscellaneous gas, and aftertaste [2–4]. The factors influencing tobacco quality are complex and varied; however, the intrinsic chemical components of tobacco leaves are the main factors determining the quality of FCT [5,6].

The content of routine chemical constituents in leaf tobacco or cut tobacco, including the total sugar, reducing sugar, nicotine, total

* Corresponding author.
** Corresponding author.
E-mail addresses: qiujun01@caas.cn (J. Qiu), pangxueli@caas.cn (X. Pang).

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nitrogen, potassium, chloride, and their derived indicators, is very important for quality monitoring and style of cigarette products [7]. Notably, the higher the total sugar and reducing sugars content, the better is the sensory quality of tobacco [8]. However, the total alkaloid content has a two-sided effect on the sensory quality of the smoke. On the one hand, a low total alkaloid content results in bland and tasteless smoke; conversely within a certain range, the higher the total alkaloid content, the more satisfied the smoker will be. However, a high total alkaloid content will increase irritation and produce a pungent taste, which is detrimental to cigarette smoking. Therefore, the total alkaloid content must have a balanced relationship with the other compound categories (e.g. sugars and acids) to ensure good smoking quality.

Sugars are the most important flavour precursors in FCT [9] owing to their high abundance (25–50 % dry weight in natural tobacco) and various existing forms (free sugars and sugar derivatives; e.g. glycosides, sugar esters, and Amadori compounds) [8]. The acceptance of tobacco smoke by consumers is directly proportional to the sugar content, and differences in sugar types and content significantly affect the sensory quality of cigarettes [8,10,11]. On the one hand, during cigarette smoking, sugars can generate acids, and play an important role in balancing the harsh taste resulting from the nitrogenous compounds, improving the strength and irritation of tobacco smoke, and increasing the filler value, flexibility and processing resistance [8]. Moreover, the reaction products, such as small carbonyl compounds, pyrroles, pyridines, pyrazines, furfural, furanones resulting from the Maillard reaction, caramelisation reaction and thermal cracking reaction of sugars can impart an agreeable caramel-like and roasted aroma to the tobacco smoke and reduce the miscellaneous gas and irritation [8,12]. Additionally, sugars (approximately 0.5 % of glucose and sucrose) can be transferred directly into the mainstream smoke during combustion, providing a sweet and luscious aftertaste [8,13]. To date, water-soluble sugars have been used in most studies on the relationship between sugar and sensory quality in FCT. Thus, a thorough investigation must be conducted to determine whether differences exist in the effects and contribution of different types of water-soluble sugars on the sensory quality and flavour style of FCT [14].

Nitrogenous compounds account for 12–25 % of the dry weight of tobacco and are closely related to the sensory quality of FCT [15]. The major nitrogenous compounds in tobacco include alkaloids, proteins, amino acids, and a small amount of volatile heterocyclic compounds. The role of amino acids in the FCT is more complex as they not only contribute to toxic compounds and irritants in smoke, but are also important flavour precursors [16,17]. More than 40 amino acids are present in FCT, with more than 20 typescommonly found in the free form. The amino acids in FCT leaves of different origins and grades were qualitatively similar but quantitatively different [18,19]. Amino acids undergo a prolonged Maillard reaction with reducing sugars, converting them into Amadori compounds and characteristic aromas, which are beneficial for the smoke quality and can modify and round the smoke and reduce irritation. Li et al. [17] determined the amino acids in Burley tobacco leaves from China and Brazil before and after curing and found that the free amino acid andvolatile pyrazine contents of the tobacco decreasedand increased, respectively. Song et al. [20] found that free amino acids in tobacco, particularly proline (Pro), aspartic acid (Asp), alanine (Ala), and threonine (Thr), were the major contributors to the roasted aroma compound 2,3-dihydro-3,5-dihydroxy-6-methy-4(H)-pyran-4-one.

Amadori compounds, which are key intermediates in the primary stage of the Maillard reaction between amines (amino acids, peptides, proteins and other free amino group-containing molecules) and carbonyl compounds (aldose) [21–23], account for approximately 2–3% of the dry weight of tobacco, and play an important role in aroma and flavour of tobacco [24–28]. Amadori compounds are normally odourless but can be cleaved during the smoking process to produce large amounts of flavour-related products, including aroma-active aldehydes, ketones, acids, pyrazines, pyridines, and other odorantsodorant [29]. The thermal degradation products of Amadori compounds formed from different amino acids in fructose demonstrated significant differences in chemical structure and aroma characteristics [29]. Thus, conducting a detailed study on the chemical composition of Amadori compounds in Shandong FCT and the relationship between the flavour quality and Amadori compounds with different amino acid moieties is urgently required.

Volatile compounds in FCT leaves can be partially or fully transferred to the smoke during combustion, thereby playing an important role in shaping the flavour characteristics of cigarettes [30]. The aroma-active compounds in FCT leaves mainly originate from the breakdown of carotenoids and the Maillard reaction during curing and aging. Carotenoid degradation products, such as damascenone and megastigmatrienone, predominantly produce floral aroma. Maillard reaction products dominated by O- and N-containing heterocyclic compounds such as pyrazines, furanones, furfural, and pyrrole, canimpart woody, roasted, and caramel-like aromas [13,31,32]. Therefore, the study of the aroma-active volatiles in FCT leaves could aid in the organoleptic quality control of tobacco. Many scholars have investigated conducted research on the relationship between the organoleptic quality and volatile compounds in FCT mainstream smoke through the combination application of gas chromatography-mass spectrometry (GC-MS) and chemometric methods [33]. However, GC-MS analysis can not provide information on the aroma properties and contributions of volatile compounds. Gas chromatography-olfactometry-mass spectrometry (GC-*O*-MS) is a technique whereincombining an olfactometer (a device in which the human nose is used to detect the odour intensity of an analyte) is combined with GC-MS. GC-*O*-MS is a powerful tool for evaluating the odour significance of an individual compound to the overall aroma and has been widely applied to many food items to rapidly map thearoma-active compounds, identify key aroma-active compounds, and determine the relationship between odorants and sensory properties [34]. However, to our knowledge, no previous studies have been conducted on the GC-*O*-based identification of aroma-active compounds in Shandong FCT leaves.

Internal sensory quality inspection of FCT is mainly achieved by human sensory and smoking tests, and the results obtained are subjective, comprehensive, and limited by the number of evaluated samples, evaluated the accurate determination of the sensory quality [35]. Instrumental measurements can provide objective data on discrete and well-defined sensory properties. Therefore, studies have been conductedto investigate the correlation between subjective sensory evaluations and objective instrument measurements. Partial least squares regression (PLSR) analysis, is a new multivariate statistical analysis, wherein the advantages of principal component analysis, typical correlation analysis, and multiple linear regression analysis are combined. PLSR is mainly

applied to the regression modelling of multiple independent variables on multiple dependent variables. It has been successfully used to predict sensory-related compositions in various types of food [36–42] as it can avoid potential problems, such as non-normal distribution of data, uncertainty of the factor structure, and unidentifiable models.

Extensive research has been conducted on the characterisation and quantification of routine chemical components, aroma precursors (water-soluble sugars, amino acids, and Amadori compounds) and volatile compounds of FCT during the production and processing of FCT leaves of different regions, varieties and positions [11,43]. PLSR has also been applied to explore the correlation between the chemical and sensory data of FCT leaves obtained using different curing methods [5], varieties [44], and production areas [1]. However, instrumental analysis was only targeted at the total concentration of certain classes of chemical constituents and lacked a refined analysis of a wider range of flavour-related chemical compositions, which is insufficient to accurately mine the chemical indicators reflecting the sensory quality of FCT.

