Article

Comparison of Different Grades of Innovative Tanyang Congou Black Tea (Minkehong) Based on Metabolomics and Sensory Evaluation

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Abstract: Innovative Tangyang Congou black tea, also known as Minkehong black tea (MKH), is of high quality and hence has gained a reputation on the market. In this study, standard samples of MKH were used as the research material, and the results of sensory evaluation showed that the overall quality of MKH decreased along with the downgrading. The radar chart showed a strong association between higher grades and specific aroma and taste character. Based on the detection of ultra-performance liquid chromatography–mass spectrometry (UPLC–MS) and related analysis, the content of both the ester-type catechins and non-ester catechins decreased along with the downgrading, and the details of their change trends were revealed. The content and dynamic changes in caffeine were also investigated. A total of 19 kinds of amino acids (AAs) were clustered; among them, 4 kinds of AAs, namely, Thr, Leu, Asp, and Ile, significantly contributed to the taste of the tea. Notably, the content of Thr had the highest correlation with the grade of MKH, and the correlation coefficient was 0.991 ($p < 0.01$). According to gas chromatography–mass spectrometry (GC–MS) detection and analysis, a total of 861 kinds of volatile compounds were detected, the aroma-active compounds in different grades were selected, and an aroma wheel of MKH was constructed. Our results found that non-volatile and volatile compounds not only contribute to defining the level of MKH standard samples but also provide a chemical basis for the measurement of flavour and quality of MKH.

Keywords: grades; catechins; amino acid; volatile; flavour-contributing metabolites

1. Introduction

Black tea, being a globally popular beverage, currently dominates over 80% of the market [1]. China is the primary production region of black tea [2]. Notably, a unique and innovative black tea has been invented in the eastern region of Fujian province and has gained popularity. This unique black tea is named "Minkehong black tea (MKH)", which has a highly floral and fruity aroma, mellow taste, and amber liquid colour (T/FACX-2018) [3]. Both tea varieties and processing methods contribute to its distinctive quality. Aroma-enriched tea cultivars like "Jinmudan", "Zimeigui", "Mingke No.1", and "Mingke No.2" are the main materials used for MKH manufacturing. However, common tea cultivars such as "Fuyun No.6" and "Tanyang Caicha" tend to be used in traditional black tea production [4,5]. Furthermore, the essential processing method of oolong tea production, known as "turning-over", has been successfully integrated into MKH processing [6,7]. The external mechanical forces and frictional forces brought by the "turning-over" process

stimulate the cells along the leaf edge, which in turn stimulates the enzyme activity, leading to the formation and accumulation of aroma-active compounds [8]. Besides the supplementary turning-over method, a longer period of withering is also needed, which will cause more water loss to tea leaves, and hence, more suitability for turning-over [9]. Furthermore, the fermentation degree of innovative black tea is generally lower than that of traditional black tea [10,11]. The advancement of both variety and processing methods contributes to the exceptional quality of innovative black tea to some extent, as represented in Figure 1.
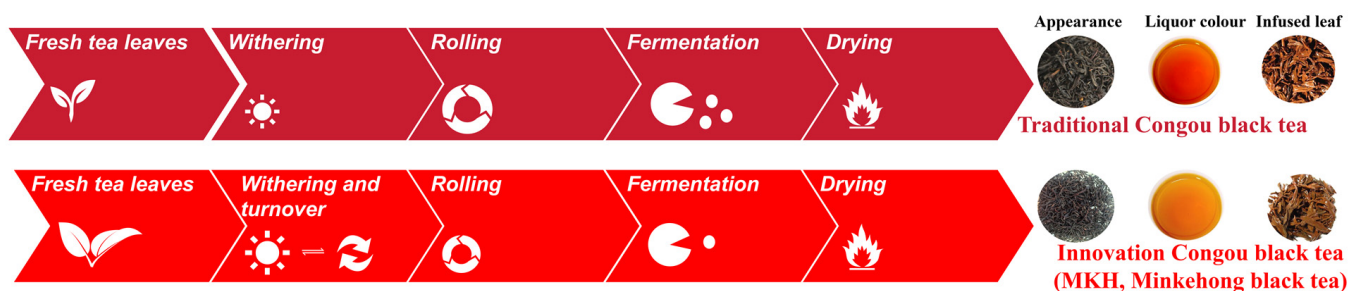

Figure 1. The difference in the manufacturing process between traditional Cougon black tea and innovative Congou black tea (Minkehong, MKH). Traditional black tea, like Tanyang Congou black tea, is typically crafted from a single bud and one or two leaves of fresh tea. After undergoing processes including withering, rolling, fermentation, and drying, traditional Congou black tea acquires its fine, tight, and uniform appearance and bright red liquor colour, as well as a sweet, mellow taste. In contrast to traditional Congou black tea, the innovative Tanyang Congou black tea (Minkehong, MKH) tends to be made from a single bud and two or three leaves. The withering process for MKH incorporates the turning-over treatment, which is followed by rolling, light fermentation, and drying. The quality of MKH is characterized by its robust appearance, orange-red liquid colour, and sweet taste with a flowery and fruity aroma.

In previous studies, it was found that the intensity of the turning-over method had a positive impact on tea quality by facilitating the degradation of fatty acids and promoting the formation of derived volatiles, which ultimately contribute to the aroma of oolong tea [12–14]. Several studies have subsequently revealed the effect of this processing method on black tea. For example, indole, phenylacetaldehyde, and δ-decalactone were identified as aroma-active compounds, with δ-decalactone being primarily responsible for the peach-like aroma in Jinmudan black tea [4]. During the Jinmudan processing procedure, jasmine lactone and other lipid-derived compounds were identified as key contributors to the fruity aroma [5]. A combination of withering and turning-over techniques was applied to produce one kind of special Hunan black tea (HBT), and, in this tea sample, indole and methyl jasmonate were identified as crucial compounds that contribute to the intensity of the floral aroma [15]. To summarize, we have demonstrated that the processing method of black tea has significantly contributed to the fruity and floral aroma characteristics of the final product.

The assessment of tea-grade samples typically relies on artificial sensory evaluation, which often involves subjective judgments. Recent advancements in metabolomics techniques have enabled more objective and reliable classification of food grades [16]. Thanks to the widespread use of metabolomics techniques, compounds that relate to tea flavour and quality can now be identified with greater depth and comprehensiveness. Zhang C et al. (2023) found that aged black tea showed a decreasing trend in the pH value in the liquid of tea along with the extension of the year, which might be related to the formation and accumulation of organic acids, such as L-ascorbic acid and salicylic acid [17]. The flavour performance of sweet aroma compounds in black tea may be due to the reduction in carbohydrates and acetylation of AAs during the period of hot-air second-drying [18]. Meanwhile, 620 differential substances were detected through metabolomics techniques during the manufacturing process of "Huangjinya" black tea [19]. Additionally, the identification of key odorants of "Baimaocha" black tea from different regions revealed that

methyl salicylate, geraniol, trans-β-ionone, and benzeneacetaldehyde were the definitive aroma compounds [20]. Through the detection of black tea during the process of manufacturing the product, the shaping of aroma quality was a continuous process, and each stage significantly affected the concentration of volatiles [21]. In addition, Wu Q et al. detected the accumulation of fatty-acid-derived volatiles in different stalk positions, suggesting that the maturity of fresh leaves would be an important factor influencing the quality of Jinmudan black tea [22].

These findings serve as a valuable reference for establishing quality standards for diverse black tea products, as well as guidance for the oriented processing of various grades of tea. A classification of tea grades was conducted in recent years, including green tea (such as Xihu Longjing tea [23] and Huangshan Maofeng tea [24]), oolong tea (such as Anxi Tiehuanyin tea [25], Wuyi Rougui Tea [26], and Yashi Xiang tea [27]), and even Sri Lankan tea products [28]. Additionally, related quality identification was also revealed based on standard tea samples. However, limited research has been conducted to investigate the relationship between metabolic compounds and the quality and grade standards of MKH.