To determine the content distribution of key intrinsic chemical components in Shandong middle FCT leaves and elucidate the influence of these flavour precursors on the honey sweetness and burnt flavour characteristics of Shandong FCT, a combination of sensory and instrumental analyses (e.g. GC, GC-MS, GC-*O*-MS, and UPLC-MS/MS) was used to obtain quantitative data on 10 smoking quality indices and five major categories of tobacco chemicals (routine chemical components, water-soluble sugars, amino acids, Amadori compounds and volatile compounds) related to smoking quality. Subsequently, PLSR was applied to conduct a correlation analysis between the chemical components and sensory quality to determine the key chemical components that are negatively or positively correlated with the honey sweetness and burnt flavour type of Shandong tobacco. We aimed to provide data support for tobacco scientists and producers for the accurate and efficient regulation of the sensory quality of Shandong tobacco and strengthen its honey sweetness and burnt flavour characteristics through agronomic management adjustment, curing process, and optimisation of aging conditions.

2. Materials and methods

2.1. Materials

2.1.1. Experimental reagents

Sixteen sugar standards (>99.0 %, Dr. Ehrenstorfer, Germany); Ala, Phe, Thr, Trp, Val, Asp, Glu, Ser, Leu, Ileu, Lys, Asn, Met, His, Arg, methoxylamine hydrochloride (98 %), 1-heptadecanol (97 %), methyl-trimethyl-silyl-trifluoroacetamide (MSTFA, 97 %) (Yuanye Bio-Technology Co., Ltd, Shanghai, China); Pro, Tyr and formic acid (J&K Scientific, Beijing, China); Glu (Acros Organics, USA); GLU (98 %, Alfa Aesar, USA); Fru-Pro (97 %), Fru-Glu (93 %), Fru-Ala (95 %), Fru-Val (95 %), Fru-Phe (95 %), Fru-Trp (95 %), Fru-Gly (96 %) (Toronto Research Chemicals, Canadian); aroma-active compound standards (Sigma-Aldrich, Saint Louis, Missouri, USA), phenethyl acetate, methanol and acetonitrile (Merck, Germany); dichloromethane (≥99.8 %, Xilongs Scientific, China); pyridine (99.5 %, Macklin Biochemical Co., Ltd, Shanghai, China).

2.1.2. Experimental materials and sample preparation

In this study, we selected 21 middle FCT leaf samples produced in 2021 from the main production areas of FCT with honey sweetness and burnt flavour in Shandong Province, China. A sensory evaluation was conducted by Sensory Committee with a national evaluation qualification certificate to evaluate the representation of these samples. Tobacco plant growth in the field was neat, uniform, and consistent, the effective number of retained leaves was 20–21, and the middle leaves were selected from 8 to 14 leaf positions.

Cigarette sample preparation: tobacco leaf samples were moisture-adjusted, de-veined, shredded, and rolled into uniformly sized sticks using an automatic cigarette rolling machine (HAUNIBABY D-7300, BGWT, Germany). Cigarette samples were equilibrated for 48 h under a condition with the temperature and humidity of 25 ± 5 °C and the 40 ± 5 %, respectively, before the sensory evaluation of smoke.

Tobacco powder preparation: thirty representative leaves were selected from each FCT sample. The samples were manually de-veined and shredded, dried at 60 °C, grounded and passed through a 40-mesh sieve, mixed thoroughly, vacuum-sealed in food grade polyethylene plastic bags, and stored at −80 °C until analysis.

2.2. Methods

2.2.1. Sensory quality assessment of cigarette samples

The panel is made up of seven experts with at least ten-year experience in cigarette sensory evaluation. Sensory evaluation was carried out in sensory room with the temperature of 18–25 °C, the relative humidity of 55–70 % and with good ventilation. The sensory quality indicators of the cigarette samples were scored on a 10-point scale for each sensory quality indicator, resulting in a total score of 100, and with higher scores indicating better sensory quality.

2.2.2. Determination of routine chemical components

The routine chemical components in FCT leaves including total/reducing sugars, total alkaloid, total nitrogen, potassium and chloride were determined using a continuous flow analyser (SAN++, Skalar, Holland) according to Peng et al. [45]. And total sugar-nicotine ratio, total nitrogen-nicotine ratio and potassium-chloride ratio are extrapolated values.

2.2.3. Determination of water-soluble sugars

The GC-MS (7890B/5977A, Agilent, USA) coupled to derivatization was used to determine water-soluble sugars in tobacco powder as described by Li et al. [46]. Twenty milligram of tobacco powder was accurately weighed into a 5 mL glass bottle with a stopper, added with 1 mL of a single-phase solvent mixture of methanol, water and trichloromethane (5:2:2, v/v/v) and 100 μL of internal standard (500 μg/mL n-heptadecanol solution dissolved in extraction solution), the extraction is then assisted by ultrasonic instruments (SB-1200 DT, SCIENTZ, Ningbo, China) for 40 min. After ultrasonication, the extract was centrifuged at 10,000 r/min for 10 min, and subsequently 100 μL of the supernatant was transferred to the GC-MS injection vial and blown to dryness using nitrogen evaporators (NEVAP 24, Organomation, USA). Then, 50 μL of methoxyamine hydrochloride solution (20 mg/mL in pyridine) was added and was vortexed for 1 min, incubated in a thermostat at 37 °C for 90 min. And then, 100 μL MSTFA was added, derivatized in a thermostat at 37 °C for 30 min and vortexed for 30 s. Lastly, the samples were allowed to stand at room temperature for at least 2 h before GC-MS analysis. Chromatographic conditions were set as follows: column: DB-5MS column (30 m × 0.25 mm × 0.25 μm, ACE, UK); inlet temperature: 290 °C; injection volume: 1 μL; programmed ramp-up conditions: 60 °C (2 min)- 8 °C/min-310 °C (10 min); inlet mode: split mode, splitting ratio: 8:1; mass spectrometry conditions: ion source temperature: 230 °C; quadrupole temperature: 150 °C; scan range: 33–510 amu.

2.2.4. Determination of free amino acids

Free amino acids in tobacco powders were determined by HPLC-MS/MS (Acquity/Qtrap 4500, Waters, Singapore) as described by Li et al. [47]. Tobacco powder sample (0.1 g) was accurately weighed into a 50 mL glass centrifuge tube, added with 10 mL extract solution (50:50 (v/v) acetonitrile/water), vortex stirred for 30 min, and centrifuged at 5000 r/min for 10 min. Then, the supernatant was passed through a membrane filter (0.22 μm, Anpel, Shanghai, China) and 1 mL of the filtrate was used for HPLC-MS/MS analysis. Chromatographic conditions: column (2.1 mm × 100 mm, 2.6 μm, Phenomenex, USA); mobile phase: 2 mM ammonium formate 95 % acetonitrile solution (A), 2 mM ammonium formate 3 % formic acid solution (B); flow rate: 300 μL/min; column temperature: 30 °C; injection volume: 2 μL; gradient elution program: 0–0.5 min 95.0 % A, 0.5–4.0 min 95.0 %–85.0 % A, 4.0–5.5 min 85.0 %–45.0 % A, 5.5–10.0 min 45.0 % A, 10.0–10.5 min 45.0 %–95.0 % A, 10.5–13.0 min 95.0 % A. Mass spectrometry conditions: ion source: electrospray ion source (ESI); scanning mode: positive ion scanning; detection mode: multiple reaction monitoring scanning mode (MRM); spray voltage: 5500 V; ion source temperature: 500 °C; curtain gas pressure: 35 psi; nebulizer gas and auxiliary gas pressure: 55 psi.