In this study, we used different grades of MKH standard samples as research materials. Sensory evaluation was conducted as the initial step. UPLC–MS and GC–MS were utilized to identify the non-volatile components (including catechins, AAs, caffeine, etc.) and volatile compounds in MKH. Furthermore, we identified the key differential compounds among different grades of MKH using stoichiometry combined with multivariate statistical analysis. This study aimed to provide molecular evaluation results for approving the classification of different grades of MKH, as well as act as a reference for standardized production methods and marketing strategies.

2. Materials and Methods

2.1. Chemicals

(-)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatachin (EGC), (-)-Epigallocatechin gallate (EGCG), (-)-epicatechin gallate (ECG), caffeine, L-Theanine (L-Thea), Arginine (Arg), Aspartic acid (Asp), γ-aminobutyric acid (γ-GABA), Glutamine (Gln), Isoleucine (Ile), Proline (Pro), Serine (Ser), Threonine (Thr), Valine (Val), Alanine (Ala), Leucine (Leu), Asparagine (Asn), Phenylalanine (Phe), Methionine (Met), Glutamine (Glu), Tyrosine (Tyr), Histidine (His), and Trypotophan (Trp) were all from Solarbio Science & Technology Co., Ltd. (Beijing, China).

Pure water was from Huarun Yibao Beverage Co., Ltd. (Shenzhen, China). Acetonitrile (HPLC grade) and methanol (HPLC grade) were from Thermo Fisher Scientific Co., Ltd. (Munich, Germany). Formic acid (HPLC grade, $\geq$98%) was from Fuchen Chemical Reagent Co., Ltd. (Tianjin, China). Ammonium acetate (HPLC grade, $\geq$99%) was from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Sodium chloride (analytically pure) was from China National Pharmaceutical Group Corporation (Beijing, China). Hexane (HPLC grade, $\geq$99%) was from Merck & Co., Inc. (Whitehouse Station, NJ, USA). 3-Hexanone (Internal standard, 50 µg/mL, HPLC grade, $\geq$99%) was from Sigma-Aldrich Corp (St. Louis, MO, USA).

2.2. Tea Samples

A selection of Minkehong black tea standard samples was classified by the Fu'an Tea Industry Association of Fujian Province, according to the quality requirements of the different grades from Chinese Consortium Standards T/FACX001 "Tanyang Congou Tea with Flowery-fruity Flavour Minkehong Black Tea" (Figure S1). The ownership of MKH standard samples was possessed by the Fu'an Tea Industry Association and entrusted to Fujian Nongken Tea Industry Co., Ltd. (Fu'an, China) for production. All the standard samples made from the fresh leaves of the tea plant (*Camellia sinensis* cv. Jinmudan), following the innovative manufacturing process based on the traditional manufacturing process (GB/T24710-2009, Production of geographical indication_Tanyang Congou black tea) [29], including withering, slight shaking, rolling, fermentation, and drying. Standard

samples of MKH are important references in the tea production and trade processes, and they are also commonly used in teaching, scientific research, and brand promotion, which is authoritative. Also, the standard samples can be separated as a basis for all tea companies or individuals to classify or study their produced MKH-related products. These tea standard samples could be used when in need through official application without any cost. To facilitate the understanding of foreign readers, in the following description, the 4 grades (Te, 1, 2, and 3) of MKH were designated MKH-0, MKH-1, MKH-2, and MKH-3 (Figure S2). The difference in the 4 standard samples of MKH was caused by differences in the maturity of fresh tea leaves, leading to differences in sensory quality [3]. The sampling of each MKH standard sample was replicated 3 times. All MKH standard samples were stored in an air-conditioned room (26 °C) until required.

2.3. Sensory Evaluation

The sensory evaluation of MKH samples was conducted by 3 tea tasters who were trained by professional organizations; all the tasters had national vocational qualification certification, and all were from the Tea Evaluation Center of Ningde City. The evaluation room located is in a clean, dry, and bright place (RT, 25 $\pm$ 2 °C). Each tea sample (3.0 g) was brewed with boiling water (150 mL) for 5 min. The tea infusion was transferred into a tea bowl (250 mL) (GB/T8313-2018) [30].

Before sensory evaluation, 3 sensory evaluators (all with national-level tea evaluator professional qualifications) participating in the evaluation and scoring discussed methods in detail, including black tea characteristics, flavour options, scoring methods, etc. At the same time, the tea evaluators promised that they would operate completely independently and would not communicate with each other throughout the entire process. After preliminary training and preparation, the panellists focused on evaluating and grading the characteristics of aroma (involving flowery, fruity, sweet, and woody) and taste (involving heavy and thick, smooth and mellow, sweet and brisk, and coarse). Each member quantified ratings based on sensory attributes of aroma and taste. The strength criteria were described as follows: very weak/only perceptible at 0–2, weak at 2–4, neutral at 4–6, strong at 6–8, and very strong at greater than 8. The data are represented as an average.

2.4. Liquor Chromaticity

Obtaining the liquor of MKH standard sample was the same as in Section 2.3. An NH310 Portable Colorimeter (ThreeNH Technology Co., Ltd., Shenzhen, China) was utilized to detect the chromaticity value including *a*, *b*, and *L* of liquor, referring to the introduction, and distilled water as a control group. Saturation (*Sab*) is a series of derived indicators that generate colours from *L*, *a*, and *b*, and its formula is as follows:

$$Sab = \sqrt{a^2 + b^2} \div L$$

2.5. Detection and Analysis of Non-Volatile Compounds by UPLC-MS
2.5.1. Catechin and Caffeine Analysis

Pre-treatment: The dried tea sample was thoroughly ground into a powder and sifted through a 0.4 mm sieve. The tea powder (200 mg) was put in a 100 mL centrifuge tube, and vortexed and mixed fully with preheated aqueous methanol (5 mL, 70% *v/v*), and then the sample was sustained at 70 °C for 10 min via a water bath, which was shaken well every 5 min. The sample was centrifuged at a speed of 3500 rpm for 10 min, and the supernatant was transferred to a 10 mL volumetric flask. Methanol was used for the re-extraction of residue. The above operation was repeated several times, and the extraction solution and the volume were brought up to 10 mL in a volumetric flask. After that, a 1 mL aliquot was transferred to a 2 mL centrifuge tube at 3500 rpm for 5 min with PSA (5 mg) and C18 (40 mg). The supernate was filtered through a 0.22 μm organic filter membrane and was subjected to UPLC–MS analysis.

UPLC–MS was utilized for catechin and caffeine analysis. A Q Exactive UPLC–MS system (Thermo Fisher Scientific, Rockford, IL, USA) was carried out on a Hypersil GOLD column (100 × 2.1 mm 1.9 μm, Thermo Fisher Scientific). The column temperature was 30 °C. The mobile phases were 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B), and the linear elution gradient program was 0–12 min, 5–17% B; 12–13 min, 17–100% B; 13–16.5 min, 100–5% B; and 16.6–20 min, 5% B. The total analysis time was 20 min, the flow rate was 0.3 mL/min, and the injection volume was 1 μL.

Mass spectrometry was performed in the ESI (Electron Spray Ionization) mode for catechins and the ESI+ mode for caffeine with the following settings: the capillary voltage was 3.5 kV (ESI+) and 3.8 kV (ESI−); the flow rates of the sheath and auxiliary gas were 20 arb and 8 arb, respectively; the source temperature and desolvation temperature were 350 °C and 400 °C; the pore gas and desolvent gas was nitrogen, and the collision gas was argon gas; the cone gas flow was 150 L/h; and the desolvation gas flow was 800 L/h. Collision energy and cone voltage were optimized for the above compounds with multiple reaction monitoring (MRM) for quantification. The optimization of MS parameters used a single standard of catechins (10 ng/mL) and caffeine (10 ng/mL) to determine characteristic parent and daughter ions [31].

2.5.2. Free Amino Acid Analysis

Tea powder (30 mg) was put in a 100 mL centrifuge tube, followed by vortexing and mixing with aqueous methanol (1 mL, 70% *v/v*), and then the sample was sustained at 25 °C for 20 min with ultrasonic extraction. The sample was centrifuged at a speed of 6000 rpm for 10 min, and the supernate was filtered through a 0.22 μm organic filter membrane and was subjected to UPLC–MS analysis [32].

A Q Exactive UPLC–MS system was carried out on an ion-exchange column (Thermo Scientific Acclaim TRINITY P1, 50 mm × 2.1 mm, 3 μm). The column temperature was 30 °C. The mobile phases were 5mM ammonium acetate solutions (A) and acetonitrile with 0.1% formic acid (B), and the linear elution gradient program was 0–5 min, 0–30% B; 5–7 min, 30–100% B; 7–11 min, 100% B; and 12–18 min, 0% B. The injection volume was 2 μL.

Mass spectrometry was performed in the ESI+ mode for AAs with the following settings: the capillary voltage was 3.5 kV (ESI+); the flow rates of the sheath and auxiliary gas were 45 arb and 15 arb, respectively; the source temperature and desolvation temperature were 270 °C and 370 °C; the pore gas and desolvent gas was nitrogen, and the collision gas was argon gas; the cone gas flow was 150 L/h; and the desolvation gas flow was 800 L/h. Collision energy and cone voltage were optimized for the above compounds with multiple reaction monitoring (MRM) for quantification. The optimization of the MS parameters used a single standard of AA (10 ng/mL) to determine characteristic parent and daughter ions.

2.6. Detection and Analysis of Volatile Compounds by GC–MS

Headspace solid-phase microextraction (SPME, Agilent, Palo Alto, CA, USA) combined with GC–MS (8890-7000D, Agilent) was used to identify metabolites in the analytical samples. Each dry tea sample was quickly removed from an ultra-low temperature refrigerator at −80 °C and thoroughly ground into powder using a ball mill (MM400, Retsch, Haan, Germany) under the protection of liquid nitrogen. Then, 500 mg of dry tea sample was weighed and placed in a 20 mL headspace bottle (Agilent) and 1 mL of saturated NaCl solution and 10 μL 3-Hexanone isotope (3-Hexanone-2,2,4,4-d4, CAS: 24588-54-3) were quickly added as the internal standard solution. Finally, the bottle cap was tightened and 3 replicates for each sample were weighed. We used CTC Analytics AG equipment (PAL RSI 120, Basel, Switzerland). Under constant temperature conditions of 60 °C, the sample was shaken for 5 min, and a 120 μm DVB/CWR/PDMS extraction head was inserted into the headspace bottle of the sample. It was extracted in the headspace for 15 min, analysed at 250 °C for 5 min, and then GC–MS separation and identification were performed. Before sampling, the extraction head was aged for 5 min at 250 °C in a Fibre Conditioning Station

(note: a new extraction head should be aged at 250 °C in a Fibre Conditioning Station for 2 h before extraction). Additionally, it used SPME Arrow, which had a sensitivity of up to 10 times that of traditional SPME fibre heads.

The identification and the quantification of the volatile components were conducted using an Agilent Model 8890 GC and a 7000 D mass spectrometer (Agilent) equipped with a 30 m × 0.25 mm × 0.25 μm DB-5MS (5% phenyl-polymethylsiloxane) capillary column. The carrier gas was helium (99.999% purity) at a flow rate of 1.2 mL/min. The injector temperature was maintained at 250 °C, and the detector was 280 °C. The oven temperature was programmed from 40 °C (3.5 min) to 100 °C increasing at 10 °C/min, and then to 180 °C at 7 °C/min, and finally, to 280 °C at 25 °C/min and kept for 5 min.

The MS operating parameters were as follows: electron impact (EI) ionisation mode, 70 eV; ion source temperature, 230 °C; quadrupole mass detector, 150 °C; and transfer line temperatures, 280 °C. The selected ion monitoring (SIM) mode between *m*/*z* 30 and 500 was used for the identification and quantification of analysis based on self-built databases (Metware Biotechnology Co., Ltd., Woburn, MA, USA).

During the analysis process, the fingerprint MS of the sample was matched and compared with the reference MS in the database for scoring and qualitative analysis, and the retention index information was referenced to increase the accuracy of qualitative analysis. MassHunter tool (Agilent) was used to process mass spectrometry files and select quantitative ions for integration and calibration processing. The retention index (RI) range was defined between (RI target− threshold) and (RI target+ threshold), and the threshold was set as 60. All the detected volatiles within the RI range were selected as candidates [4].

2.7. Statistical Analysis

All experimental data were presented as mean ± standard deviation. Differences in the content of non-volatile and volatile compounds were determined using Tukey's honest significant difference (HSD) test as described in the reference. The correlation between the content of non-volatile and volatile compounds and MKH grade was determined using Spearman correlation analysis performed by SPSS software (PASW Statistics Base 18, IBM, Chicago, IL, USA). Partial least squares discriminant analysis (PLS–DA) was conducted using SIMCA–P 14.1 software from Umetric (Umea, Sweden). Bioinformatic analysis, including flavour radar chart, heatmap, and network diagram generation, was carried out using the OmicStudio tools (Available online: https://www.omicstudio.cn/toolm accessed on 29 January 2020) [33].

3. Results

3.1. The Sensory Evaluation of Different Grades of MKH

The evaluation of MKH was conducted by measuring the appearance and quality. The lower grade of MKH possessed higher fatness and boldness in appearance and a darker colour of dry tea. Furthermore, there was a decrease in liquid brightness, the fragment of brewed leaves, the persistence of aroma, the heaviness, and the thickness of taste (Figure 2A), which indicated that the overall quality decreased as the tea downgraded. Taking into account these findings, we constructed a flavour radar chart and utilized multiple factors for evaluation. In terms of aroma, we utilized the characteristics of flowery, fruity, sweet, and woody as measures. We observed a strong correlation among the characteristics including fruity, flowery, and sweet with various tea grades, whereas the woody characteristic was primarily associated with lower tea grades (Figure 2B). For taste evaluation, we took the characteristics including heavy and thick, coarse, smooth and mellow, sweet and brisk into account. Our findings indicated that heavy and thick, smooth and mellow, and sweet and brisk were strongly associated with tea grades except for the coarse characteristic (Figure 2C).

When considering the measurement of tea liquid colour, the chromatism of the *L*, *a*, and *b* values were implemented to reflect the bright, redness and greenness, yellowness, and blueness, respectively. The *L*, *a*, and *b* values of MKH-1 were significantly higher