2.2.5. Determination of Amadori compounds

The HPLC-MS/MS (Waters, Acquity/Qtrap 4500, Singapore) method was used to detect Amadori compounds in tobacco powders as described by Liu et al. [48]. Tobacco powder sample (0.1 g) was weighed accurately into a 50 mL glass centrifuge tube, added with 20 mL of 30:70 (v/v) methanol/water extract, vortex stirred for 10 min, and then centrifuged at 5000 r/min for 10 min. Then, the supernatant was passed through a membrane filter (0.45 μm, Anpel, Shanghai, China), and then 100 μL of the filtrate was transferred to a 10 mL volumetric flask (diluted 100 times), diluted to volume with methanol (HPLC grade), and used for HPLC-MS/MS analysis after thorough mixing. Chromatographic parameters are as follows: column: ACE Excel 1.7C_{18}-Amide column (2.1 mm × 100 mm, 5 μm, ACE, UK); mobile phase: 2 % formic acid solution (A), acetonitrile (B); flow rate: 350 μL/min; column temperature: 30 °C; injection volume: 2 μL; gradient elution program: 0~0.1 min 89.5 % Ã78.0 % A, 0.1–10.0 min 78.0 % A. Each sample was equilibrated with the initial mobile phase for 8 min. Mass spectrometry conditions: ion source: electrospray ion source (ESI); scan mode: positive ion scan; detection mode: multiple reaction monitoring scan mode (MRM); spray voltage: 5500 V; ion source temperature: 550 °C; nebulizer gas pressure 7 psi; air curtain gas pressure: 15 psi; nebulizer pressure: 60 psi; auxiliary gas pressure: 75 psi.

2.2.6. Screening and identification of aroma-active compounds in tobacco leaves by GC-O-MS

The SPME-GC-*O*-MS (7890B/5977A, Agilent, USA) method was used for the identification of aroma-active compounds in tobacco powders, following the method of Cristina et al. [49] with dome modifications. Tobacco powder sample (0.5 g) was accurately weighed into a 30 mL headspace vial added with 2 μL of internal standard (105 ppm phenethyl acetate in methanol), and then the vial was screwed tightly and equilibrated at 70 °C for 30 min and was extracted and sorbed at 70 °C for 30 min using a DVB/CAR/PDMS fiber and then directly injected for GC-MS/MS analysis. Chromatographic conditions: column: BD-Wax column (30 m × 0.25 mm × 0.25 μm, Agilent, USA) and DB-5 column (30 m × 0.25 mm × 0.25 μm, Agilent, USA); injection port temperature: 280 °C; thermal desorption time: 10 min; programmed oven temperature: 40 °C (2 min) - 6 °C/min - 230 °C (10 min). Mass spectrometry conditions: ion source temperature: 230 °C; quadrupole temperature: 150 °C; scan range: 33–325 amu. Sniffing port: interface temperature 230 °C, MS to sniffer distribution ratio 1:1. The GC-O experiments were carried out by 2 experienced sniffers who were required to record the time of appearance, intensity and odour properties of the odour and each sample was repeatedly sniffed 4 times by each sniffer.

Qualitative and quantification of aroma-active compounds: The compounds were accurately characterised by comparing LRI (liear retention index), MS (NIST 14 library search), odour property and S (reference standards) of the target and standards on two columns of different polarities. Semi-quantitative analysis of volatile aroma compounds using internal standards combined with quantitative ion peak areas.

2.2.7. Data analysis

Significance analysis was performed using IBM SPSS Statistics software (version 27, IBM, USA). Figures were drawn using Origin software (version 2021, OriginLab, USA). PLSR analysis was performed with Unscrambler software (version X 10.4, CAMO, Norway). In the PLSR analysis, all data were centralised and standardised (1/Sdev).

3. Results and discussion

3.1. Sensory quality evaluation of middle leaves of Shandong FCT

The 21 samples were classified into three quality grades according to the results of the sensory quality evaluation: good (aroma quality >7.40, aroma quantity >7.95, total score >68.50), medium (7.15< aroma quality <7.40, 7.85< aroma quantity <7.95, 65.50< total score <68.50), and poor (aroma quality <7.15, aroma quantity <7.85, total score <65.50). Significant differences were observed in the sensory quality profiles among the three quality grades of Shandong FCT (Fig. 1). Cigarettes made from high-quality leaves showed better gas diffusiveness, better agglomeration, purer aroma, fuller aroma, more pronounced sweetness, softer taste, less irritation, more pleasant aftertaste, and fewer miscellaneous gases. Cigarettes made with medium-quality leaves showed the second highest scores for several sensory quality indicators, whereas those made with poor-quality leaves had the lowest sensory quality scores for all sensory indices. Additionally, the scores for the 10 organoleptic indicators showed the same trend as the overall score, indicating a certain harmony between the different indicators of organoleptic quality.

3.2. Distribution characteristics of non-volatile aroma precursors in Shandong FCT leaves of different quality grades

3.2.1. Variance analysis of routine chemical components in Shandong FCT leaves of different quality grades

Routine chemical component contents in Shandong middle FCT leaves varied greatly among the different quality grades (Fig. 2A). Similar to that in the Chinese FCT [13], the sugar content in Shandong FCT covered a wide range, wherein the greatest variation in total and reducing sugar content was found in medium-grade leaves. The total sugar content of Shandong middle FCT leaves ranged from 17.10 to 30.33 %, whereas that in natural tobacco can reach up to 20 % according to Talhout et al. [8]. Therefore, the total sugar content of Shandong middle FCT leaves is relatively high. The content of reducing sugars ranged from 16.31 to 28.10 %, which is in accordance with a previous study, wherein reducing sugars were found to account for 22 % of the FCT leaves [50]. Sugars in tobacco are formed through the enzymatic hydrolysis of starch during the period after priming (harvesting) and the early stages of the curing process [51]. The sugar content of tobacco types is highly variable but primarily depends on the method of curing. For instance, sugars are largely metabolised during air-curing (Burley, Maryland, and cigars) [51]. In contrast, flue- and sun-cured tobacco leaves contain higher sugar levels as the metabolising enzymes are rapidly inactivated at the relatively high temperatures employed during this curing [52]. The total nitrogen, total alkaloid, chloride and potassium contents were 1.48–2.30, 1.56–3.08, 0.20–0.93 and 1.04–1.88 %, respectively, which is in agreement with those of a previous study, wherein Peng et al. [45] found that the total nitrogen, total alkaloids, chloride, and potassium contents in tobacco were 1.63–2.42, 1.48–3.27, 0.07–1.10 and 1.36–2.89 %, respectively.

Significant differences ($P < 0.05$) were observed in the total sugars, reducing sugars, total nitrogen, total alkaloids, total sugar-to-nicotine ratio and total nitrogen-to-nicotine ratio among the samples of different quality grades. The total sugar and reducing sugar contents in leaves of different quality grade showed the following order: good > medium > poor; the total nitrogen and total alkaloid

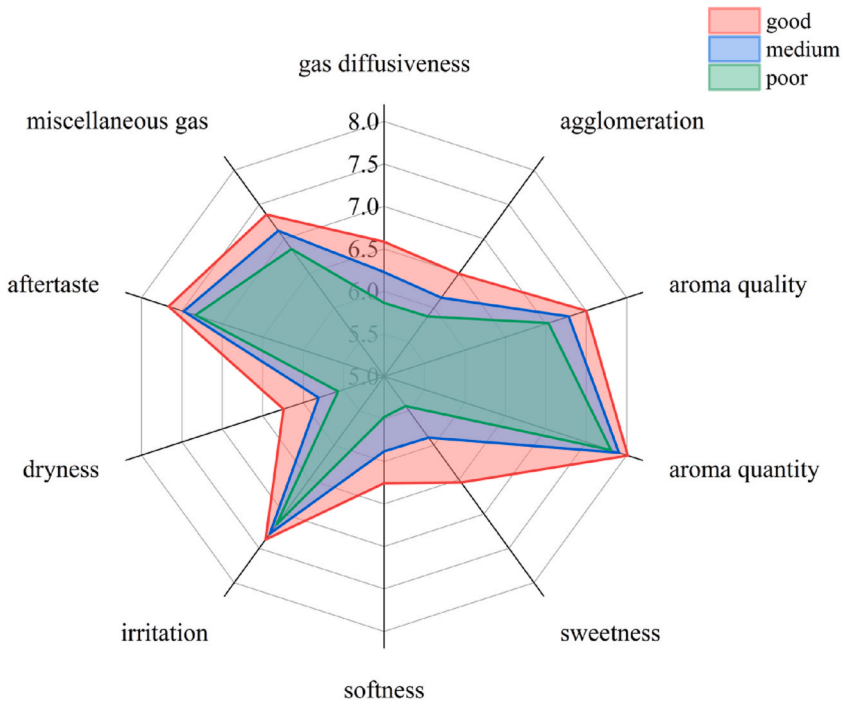

Fig. 1. Sensory quality profile of Shandong FCT middle leaves of different quality grades.

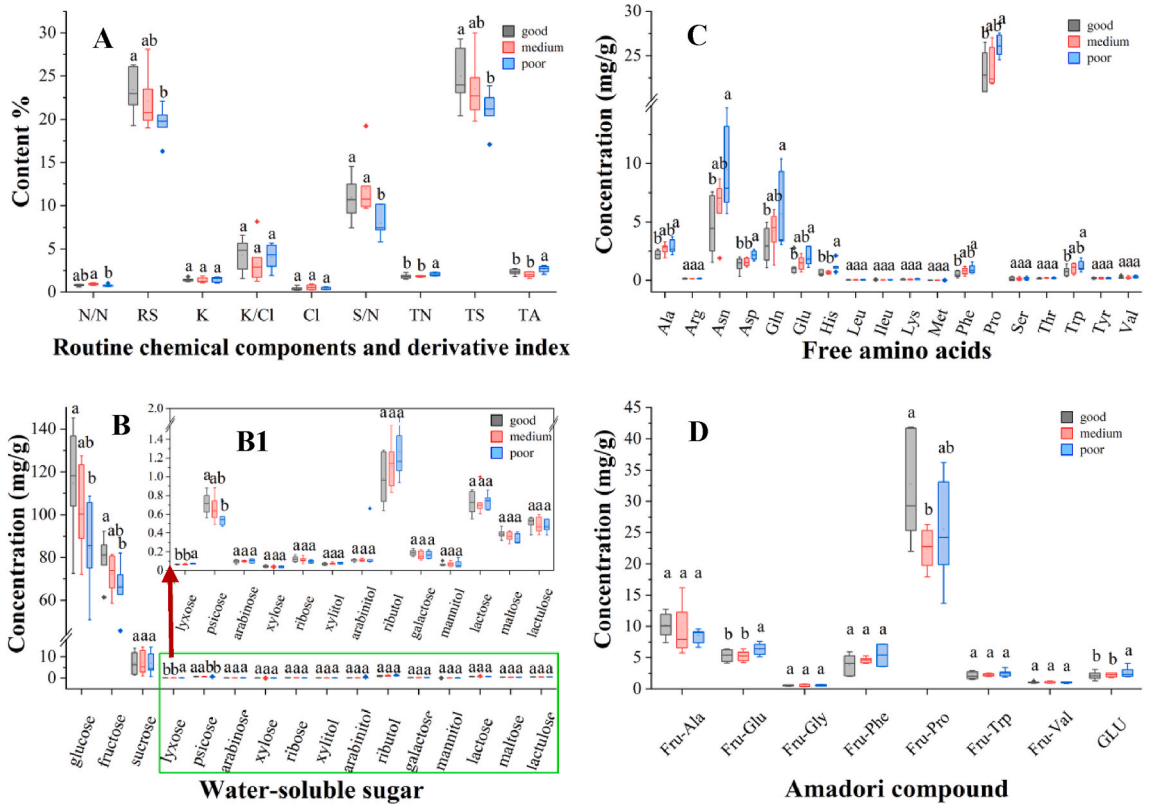

Fig. 2. Box line diagram of the chemical components of different quality grades of Shandong TCT middle leaf. A: routine chemical components. B: water-soluble sugars (B1 is an enlargement of the green boxed area in Fig.2B). C: free amino acids. D: Amadori compounds. (*Note: Different letters in the graph between different grades of the same chemical components represent significant differences at the P<0.05 level. TS: total sugar, RS: reducing sugar, TN: total nitrogen, TA: total alkaloid, S/N: total sugar-nicotine ratio, N/N: total nitrogen-nicotine ratio, K/Cl: potassium-chlorin ratio, K: potassium, Cl: Chlorin. Ala: Alanine; Arg: Arginase; Met: Methionine; Phe: Phenylalanine; Pro: Proline; Thr: Threonine; Trp: tryptophan; Tyr: Tyrosine; Val: Valine; Asp: Asparagine; Glu: Glutamine; His: Histidine; Ser: Serine; Leu: Leucine; Ileu: Isoleucine; Gln: Glutamine; Lys: Lysine; Asn: Asparagine. Fru-Ala: 1-deoxy-1-L-alanine mono-D-fructose; Fru-Glu: N-(1-deoxyfructos-1-yl) glutamic acid; Fru-Gly: 1-deoxy-1-L-glycine-D-fructose; Fru-Phe: 1-deoxy-1-L-phenylalanine-D-fructose; Fru-Pro: N-(1-deoxy-β-d-fructopyranos-1-yl)-L-proline; Fru-Trp: 1- (N-L-tryptophan)-1-deoxy-D-fructose; Fru-Val: 1-deoxy-1-L-valine-D-fructose; GLU: glucosamine.*). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

contents in poor-quality leaves were significantly higher than those in medium-and good-quality leaves. The total sugar-nicotine and total nitrogen-nicotine ratios were higher in good-quality leaves than in the poor-quality leaves, with no significant difference between the total sugar-nicotine ratio in good- and medium-quality leaves. During smoking, a larger portion of the sugars will combust, pyrolyse or participate in pyrosynthesis processes. The products formed through the caramelisation of sugars and their reaction with amines (Maillard reaction) contribute to the improvement of the flavour of tobacco smoke [8]. For example, sugar produces caramel flavours that give tobacco a sweet taste, thereby masking the adverse bitter taste of cigarette smoke. Moreover, during smoking, sugars generate acids that decrease the smoke pH, which in turn decreases the level of free-base nicotine in mainstream smoke and irritation caused by nicotine [10]. Many tobacco companies add sugar additives to tobacco products. Additionally, better FCT samples showed higher sugar content.

3.2.2. Variance analysis of water-soluble sugars in Shandong FCT leaves of different quality grades

As shown in Fig. 2B, the concentrations of the 16 water-soluble sugars widely varied, with glucose showing the highest concentration (51.00–145.13 mg/g), followed by fructose (45.88–92.23 mg/g), sucrose (0.74–14.64 mg/g) and other water-soluble sugars and sugar alcohols with concentrations below 1 mg/g. In accordance with the results of our study, those of Clarke et al. [53] indicated that the most abundant natural sugars in dried tobacco leaves were glucose, fructose, and sucrose. Additionally, these three reducing sugars are used as cigarette additives to enhance the cigarette flavour [14]. Glucose showed the greatest variation in concentration among all reducing sugars, and was the major water-soluble sugar responsible for the large variation in total and reducing sugars between the leaves of different grades. Significant differences ($P < 0.05$) were observed in glucose, fructose, psicose, and lyxose between samples of different quality grades, wherein glucose, fructose, and psicose concentrations showed the following order: good > medium > poor; and the lyxose was higher in poor-grade than in medium- and good-grade leaves. In contrast to previous studies, wherein the sucrose concentrations significantly differed between different positions on the FCT leaf, sucrose concentrations showed

no significant difference between FCT leaves of different quality grades [54]. Differences in the tobacco variety, production region, agronomic management, and curing parameters may be responsible for this inconsistency.