and distinct from MKH-2 and MKH-3, with minimal differences observed in comparison to MKH-0. It was noteworthy that along with the upgrading, the colour saturation (*Sab*) increased, illustrating that this factor could be one of the references used to distinguish MKH quality (Figure 2D). Interestingly, the raw materials used for MKH-0, which was designated as the highest grade, exhibited greater tenderness compared with the other groups. Consequently, MKH-0 was rich in substances, resulting in a redder and more brilliant liquid colour. These factors might have contributed to the reduced differences in chromatism values between MKH-0 and the other groups.
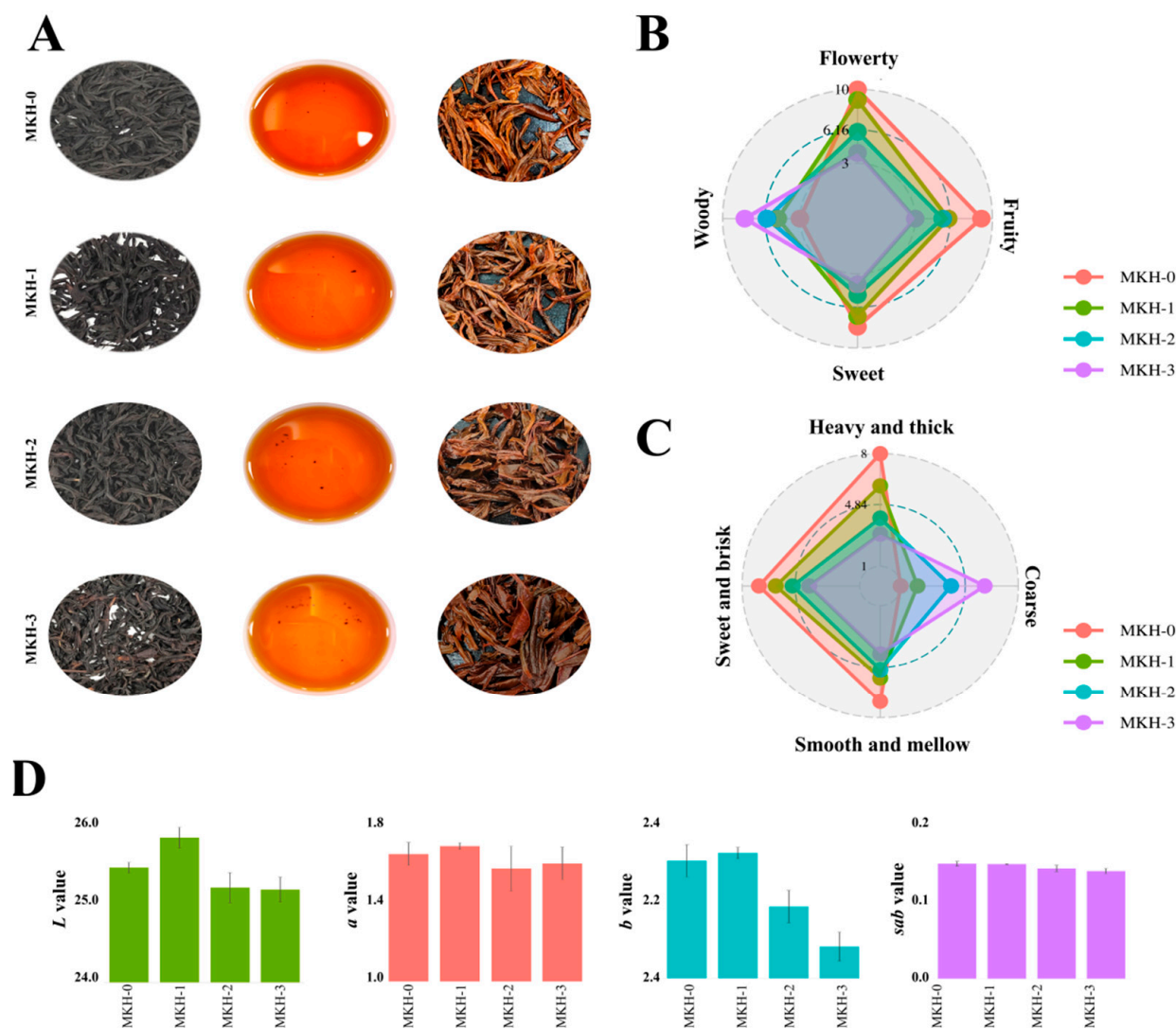

Figure 2. Sensory evaluation of different grades of MKH. (**A**) The appearance, liquor colour, and infused leaf of various grades of MKH. (**B**) Radar chart representing sensory scores for the aroma of MKH using the infused leaf. (**C**) Radar chart representing sensory scores for the taste of MKH using liquid tea after infusion. (**D**) The chromaticity parameters, specifically, the values of *L*, *a*, *b*, and *Sab*, for tea liquid from various grades of MKH.

3.2. The Analysis of Catechins and Caffeine Content among Different Grades of MKH

Catechins are the contributors to the bitter and astringent taste of tea and can be divided into two categories including ester-type catechin and non-ester catechin according to their chemical structure [34]. In this study, a total of five kinds of catechins were detected, including three kinds of non-ester catechins (C, EC, and EGC) and two kinds of ester catechins (EGCG and ECG) (Table 1). Among the different grades of MKH, the ester catechin possessed the majority proportion (nearly 70%), and among ester catechins, EGCG

possessed a higher abundance than ECG. Both these two compounds showed a generally decreasing trend along with the downgrading, and there also existed significant differences among the different grades of MKH. Regarding the non-ester catechins, all three non-ester catechins including C, EC, and EGC were decreased along with the downgrading generally, while in MKH-3, the content of C and EC fluctuated and increased slightly.

Table 1. Abundance (mg/g, DW) of catechin and caffeine caffeine in relation to different grades of MKH.

Compound/mg/g	MKH-0	MKH-1	MKH-2	MKH-3
C	8.991 ± 0.570 [a]	6.448 ± 0.254 [b]	5.408 ± 0.102 [c]	5.490 ± 0.122 [c]
EC	12.105 ± 1.471 [a]	6.701 ± 0.449 [b]	3.521 ± 0.236 [c]	4.932 ± 0.222 [bc]
EGC	76.807 ± 4.638 [a]	57.608 ± 2.281 [b]	29.617 ± 2.653 [c]	25.538 ± 1.098 [c]
EGCG	133.316 ± 8.235 [a]	83.953 ± 1.394 [b]	33.508 ± 3.130 [c]	33.732 ± 4.198 [c]
ECG	25.650 ± 1.199 [a]	16.652 ± 0.857 [b]	7.993 ± 0.355 [d]	11.597 ± 1.377 [c]
Caffeine	24.455 ± 1.416 [a]	22.576 ± 0.598 [ab]	21.081 ± 1.081 [b]	22.427 ± 1.357 [ab]

Note: different lowercase letters (a–d) represent significant differences at $p < 0.05$.

Analogous to catechins, the other compound, namely, caffeine, also contributes to the bitter taste of tea. In this study, its content in different grades of MKH was revealed. This compound achieved its maximum in MKH-0, its content fluctuated slightly along with the downgrading, and there existed less difference between samples.

It is noteworthy that catechins not only contribute greatly to the taste quality of tea but also influence the intensity of the thick character of tea liquid [35]. In this study, the content of both ester catechins and non-ester catechins in MKH-0 was higher than others, which was consistent with the sensory evaluation results that this tea sample possessed an outstandingly thick and mellow taste character.

3.3. The Analysis of AA Content among Different Grades of MKH

As the principal non-volatile compound in tea, free AAs mainly contribute fresh and sweet taste to the flavour quality of Congou black tea. Therefore, absolute quantifications of 19 free AAs were applied to determine the characteristic AA that could help to distinguish among the grades of MKH as quality reference. The results showed that theanine accounted for the major proportion of AAs, followed by Ser and Val. In detail, the total content of AAs in MKH-0 was significantly higher than in the other grade samples, which was consistent with the sensory evaluation results that this tea sample possessed a fresher and sweeter taste than the others. As for the other MKH tea samples, the content of AAs fluctuated and decreased along with the downgrading.