3.2.3. Variance analysis of free amino acids in Shandong FCT leaves of different quality grades

Among 18 free amino acids detected in Shandong FCT leaves, Pro (15.00–30.00 mg/g), asparagine (Asn) and glutamine (Gln) showed higher concentrations (1.00–15.00 mg/g), followed by Ala, glutamic acid (Glu), Asp, histidine (His), phenylalanine (Phe), tryptophan (Trp) (0.20–4.00 mg/g), and the concentrations of other free amino acids were below 0.5 mg/g (Fig. 2C). In agreement with our study, Li et al. [19] found that Pro was the richest amino acid in Virgina tobacco (5.63 mg/g) and Asn was the most abundant amino acid in the Burley tobacco sample (5.48 mg/g), whereas tyrosine (Tyr) and cysteine (Cys) showed the lowest concentrations in both Chinese and American tobacco samples (<0.01 mg/g). Significant differences ($P < 0.05$) were observed in Pro, Asn, Gln, Ala, Glu, Asp, His, Phe and Trp between the Shandong FCT samples of different quality grades, wherein the concentrations of Pro, Asn, Gln, Ala, Glu, Phe and Trp showed the following order: poor > medium > good quality.

To determine the correlation between amino acids and quality grade, free amino acids in Shandong FCT were classified into eight categories according to their side chain structure: aromatic, acidic, basic, aliphatic, hydroxyl-containing, sulphur-containing, amide and sub-amino acids (Pro). As shown in Fig. 3, Pro showed the highest concentration, accounting for 40–70 % of the total free amino acids, followed by amide amino acids, accounting for 10–40 %, whereas the content of the other amino acids was relatively low (<10 %). The percentage of free amino acids varies significantly among different quality grades of tobacco, with a higher percentage of Pro and sulphur-containing amino acids in good-quality tobacco, but a higher percentage of basic and amide amino acids in lower-grade tobacco. An important pathway involved in aroma formation during tobacco curing is the Millard reaction, which uses free amino acids and reducing sugars as the main substrates. A large number of flavour-related products of the Maillard reaction were produced during the middle and late stages of tobacco curing, and that the content of basic amino acids and aromatic amino acids, which are highly reactive in the Maillard reaction, decreased greatly, while the proline is relatively less reactive, and would increase or remained relatively stable [55,56]. Therefore, it is reasonable that the content of basic amino acids was lower in good quality leave due to their participation in Maillard reaction and therefore contributed more flavour-related products in high quality grades of FCT.

3.2.4. Variance analysis of Amadori compounds in Shandong FCT leaves of different quality grades

Among the eight Amadori compounds detected in Shandong FCT, fructosyl-proline (Fru-Pro) showed the highest abundance (14.69–44.18 mg/g), followed by Fru-Ala, Fru-Glu, Fru-Phe, glucosamine (GLU), and Fru-Trp (1–20 mg/g), and fructosyl-valine (Fru-Val) and fructosyl-glycine (Fru-Gly) (<1 mg/g) (Fig. 2D). To our knowledge, this is the first report of Amadori compounds in the leaves of Shandong FCT. The total percentage of Fru-Pro and Fru-Ala studied in this study was 60–80 % of the eight Amadori compounds and agreed well with the results of Liu et al. [48], wherein Fru-Asn, Fru-Pro and Fru-Ala in FCT in Yunnan, Guizhou and Henan were all at the 'mg/g' level and their total amount account for more than 80 % of the total Amadori content. Significant differences ($P < 0.05$) were observed in Fru-Pro, Fru-Glu and Glu among the samples of different grades, wherein Fru-Pro concentrations showed the following decreasing order: higher > poor > medium grade; and Fru-Glu and Glu concentrations showed the following order: poor > medium ≈ good grade. Moreover, the order of Amadori compound concentrations in the different grades of leaves was the same as the concentration trends of their corresponding amino acids, which is consistent with the findings of Liu et al. [48].

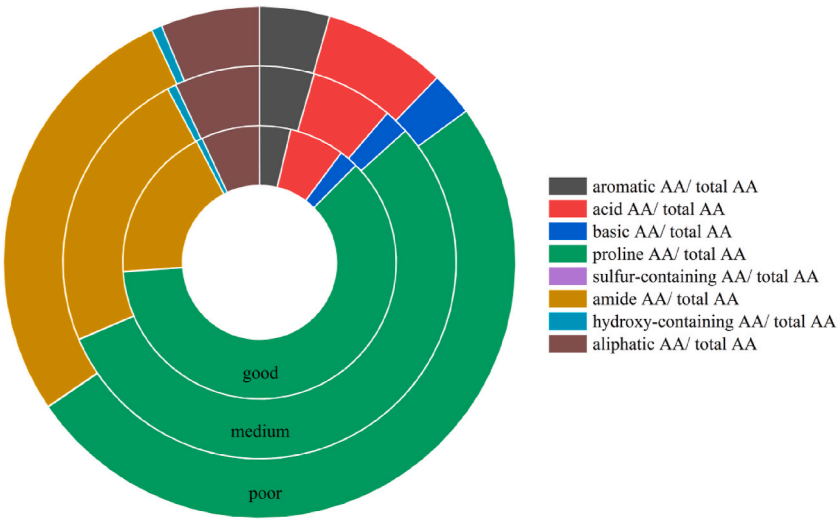

Fig. 3. Ring chart of the percentage of various amino acids of different quality grades of Shandong FCT middle leaf.. (Note: AA: amino acids.)

Table 1
Predominant aroma-active compounds identified in Shandong FCT based on GC-*O*-MS analysis.

No	Name	CAS	LRI-WAX[a]	LRI-DB5[b]	Quan-Ion[c]	Qua-Ion[d]	Odour[e]	Odour Class	Chemical Category[f]
1	2,3-Butanedione	431-03-8	977	595	86	43,87	Yogurt	Sweet	AK
2	Pentanal	110-62-3	982	699	58	57,86	Green	Green	AALD
3	Methyl butanoate	623-42-7	992	722	87	59,74	Alcoholic	Floral	ARO
4	1-Penten-3-one	1629-58-9	1024	681	55	57,84	Green	Green	AK
5	2,3-Pentanedione	600-14-6	1056	698	100	43,57	Sweet	Sweet	AK
6	Hexanal	66-25-1	1084	800	82	56,72	Green	Green	AALD
7	Methyl valerate	624-24-8	1085	823	74	59,85	Fruity	Fruity	AE
8	1,3-Dimethylbenzene	108-38-3	1149	866	91	77,106	Fried	Nutty	ARO
9	1,2-Dimethylbenzene	95-47-6	1192	887	91	77,106	geranium	Roasted	ARO
10	2,3-Dihydro-4-methylfuran	34314-83-5	1170	761	84	55,83	Roasted	Roasted	FUR
11	2-Pentylfuran	3777-69-3	1239	993	81	82,138	Coffee	Roasted	FUR
12	(Z)-4-Hepten-1-al	6728-31-0	1246	900	68	55,84	Stale	Earthy	AALD
13	Methyl heptanoate	106-73-0	1289	1023	74	87,113	Fruity	Fruity	AE
14	Octanal	124-13-0	1298	1003	84	69,100	Citrus	Citrus	AALD
15	1-Octen-3-One	4312-99-6	1308	979	70	55,83	Mushroom	Earthy	AK
16	6-Methyl-5-hepten-2-one	110-93-0	1340	986	108	111,126	Fresh	Citrus	AK
17	2-Acetyl-1-pyrroline	85213-22-5	1350	922	83	69,111	Rice	Roasted	PYRI
18	2,6-Dimethyl-5-heptenal	106-72-9	1356	1052	82	67,69	Melon	Green	AALD
19	Nonanal	124-19-6	1401	1104	98	70,82	Nutty	Nutty	AALD
20	1,2,3,5-Tetramethylbenzene	527-53-7	1421	1117	119	91,134	Green	Green	ARO
21	Acetic acid	64-19-7	1444	610	60	43,45	Vinegar	Acidic	AACD
22	2-Ethylhexanol	104-76-7	1479	1030	57	70,83	Green	Green	AALC
23	3,5-Octadien-2-one	38284-27-4	1529	1091	95	81,124	Mushroom	Earthy	AK
24	Propanoic acid	79-09-4	1538	700	74	45,73	Rancid	Acidic	AACD
25	γ-Pentalactone	108-29-2	1636	958	85	56,100	Sweet	Sweet	LACT
26	Butyrolactone	96-48-0	1653	915	86	42,56	Caramel	Sweet	LACT
27	2-Furanmethanol	98-00-0	1651	859	98	81,97	Caramel	Sweet	FUR
28	3-Methylbutanoic acid	503-74-2	1673	863	60	45,87	Cheese	Acidic	AACD
29	2-Methylbutanoic acid	116-53-0	1678	861	74	57,87	Sweaty	Acidic	AACD
30	4-Oxoisophorone	1125-21-9	1710	1144	68	96,152	Must	Earthy	TER
31	Pentanoic acid	109-52-4	1746	903	60	55,73	Cheese	Acidic	AACD
32	3,4-Dimethyl-2,5-furandione	766-39-2	1753	1038	82	54,126	Oat	Roasted	FUR
33	Methyl phenylacetate	101-41-7	1772	1178	91/55	65,150	Jasmine	Floral	ARO
34	δ-Hexadecalactone	7370-44-7	1787	2148	99	83,114	Coconut	Sweet	LACT
35	3-Methylpentanoic acid	105-43-1	1794	947	60	57,87	Cheese	Acidic	AACD
36	β-Damascenone	23726-93-4	1825	1386	121	105,190	Floral	Floral	TER
37	Hexanoic acid	142-62-1	1852	990	60	73,87	Fermented	Aciidc	AACD
38	trans-Geranylacetone	3796-70-1	1872	1453	69	136,151	Magnolia	Floral	TER
39	Benzyl alcohol	100-51-6	1884	1036	108	79,107	Floral	Floral	ARO
40	γ-Butylbutyrolactone	104-50-7	1939	1261	85	56,100	Coconut	Sweet	LACT
41	β-Ionone	14901-07-6	1970	1491	177	91,135	Violet	Floral	TER
42	β-Ionon-5,6-epoxide	23267-57-4	2020	1473	94/123	107,135	Wood	Woody	TER
43	1,3,7,7-Tetramethyl-2-oxabicyclo[4.4.0]-5-decen-9-one	20194-67-6	2040	1541	193	109,208	Tobacco	Roasted	PYR
44	2-Pyrrolecarbaldehyde	1003-29-8	2040	1015	95	66,94	Baked	Roasted	PYR
45	3-Ethyl-4-methyl-1H-pyrrole-2,5-dione	20189-42-8	2288	1239	139	96,124	Baked	Roasted	PYR
46	Pyranone	28564-83-2	N.A.[g]	1151	144	72,101	Baked	Roasted	PYA