As shown in Figure 3, 19 kinds of AAs could be clustered into three categories. The first categories included eight kinds of AAs including Asn, Arg, Ile, Thr, Thea, Ser, Pro, and Val. These eight compounds all showed a maximum content in MKH-0, and their content gradually decreased along with the downgrading. Moreover, there also existed a significant difference in specific AAs among the four grades of tea. Secondly, a total of four kinds of AAs made up the next categories, which were Ala, Trp, Met, and Glu. In this category, there was little difference among the different grades of tea. The final categories consisted of seven kinds of AAs, namely, Leu, Asp, Phe, Tyr, Gln, GABA, and His. The changing trend in this category was similar to the first category. However, most of the compounds in this part possessed less content, and there existed less difference among different grades of MKH.
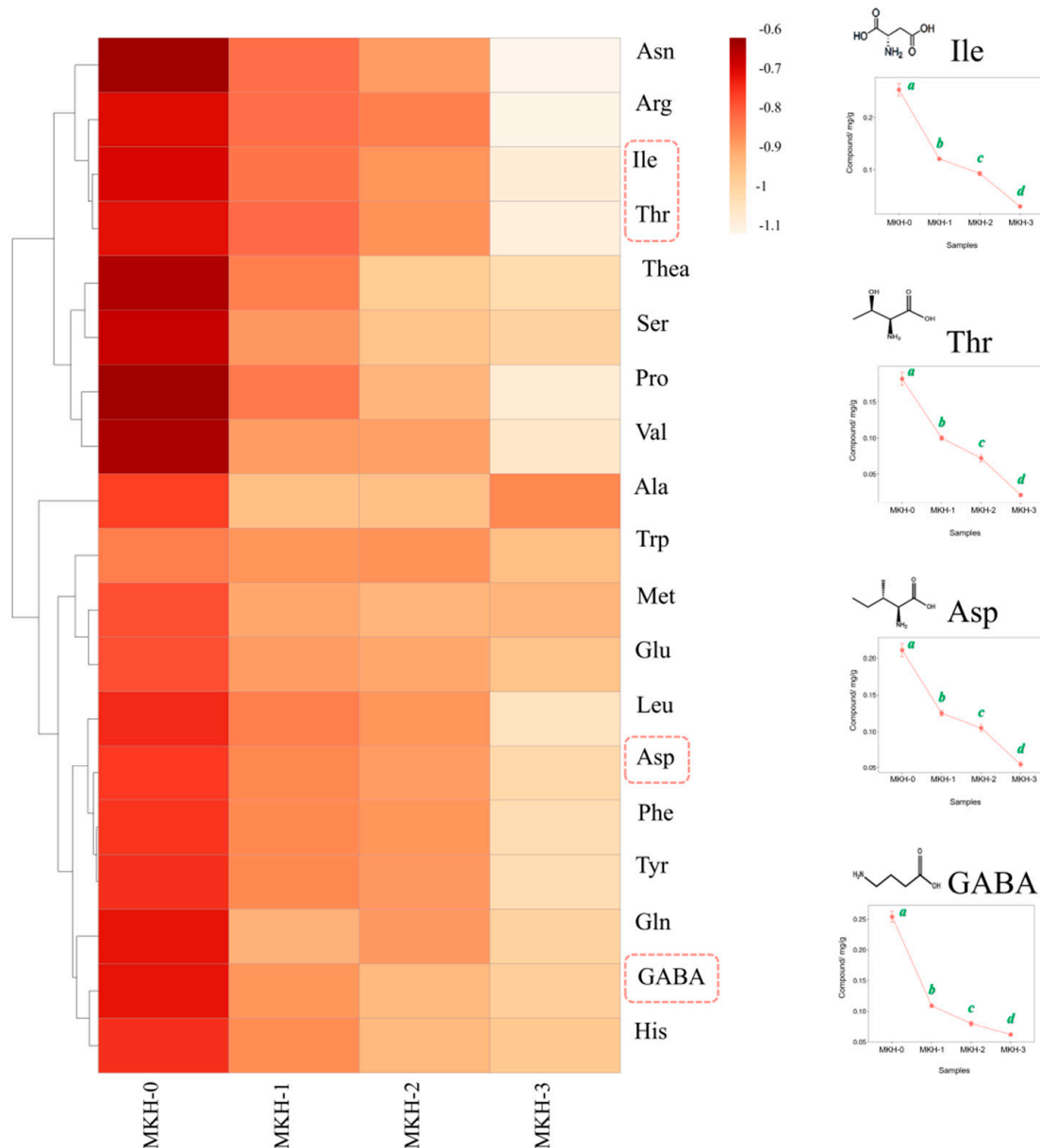
Figure 3. Abundance (mg/g, DW) of AAs detected in relation to different grades of MKH. The AA components (Ile, Thr, Asp, and GABA) highlighted by the red dashed line refer to the 4 levels, where their content decreases with the decrease in energy level content, and there was a significant difference between 2 pairs, displayed in the form of a line chart. Note: Different lowercase letters (a–d) represent significant differences at $p < 0.05$.

3.4. The Analysis of Volatilesamong Different Grades of MKH

In total, 861 volatile compounds were detected including 159 esters, 146 heterocyclic, 195 terpenoids, 72 hydrocarbons, 83 ketones, 61 aromatics, 61 alcohols, 61 aldehydes, and 23 acids (Figure 4A). A heatmap integrated with a hierarchical clustering analysis (HCA) was produced, rooted in the selected 45 volatile compounds under the PLS-DA model [8,36] (Figure 4B), to develop an evident visual comparison of the different MKH samples, and a permutation test was used to validate the stability and effectiveness of the PLS-DA model ($R2 = 0.229$, $Q2 = -0.340$) (Figure S3). There were 45 kinds of volatile compounds selected, whose Variable Important Projection (VIP) values were more than 1.0, and their CAS number, class, NIST RI, odour description, and average contents in different grades of MKH are presented in Table 2. The odour descriptions of the compounds were

taken from the Flavor and Extracts Manufacturers Association (FEMA) database and a resource to explore flavour molecules (FlavorDB). The heatmap shows the intensity of the selected compounds visualized on a normalized scale from a minimum of −2.0 (green) to a maximum of 2.0 (red), indicating the relative content of each compound [37]. The MKH samples were analysed by HCA, and the different grade samples were clearly separated (Figure 4B). According to the HCA results, the volatile compounds were classified into four groups (I, II, III, and IV) based on the dendrogram (Figure 4C).
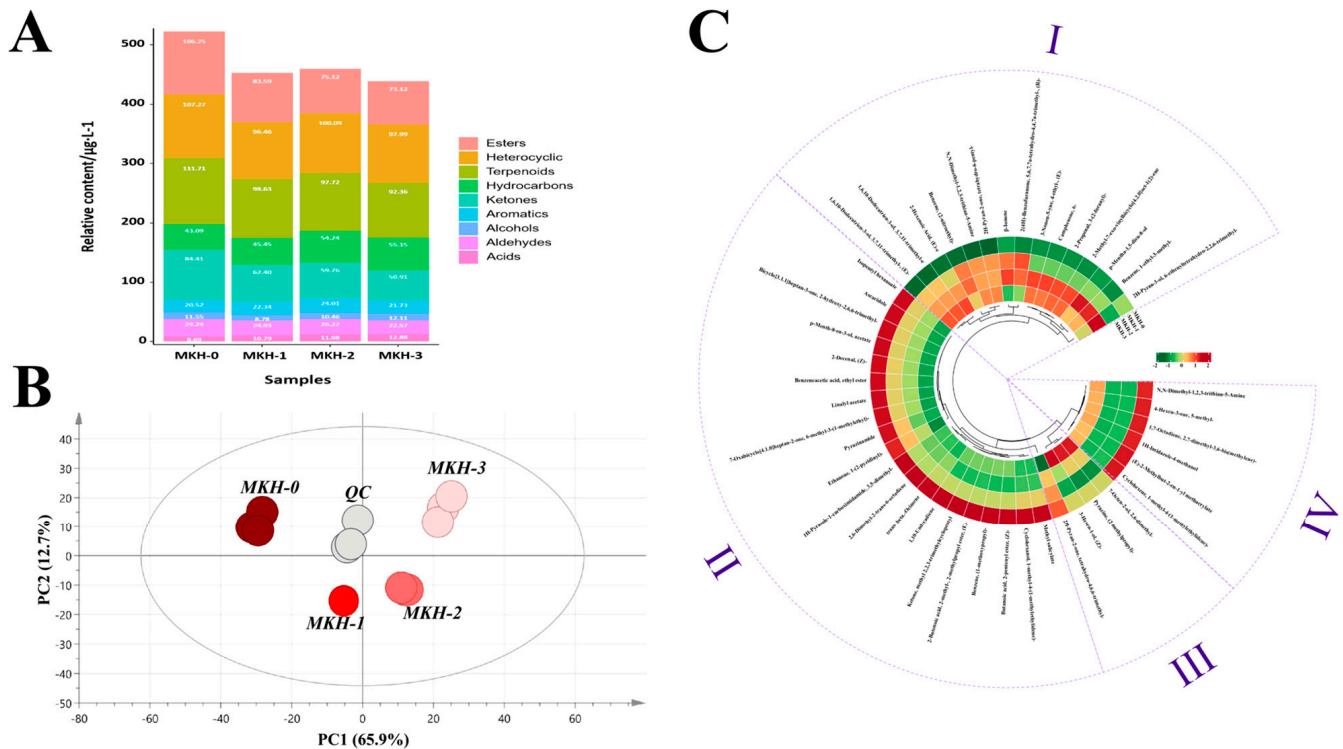

Figure 4. Comparison of volatile compounds in MKH. (**A**) Proportion chart of volatile compounds of different grades of MKH. (**B**) PLS-DA score plots of different grades of MKH, QC indicates quality control. PC1: principle component 1; PC2: principle component 2. (**C**) Heatmap of differential substances (VIP > 1), with relative concentrations greater than 0.1 µg/L. Volatile compounds were classified into 4 groups. Group-I comprised 15 compounds that were more abundant in the MKH-2 sample, group-II and group-IV comprised 26 compounds that were all more abundant in the MKH-0 sample, and group-III comprised 4 compounds that were more abundant in the MKH-3 sample.

3.5. Association Analysis of Non-Volatile and Volatile Compounds among Different Grades of MKH

When the association analysis was applied, the results revealed a close relationship between compounds and flavour (taste and aroma) as well as grades. As Figure 5A shows, there was a positive correlation between EGCG and heavy/thick taste and also between EGC and sweet/brisk taste. This suggested that the thickness and freshness of MKH's taste were closely linked to EGCG and EGC. Moreover, according to the Venn analysis, there were four kinds of compounds (Thr, Leu, Asp, Ile) that played a significant role in determining different tastes. It was also pointed out that EGC and EGCG were notable contributors to sweet, brisk, heavy, and thick tastes; Asn was recognized to play a pivotal role in aroma characters. Eight distinct compounds, including Phe, His, GABA, Tyr, and Thea, were identified as specifically associated with fruity aroma characters. Based on aroma analysis, AAs were established as influencing the fruity fragrance of MKH to some extent. Similar to AAs, the augmentation of catechins was observed to enhance the viscosity of tea liquid and improve the sensory experience of floral and sweet aroma characters (Figure 5B,C).

Table 2. The concentration of 45 key volatile compounds (VIP > 1) in different grades of MKH.

No	Compounds	CAS Number	Class	NIST RI	Odor Description	Relative Mean Content (µg/g)			
						MKH-0	**MKH-1**	**MKH-2**	**MKH-3**
v1	6-Methyl-3-(1-methylethyl)-7-oxabicyclo [4.1.0]heptan-2-one	5286-38-4	Ketone	1256	-	0.999 ± 0.146	0.629 ± 0.050	0.552 ± 0.017	0.373 ± 0.090
v2	Methyl salicylate	119-36-8	Ester	1192	Wintergreen, minty	1.000 ± 0.081	0.652 ± 0.036	0.493 ± 0.013	0.483 ± 0.055
v3	Linalyl acetate	115-95-7	Terpenoids	1257	Orange	0.270 ± 0.046	0.165 ± 0.013	0.143 ± 0.005	0.096 ± 0.024
v4	(Z)-2-Decenal	115-95-7	Terpenoids	1257	Citrus, floral	0.253 ± 0.040	0.153 ± 0.012	0.129 ± 0.004	0.083 ± 0.023
v5	2-Methyl-7-exo-vinylbicyclo [4.2.0]oct-1(2)-ene	107914-89-6	Hydrocarbons	1112	-	0.440 ± 0.044	0.499 ± 0.031	0.628 ± 0.013	0.612 ± 0.061
v6	(Z)-Butanoic acid, 2-pentenyl ester	42125-13-3	Ester	1091	Fruity, honey	0.190 ± 0.007	0.101 ± 0.006	0.069 ± 0.002	0.084 ± 0.005
v7	Ethanone	1122-62-9	Heterocyclic compound	1034	Balsamic, coffee	0.199 ± 0.015	0.163 ± 0.010	0.147 ± 0.002	0.113 ± 0.009
v8	Ascaridole	512-85-6	Terpenoids	1244	-	0.097 ± 0.020	0.052 ± 0.006	0.043 ± 0.002	0.024 ± 0.010
v9	Pyrazinamide	98-96-4	Heterocyclic compound	1250	bitter	0.061 ± 0.020	0.039 ± 0.005	0.026 ± 0.009	0.020 ± 0.011
v10	Tetrahydro-6-pentyl-2H-pyran-2-one	705-86-2	Ester	1497	Coconut, creamy	0.046 ± 0.017	0.119 ± 0.011	0.116 ± 0.003	0.123 ± 0.049
v11	(E)-2-Hexenoic acid	13419-69-7	Acid	982	Fruity	0.057 ± 0.002	0.088 ± 0.008	0.102 ± 0.002	0.114 ± 0.007
v12	Isopentyl hexanoate	2198-61-0	Ester	1250	Fruity, apple	0.071 ± 0.015	0.037 ± 0.005	0.029 ± 0.001	0.014 ± 0.008
v13	Methyl 2,2,3-trimethylcyclopentyl-ketone	17983-22-1	Ketone	1092	-	0.071 ± 0.003	0.034 ± 0.002	0.023 ± 0.001	0.028 ± 0.002
v14	1-Methyl-4-cyclohexanol	586-81-2	Terpenoids	1197	Camphor	0.063 ± 0.007	0.034 ± 0.003	0.021 ± 0.001	0.020 ± 0.005
v15	1-Methoxypropyl-benzene	59588-12-4	Aromatics	1104	Floral	0.059 ± 0.003	0.025 ± 0.002	0.013 ± 0.001	0.019 ± 0.002
v16	2-Hydroxy-2,6,6-trimethyl-bicyclo [3.1.1]heptan-3-one	10136-65-9	Ketone	1256	Minty	0.057 ± 0.012	0.029 ± 0.004	0.022 ± 0.001	0.010 ± 0.007
v17	1-Ethyl-3-methyl-benzene	620-14-4	Aromatics	957	Aromatic	0.104 ± 0.004	0.124 ± 0.005	0.163 ± 0.002	0.142 ± 0.007
v18	α-Farnesene	40716-66-3	Terpenoids	1564	Floral, woody	0.006 ± 0.007	0.038 ± 0.006	0.045 ± 0.001	0.052 ± 0.020
v19	p-Menth-8-en-3-ol acetate	89-49-6	Ester	1259	Sweet, woody, minty	0.037 ± 0.009	0.015 ± 0.003	0.011 ± 0.001	0.002 ± 0.005
v20	Benzeneacetic acid ethyl ester	101-97-3	Ester	1247	Floral, sweet	0.040 ± 0.009	0.019 ± 0.002	0.014 ± 0.001	0.005 ± 0.005
v21	(E)-2-Butenoic acid, 2-methyl-2-methylpropyl ester	61692-84-0	Ester	1093	Fruity	0.035 ± 0.002	0.015 ± 0.002	0.009 ± 0.001	0.012 ± 0.001
v22	N,N-Dimethyl-1,2,3-trithian-5-amine	31895-21-3	Heterocyclic compound	1491	-	0.016 ± 0.009	0.050 ± 0.005	0.049 ± 0.002	0.052 ± 0.024
v23	(E)-Linalool oxide	34995-77-2	Heterocyclic compound	1086	Floral, woody, camphor	0.313 ± 0.012	0.255 ± 0.012	0.255 ± 0.004	0.290 ± 0.015
v24	2-Nitroethyl-Benzene	6125-24-2	Aromatics	1304	Bitter, almond	0.009 ± 0.006	0.047 ± 0.005	0.049 ± 0.002	0.036 ± 0.014
v25	(E)-β-Ocimene	3779-61-1	Terpenoids	1049	Sweet, herbal	0.029 ± 0.010	0.010 ± 0.002	0.008 ± 0.001	0.004 ± 0.002
v26	1-Methyl-4-(1-methylethylidene)-cyclohexene	586-62-9	Terpenoids	1088	Citrus, woody, sweet	0.232 ± 0.012	0.189 ± 0.010	0.186 ± 0.004	0.210 ± 0.012