[a] LRI-WAX, linear retention index on polar WAX column.
[b] LRI-DB5, linear retention index on nonpolar DB5 column.
[c] Quan-Ion, characteristic ions of targeted odorants selected used for quantitation.
[d] Qua-Ion, characteristic ions of targeted odorants selected used for qualification during quantitative analysis.

[e] Odour, odour property detected by the panelist during GC-O analysis.
[f] AACD: aliphatic acid; AALC: aliphatic alcohol; AALD: aliphatic aldehyde; AE: aliphatic ester; AK: aliphatic ketone; ARO: aromatics; FUR: furans; LACT: lactones; PYA: pyranone; PYR: pyrrole; PYRI: pyrroline; TER: terpene.
[g] not available.

3.3. Distribution characteristics of aroma-active compounds in Shandong FCT leaves of different quality grades

3.3.1. Composition and variance analysis of aroma-active compounds in Shandong FCT leaves of different quality grades

A total of 46 aroma-active compounds were identified in different quality grades of Shandong FCT leaves (Table 1). These odorants belong to multiple chemical categories, including aliphatic alcohols, aliphatic esters, lactones, aromatics, aliphatic acids, aliphatic aldehydes, terpenes, aliphatic ketones, and heterocyclics compounds. These aroma-active compounds have various odour properties, including sweety, nutty, roasted, floral, fruity, citrus, green, earthy, acidic, and woody properties. Most odorants identified herein were previously reported as volatiles in FCT leaves [49,57,58], whereas seven odorants, namely, 1-octen-3-one, 2,6-dimethyl-5-heptenal, 1,2,3,5-tetramethylbenzene, 3,4-dimethyl-2,5-furandione, δ-hexadecalactone, 1,3,7,7-Tetramethyl-2-oxabicyclo[4.4.0]-5-decen-9-one, and 2-pyrrolecarbaldehyde, were identified for the first time as aroma-active compounds in Shandong FCT. Moreover, the frequently reported volatile compounds in tobacco, such as solanone, megastigmatrienone, and neophytadiene, were not detected by GC-O as odour-active compounds in Shandong FCT leaves. These differences may result from geographical and varietal differences, as well as differences in aroma extraction methods.

Cluster analysis (Fig. 4) of the 46 aroma-active compounds in Shandong FCT showed that some aroma compounds, including 2-pentylfuran, (Z)-4-hepten-1-al, β-ionone, β-ionone-5,6-epoxide, and 3-ethyl-4-methyl-1H-pyrrole-2,5-dione, among others, are not detected in the medium-quality tobacco. Some odorants, such as 1-penten-3-one and propanoic acid showed the highest abundance in good-quality tobacco, whereas other odorants such as 1,3-dimethylbenzene, 1,2-dimethylbenzene, 2-ethylhexanol, and 2,3-dihydro-4-methylfuran, showed the highest contents in medium-quality tobacco. Poor-quality tobacco is characterised by a high relative concentration of 1,2,3,5-tetramethylbenzene, pentanoic acid, and *trans*-geranylacetone. To further clarify the differences in aroma-active compounds between the different quality grades of Shandong FCT, 13 odorants showing significant differences ($P < 0.05$) among the different quality grades were grouped according to their odour properties and plotted as bubble diagrams (Fig. 5). We found that 2,3-pentanedione, 2,3-butanedione, butyrolactone, 2-furanmethanol with a sweet aroma, 2-pentylfuran with a roasted aroma, and 1-

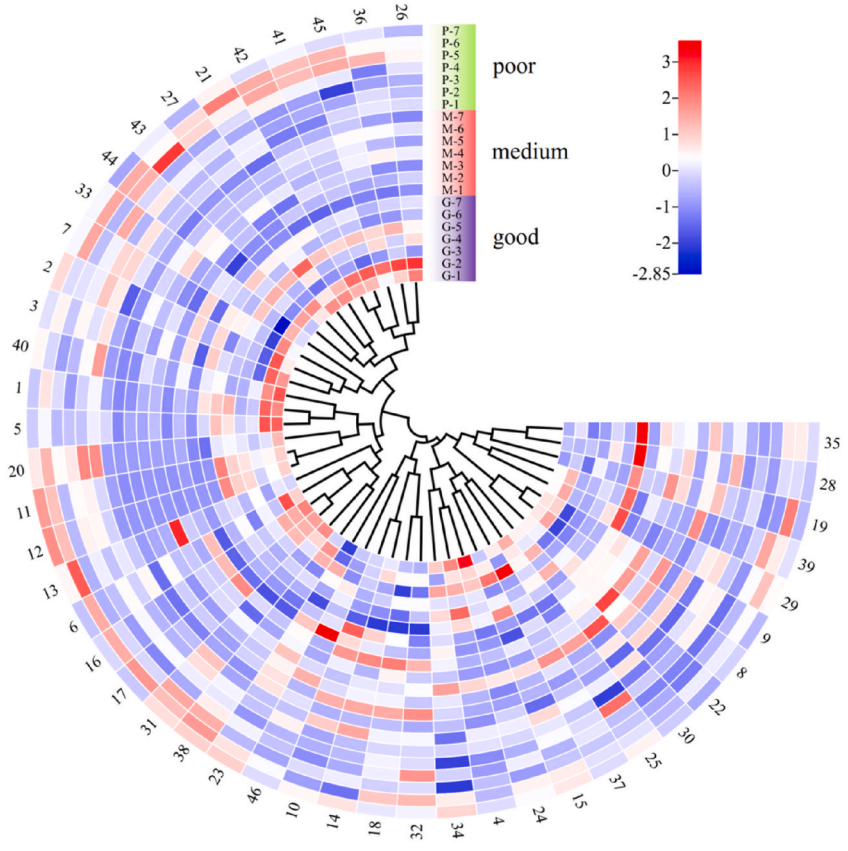

Fig. 4. Heat map of key aroma compounds in Shandong FCT middle leaf of different quality grades.. (Note: Numbers 1–46 are in the same order as compound numbers in Table 1.)