Table 2. *Cont.*

No	Compounds	CAS Number	Class	NIST RI	Odor Description	Relative Mean Content (µg/g)			
						MKH-0	MKH-1	MKH-2	MKH-3
v27	1,10-Undecadiene	13688-67-0	Hydrocarbons	1095	-	0.024 ± 0.001	0.007 ± 0.001	0.001 ± 0.000	0.003 ± 0.001
v28	2,6-Dimethyl-2-trans-6-octadiene	2609-23-6	Terpenoids	993	Citrus	0.022 ± 0.008	0.006 ± 0.002	0.003 ± 0.001	0.000 ± 0.002
v29	3,5-Dimethyl-1H-pyrazole-1-carboximidamide	22906-75-8	Heterocyclic compound	1096	-	0.036 ± 0.002	0.022 ± 0.002	0.020 ± 0.001	0.019 ± 0.002
v30	2-Propenal	623-30-3	Heterocyclic compound	1111	Pungent	0.028 ± 0.005	0.033 ± 0.003	0.046 ± 0.002	0.044 ± 0.006
v31	p-Mentha-1,5-dien-8-ol	1686-20-0	Terpenoids	1167	-	0.015 ± 0.002	0.021 ± 0.003	0.036 ± 0.002	0.026 ± 0.003
v32	(E)-Nerolidol	7212-44-4	Terpenoids	1563	Floral, citrus	0.006 ± 0.007	0.038 ± 0.006	0.045 ± 0.001	0.052 ± 0.020
v33	5-Methyl-4-hexen-3-one	13905-10-7	Ketone	1080	Floral	0.075 ± 0.003	0.058 ± 0.003	0.058 ± 0.001	0.068 ± 0.004
v34	6-Camphenone	55659-42-2	Ketone	1114	-	0.005 ± 0.002	0.007 ± 0.001	0.015 ± 0.000	0.013 ± 0.003
v35	4-Ethyl-(E)-3-nonen-5-yne	74744-60-8	Hydrocarbons	1117	-	0.008 ± 0.003	0.010 ± 0.002	0.017 ± 0.001	0.016 ± 0.003
v36	2,7-Dimethyl-3,6-bis(methylene)-1,7-octadiene	16714-60-6	Hydrocarbons	1083	-	0.058 ± 0.003	0.045 ± 0.003	0.044 ± 0.001	0.052 ± 0.003
v37	β-Ionone	14901-07-6	Terpenoids	1491	Dry, powdery, floral	0.109 ± 0.029	0.146 ± 0.010	0.154 ± 0.004	0.116 ± 0.033
v38	(Z)-3-Hexen-1-ol	928-96-1	Alcohol	858	Fresh, green, leafy	0.016 ± 0.001	0.001 ± 0.001	0.013 ± 0.001	0.042 ± 0.004
v39	(E)-2-Methylbut-2-en-1-yl methacrylate	88142-95-4	Ester	1088	-	0.041 ± 0.002	0.031 ± 0.002	0.031 ± 0.001	0.035 ± 0.003
v40	Dihydroactinidiolide	17092-92-1	Heterocyclic compound	1532	Ripe, apricot, fruity	0.019 ± 0.009	0.041 ± 0.004	0.039 ± 0.001	0.027 ± 0.013
v41	1H-Imidazole-4-methanol	822-55-9	Heterocyclic compound	1080	-	0.049 ± 0.003	0.038 ± 0.003	0.037 ± 0.001	0.044 ± 0.003
v42	2-Methylpropyl-pyrazine	29460-92-2	Heterocyclic compound	1074	Fruity	0.119 ± 0.005	0.103 ± 0.006	0.121 ± 0.002	0.141 ± 0.007
v43	6-Ethenyltetrahydro-2,2,6-trimethyl-2H-Pyran-3-ol	14049-11-7	Heterocyclic compound	1173	Floral, honey	0.109 ± 0.008	0.100 ± 0.006	0.137 ± 0.001	0.113 ± 0.010
v44	2,6-Dimethyl-7-octen-2-ol	18479-58-8	Terpenoids	1064	Citrus	0.106 ± 0.005	0.093 ± 0.005	0.108 ± 0.002	0.126 ± 0.007
v45	Tetrahydro-4,6,6-trimethyl-2H-Pyran-2-one	20628-36-8	Heterocyclic compound	1141	-	0.018 ± 0.003	0.017 ± 0.001	0.016 ± 0.001	0.010 ± 0.002

RI: retention index; CAS number: Chemical Abstracts Service Registry Number; Odor descriptions of compounds were from the Flavor and Extracts Manufacturers Association (FEMA) database and a resource to explore flavour molecules (FlavorDB).

The majority of volatile compounds exhibited a positive correlation with aroma sensory characteristics. Thirty-two compounds exhibited an extremely significant positive correlation, while four compounds had a significant positive correlation, and only nine compounds had minimal correlation with the aroma character. Similarly, 25 compounds exhibited a strong positive correlation with grades, 9 compounds had a significant positive correlation with grades, and 11 compounds had a negligible correlation with grades (Figure 5D).
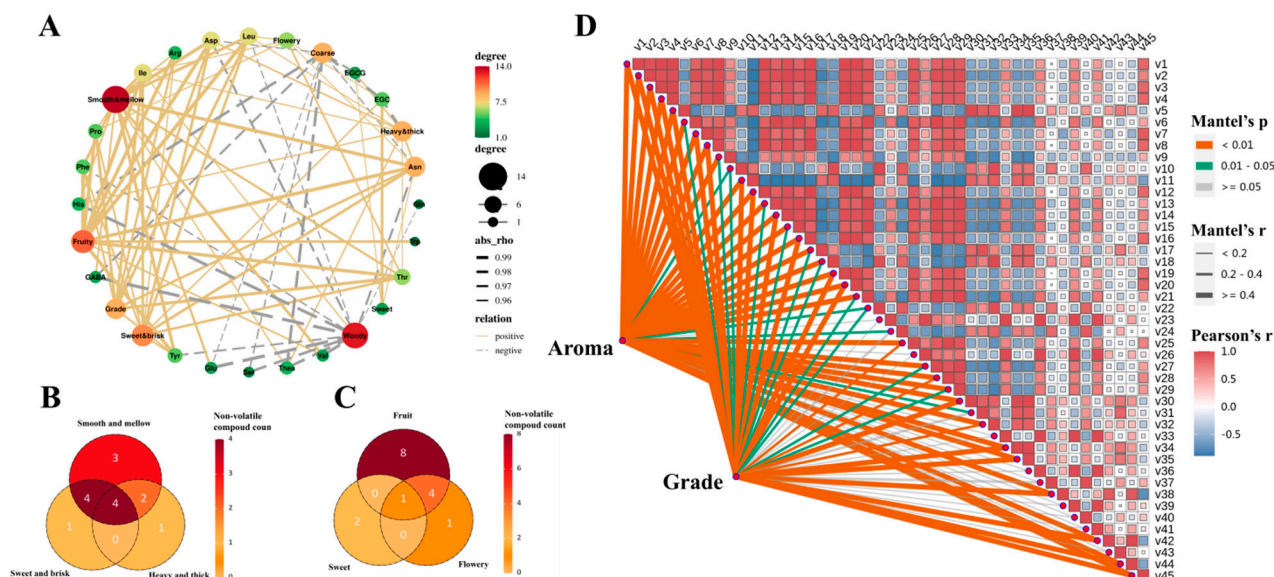
Figure 5. Volatile and non-volatile compound analysis of different grades of MKH. (**A**) The correlation network diagram focuses on the relationship among non-volatile components, grades, and taste attributes. The solid and dashed lines indicated positive and negative correlations, respectively; the thickness of the lines represents the magnitude of the correlation coefficient; and the size of the dots represents the amount of non-volatile components that are associated with taste characters. (**B**) The Venn diagram representing the potential positive relationships between non-volatile compounds and the attributes of aroma. (**C**) The Venn diagram representing the potential positive relationships between non-volatile compounds and the attributes of aroma. (**D**) A heatmap illustrating the correlation between the selected volatile compounds (v1–v45, VIP > 1) and both grade and aroma characteristics. The thickness of the lines represents the magnitude of the correlation coefficient. Different colours are used to represent the correlation between grades and aroma: orange indicates an extremely significant positive correlation ($p < 0.01$), green represents a significant positive correlation ($p < 0.05$), and grey signifies little to no correlation ($p > 0.05$).