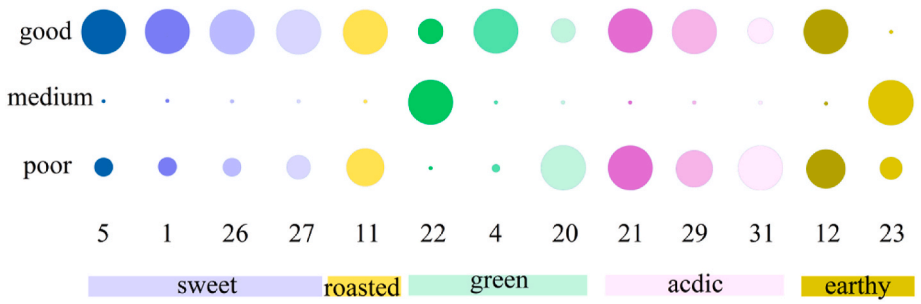

Fig. 5. Concentration bubble plot of different odour groups of aroma-active compounds in Shandong middle FCT leaves with different quality grades. (Note: The Arabic numbers representing the aroma compounds are the same as compound numbers in Table 1, the size of the dots in the graph represents the relative concentrations (average values) and the same aroma compounds are comparable between different quality grades. The colors of the blocks under the numbers in the horizontal coordinates represent the odour property of these compounds.). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

penten-3-one with a green note were significantly higher in good-quality tobacco, whereas 1,2,3,5-tetramethylbenzene, with a greenish odour, and pentanoic acid with a pungent and acidic odour, were significantly more concentrated in the poor-quality tobacco. Medium-quality tobacco was characterised by a higher contribution of 2-ethylhexanol (green) and 3,5-octadien-2-one (earthy). According to Talhout et al. [8], 2-furanmethanol can be produced from sucrose, 2,3-pentanedione and 2,3-butanedione from glucosylalanine, and the addition of sugars to tobacco primarily enhances the aldehyde and ketone levels. These findings indicate that the sources of these compounds in FCT may be sugars, amino acids, and Amadori compounds related to the raw materials of FCT. Studies on the sources of these compounds can provide clues to better understand the formation of specific odours and flavours in FCT. However, further research is required to confirm the pathways through which these compounds are generated. The levels of these compounds in FCT result in different odour and flavour profiles. Notably, odours and flavours in FCT are produced by the interaction of a wide range of compounds, among which the aforementioned compounds are only a few and do not fully account for all the odour and flavour characteristics.

3.4. PLSR analysis between chemical and sensory data of Shandong FCT leaves

To further investigate the influence of chemical components on the sensory quality of Shandong FCT leaves, PLSR was conducted to determine the correlation between the sensory (10 sensory quality indicators) and instrumental data based on the five categories of flavour-related chemical components (routine chemical composition, water-soluble sugars, free amino acids, Amadori compounds, aroma-active compounds, and derived indicators, including the total sugar-nicotine ratio, total nitrogen-nicotine ratio, proportion of basic amino acids, proportion of Pro, proportion of sulphur-containing amino acids and proportion of amide-containing amino acids).

Table 2
Chemical components and sensory quality indicators in Shandong middle FCT leaves used for PLSR analysis.

No.	Indicator	No.	Indicator	No.	Indicator
A-1[h]	reducing sugar	C-8	Gln	E−9	3,5-Octadien-2-one
A-2	total sugar	C-9	Asn	E−10	Butyrolactone
A-3	total alkaloid	D-1[k]	Fru-Glu	E−11	2-Furanmethanol
A-4	total nitrogen	D-2	Fru-Pro	E−12	2-Pentylfuran
A-5	total sugar-nicotine ratio	D-3	GLU	E−13	(Z)-4-Hepten-1-al
A-6	total nitrogen-nicotine ratio	D-4	basic AA/total AA	a	gas diffusiveness
B-1[i]	lysose	D-5	Pro AA/total AA	b	agglomeration
B-2	fructose	D-6	sulphur-containing AA/total AA	c	sweetness
B-3	psicose	D-7	amide AA/total AA	d	dryness
B-4	glucose	E−1[l]	1,2,3,5-Tetramethylbenzene	e	softness
C-1[j]	Ala	E−2	2,3-Pentanedione	f	aroma quality
C-2	Phe	E−3	2,3-Butanedione	g	aroma quantity
C-3	Pro	E−4	2-Ethylhexanol	h	aftertaste
C-4	Trp	E−5	Pentanoic acid	i	miscellaneous gas
C-5	Asp	E−6	1-Penten-3-one	j	irritation
C-6	Glu	E−7	Acetic acid		
C-7	His	E−8	2-Methylbutanoic acid		

[h] A-1, routine chemical components.
[i] B-1, water-soluble sugars.
[j] C-1, free amino acids.
[k] D-1, the percentage of various amino acids.
[l] E−1, Amadori compounds.

The indicators of chemical components and sensory quality are listed in Table 2. Two optimal principal components (PC_1 and PC_3), obtained based on cross-validity, explained 79 % of the self-cross-validation variance, indicating that they are adequate to explain the total information of the samples. As shown in Fig. 6A, the variables fell between two ellipses ($r^2 = 0.5$ and $r^2 = 1.0$), indicating that these variables were well explained by the PLSR model.

As shown in Fig. 6, among the variables explained by the first principal component (PC_1), all sensory quality indicators, as well as the total sugars, reducing sugars, sugar/nicotine ratio, glucose, fructose, psicose, Fru-Pro, Fru-Ala, proportion of sulphur-containing amino acids, proportion of proline, 2-pentylfuran, (Z)-4-hepten-1-al, 2,3-pentanedione, 2-ethylhexanol, 2,3-butanedione, 1-penten-

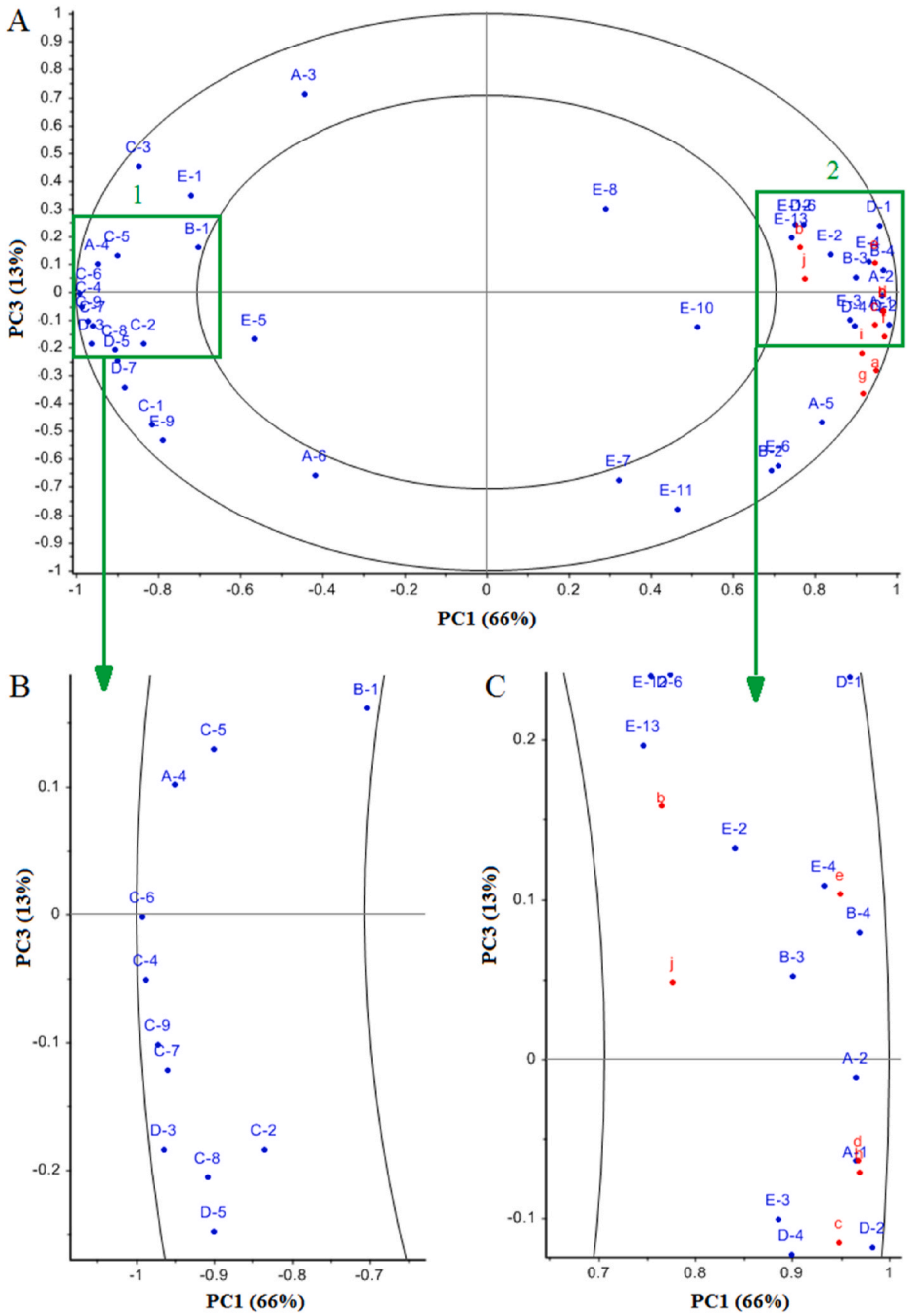

Fig. 6. PLSR correlation analysis between chemical components indicators and sensory quality indicators of Shandong FCT middle leaves. (Note: Fig. 6 B and C is a magnified view of the leftside green Box ad rightside greenbox in Fig. 6 A, respectively.). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3-one, 2-furanmethanol, and acetic acid were located on the right side of the loading plot and fell between the two ellipses. These indicated that these chemical indicators were positively correlated with gas diffusiveness, agglomeration, aroma quality, aroma quantity, sweetness, softness, and aftertaste sensory quality indicators, and negatively correlated with miscellaneous gas and irritation (the higher the score, the lighter is the miscellaneous gas and lesser is the irritation). Tobacco with a high sugar content is often preferred by tobacco producers and consumers as the preference for tobacco products is directly proportional to the sugar content [8]. Sucrose, glucose and fructose have been extensively used in American blended cigarettes to replenish the sugars lost during the Burley tobacco curing and maintain a balanced flavour [13]. Lyxose, total alkaloid, total nitrogen, total nitrogen-nicotine ratio, Phe, Ala, Gln, Asp, Asn, His, Trp, Glu, Pro, Fru-Glu, Glucosamine (GLU) percentage of basic amino acids, percentage of amide amino acids, 1,2,3, 5-tetramethylbenzene, and 3,5-octadien-2-one were located on the left side of the loading plot and fell between the two ellipses. This indicated that these chemical indicators were negatively correlated with gas diffusiveness, agglomeration, aroma quality, aroma quantity, sweetness, softness, and aftertaste sensory quality indicators and positively correlated with miscellaneous gas and irritation. This result was in agreement with that of a previous study indicating that sugars, except glucose, xylose, fructose, and maltose and amino acids, such as Asp negatively contribute to freshness and flowery sensory attributes [11]. Notably, tetramethylbenzene and 3, 5-octadien-2-one were deemed to be negatively correlated with sensory quality, considering that they have camphor and mushroom-like odours, respectively, and have been reported as off-flavour compounds in food [59]. Based on the PLSR analysis results, we concluded that an increase in sugar content, especially glucose, fructose, and allulose, and a decrease in the content of nitrogenous compounds, especially free amino acids, except for sulphur-containing amino acids and Pro, could potentially result in better gas diffusiveness, better agglomeration, purer aroma, more aromatic quantity, more pronounced sweetness, softer taste, drier leaves, more pleasant aftertaste, lighter miscellaneous gas, and less irritation.

4. Conclusion

First, considering the research object, this is the first study conducted to investigate the FCT leaves with honey-sweet and burnt aroma characteristics. Second, considering the substance categories, we conducted a more detailed analysis and our study involved a greater variety of substances. Finally, the contributing aromatic components were examined. The sensory quality of smoke and five categories of major flavour-related compounds in the leaves of 21 representative Shandong middle FCT samples of different quality grades were quantitatively assessed by sensory evaluation, metabolomics approaches, and GC-O technique to provide combined information on chemical characterisation and odour perception. Chemical indicators that were positively or negatively associated with the 10 sensory indicators of Shandong middle FCT leaves were selected based on PLSR analysis. We also demonstrated that Shandong middle FCT leaves of premium quality were characterised by a high levels of psicose, proline and sulphur-containing amino acids, whereas lower quality grade FCT leaves were characterised by a high content of lyxose, free amino acids (Phe, Ala, Gln, Asp, Asn, His, Trp, Glu, and Pro), percentage of basic amino acids, percentage of amide amino acids, Fru-Glu, and GLU. The predominant odorants that characterize the honey sweetness and burnt flavour of Shandong FCT leaves include 2,3-pentanedione, 2,3-butanedione, butyrolactone and 2-furanmethanol with a sweet aroma, 2-pentylfuran with a toasted aroma and 1-penten-3-one with a green odour. PLSR analysis between the flavour-related chemical and sensory data was conducted to screen the potential chemical indicators that could be used for quality predication and evaluation. We found that the following could probably improve the gas diffusiveness, agglomeration, aroma quality and quantity, sweetness, taste, aftertaste, and weaken the miscellaneous gas and irritation of Shandong middle FCT: increasing the content of Fru-Pro, Fru-Ala, 2-pentylfuran, (Z)-4-hepten-1-al, 2,3-pentanedione, 2-ethylhexanol, 2,3-butanedione, 1-penten-3-one, 2-furanmethanol, acetic acid, total sugars, reducing sugars especially glucose, fructose, and psicose; increasing the proportions of Pro, and sulphur-containing amino acids; and decreasing the proportions of lyxose, 1,2,3,5-tetramethylbenzene, 3,5-octadien-2-one, and nitrogenous compounds, especially the reduction of total alkaloids, total nitrogen, Fru-Glu, Glu, Phe, Ala, Gln, Asp, Asn, His, Trp, Glu, Pro, and other free amino acids, as well as those of basic and amide amino acids. Further studies should be conducted to verify the actual role of these potential quality indicators, identify their pyrolysis products that could directly contribute to the honey sweetness and burnt flavour characteristics of Shandong FCT. The regulatory effects of different agronomic measures and aging processes on the accumulation of key chemical quality indicators must also be systematically investigated in depth in future studies.

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Ethics declarations

Review and/or approval by an ethics committee was not needed for this study because no animal experiments and human and behavioural studies were involved in this study.

Informed consent was not required for this study because no animal experiments and human and behavioural studies were involved in this study.

Data availability statement

Data associated with this study has not been deposited into a publicly available repository due to that the authors do not have permission to share data.

CRediT authorship contribution statement

Jing Li: Writing – original draft. **Zhiyuan Ma:** Funding acquisition. **Huawei Dai:** Investigation. **Hu Li:** Resources. **Jun Qiu:** Investigation. **Xueli Pang:** Writing – review & editing, Supervision, Project administration, Formal analysis.

Declaration of competing interest

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

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