The differential metabolites among the levels of standard samples are significant potential marker compounds for grading. In terms of the relationship among the non-volatile compounds and grades, eight compounds exhibited strong positive correlations, including Thr, Ile, Pro, Leu, Asn, Asp, Arg, Phe, and Tyr. Among these compounds, the correlation coefficient between Thr and grades amounted to 0.991 (Figure 6A). The selected aroma-active compounds are shown in the form of an aroma wheel (Figure 6B). At the super grade, the compounds (*E*)-*β*-ocimene and 2,6-dimethyl-2-trans-6-octadiene were identified based on their sweet, herbal, and citrus odour, respectively. In the first grade, the compound named 1-methoxypropyl-benzene possessed a highly floral odour. As for the second grade, additional volatile compounds were selected, which subsequently influenced the sensory evaluation results. In detail, (*E*)-2-hexenoic acid contributed to fruity odour, (*Z*)-3-hexen-1-ol contributed to fresh, green, leafy odour, (*E*)-nerolidol contributed to floral and citrus odour, and methyl salicylate contributed to wintergreen and minty fragrance. Finally, in the third grade, (*Z*)-butanoic acid, 2-pentenyl ester contributed to fruity, honey-odour fragrances.
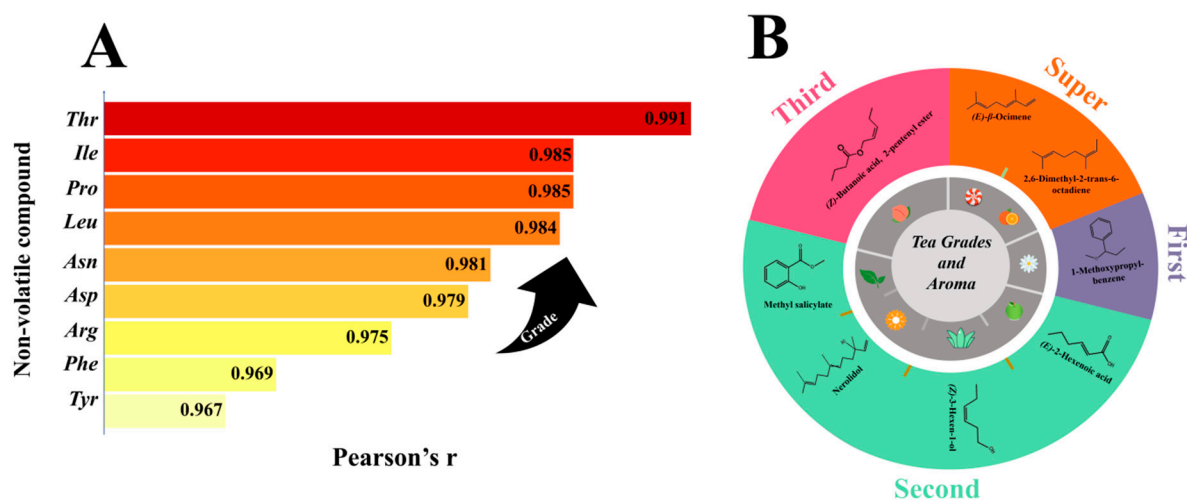
Figure 6. Potential marker compounds selection of different grades of MKH. (**A**) The correlation coefficient of non-volatile and volatile compounds demonstrating a highly significant positive correlation across all grades. (**B**) The aroma wheel representing different grades of MKH. A total of 45 volatile (v1 to v45) were selected based on a VIP value greater than 1.0, as exhibited in Table 2.

4. Discussion

4.1. The Selection of Non-Volatile Compounds and Their Correlation among Different MKH Grades

Catechins, AAs, caffeine, and other non-volatile compounds are the key taste-active compounds that contribute astringent, bitter, and delicate characteristics to the taste of tea, respectively [38,39]. The sensory evaluation results demonstrated that the taste characteristic underwent a transition from "mellow" to "mellow and thick" and then to "mellow and strongly thick" during the upgrading process, which aligned with the changing trend in catechins. During the upgrading process, the content of four AAs (Ile, Thr, Asp, and GABA) decreased. These AAs possessed the following distinct taste characteristics: Ile had a bitter taste, Thr had a sweet taste, GABA had a delicate taste, and Asp had a sour taste [40,41]. It was interesting to note that although AAs contributed to bitter and sour tastes, which were more prevalent in higher grades of MKH, they had less influence on tea flavour compared with AAs, which impart sweet and delicate tastes. This could be attributed to the difference in their taste thresholds [42]. Furthermore, AAs with bitter tastes tended to have higher thresholds, which means they play less of a role in contributing to tea flavour compared with AAs with sweet and delicate tastes [43]. Additionally, the collaborative interactions between compounds also impacted the balance and diversity of flavour.

The AAs that possessed aroma characteristics, such as Val, Leu, Phe, and Arg, were present at higher concentrations in higher grades of MKH. During the sensory evaluation, when the liquid of tea was being judged, these non-volatile compounds interacted with aroma receptor proteins in the oral cavity, which is the functional area for taste and smell. The resulting signal was subsequently transmitted to the brain of the quality evaluator, which is similar to the mechanism when volatility occurs [44]. This may help explain why the liquid of higher-grade tea samples exhibited a stronger fragrance not only when it was being smelled and drunk. In terms of the correlation between non-volatile compounds and MKH quality, several AAs and signal catechins were strongly associated with sensory characteristics. In particular, Asn was highly associated with grade classification, as well as smooth, mellow taste and even fruity aroma. Previous studies have also demonstrated that a high content of Asn was one of the factors that contributed to the formation of excellent-quality Congou black tea [45]. This suggested that Asn could be used as an essential metabolite contributing to the taste quality of MKH.

4.2. The Selection of Volatile Compounds and Their Correlation among Different MKH Grades

Differing from traditional black tea, the innovative black tea named MKH had distinctive cultivar aroma and taste. These characteristics were primarily attributed to its unique processing methods and original materials [46–48]. In this study, it was found that floral, fruity, and sweet fragrances were the main aroma sensory character of MKH, and several compounds like trans-*β*-ocimene in the super grade, (*E*)-2-hexenoicacid, methyl salicylate and (*Z*)-3-hexen-1-ol in the second grade were characterized as key aroma-active compounds for classifying the grade of MKH. Jiang et al. (2023) reported that the concentration of (*E*)-*β*-ocimene decreased during the processing of Sichuan black tea [49], and this compound still kept a higher content in MKH. The primary reason behind this difference is the distinct processing methods used for these two types of Chinese black tea. When compared with Sichuan black tea, MKH exhibited a higher degree of oxidation during withering with a lower degree of fermentation. As a result, besides trans-*β*-ocimene, low-boiling compounds that primarily contribute to grassy aroma were retained. In terms of the impact of processing raw materials on differences, numerous studies have established that the optimal maturity of tea leaves is the primary determinant of the aroma of tea [7,16]. The second grade of MKH was taken as an example. Its plucking standard was set as "one bud and two to three leaves", containing tender and mature leaves. While over-aged mature leaves had a tendency to contribute to a higher and more long-lasting fragrance in tea, they also led to a coarse and ageing odour, as well as lower taste quality [11,50]. During the sensory evaluation period, it was practical to observe the appearance of tea, as it aids in the initial discrimination of tea quality based on factors such as evenness and clarity. Despite being produced from mature tea leaves of elderly tea plants, the third grade of MKH exhibited a pleasant woody fragrance, which was primarily attributed to the presence of (*Z*)-butanoic acid, 2-pentenyl ester, and these compounds have received little attention in the literature. The precursors of (*Z*)-butanoic acid, 2-pentenyl ester, specifically, (*E*)-2-pentenal and (*Z*)-2-penten-1-ol, have been identified as being significantly decreased during solar withering processing in black tea manufacturing [51]. This finding suggested that the accumulation of (*Z*)-butanoic acid, 2-pentenyl ester in the third grade of MKH might be closely linked to the high-intensity withering method used in its production.

5. Conclusions

In this research, volatile and non-volatile compounds were quantitatively analysed using sensory evaluation, GC–MS, and UPLC–MS technology combined with multivariate statistical analysis, and the key compounds contributing to the grade of MKH standard samples were identified. Furthermore, we investigated the biochemical basis of the aroma and taste quality performance of MKH standard samples. The following conclusions were drawn:

(1) The MKH standard sample was generally positively correlated with the content of non-volatile compounds including AAs, catechins, and caffeine, and Ile, Thr, Asp, and GABA show significant differences between grades and are potential biomarker components for MKH.

(2) (*E*)-*β*-Ocimene, 2,6-dimethyl-2-trans-6-octadiene, and 1-methoxypropyl-benzene were the most abundant volatile compounds in the higher grade of MKH, being conducive to the formation of the delicate sweet and floral aroma, while (*Z*)-3-hexen-1-ol, nerolidol, and (*Z*)-butanoic acid, 2-pentenyl ester were the most abundant volatile compounds in the second and third grade of MKH, being conducive to the formation of the rich fruity aroma.

(3) The maturity of fresh tea leaf raw materials was negatively correlated with the grade of MKH, which was the main internal factor for the difference between volatile and non-volatile compounds in the sample grade.

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