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Effect of Different Harvesting Dates on Tea Quality of Five Mulberry Varieties in Turpan City

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Abstract

This study aimed to investigate the influence of different harvest dates on the chemical composition of *Morus nigra* leaf tea in Turpan City, Xinjiang Province. *Morus* (mulberry) varieties belong to the order Rosales, family Moraceae and genus *Morus*, and are widely cultivated around the world. Mulberry leaf tea is made from the processing of mulberry leaves and is a primary way of high-value utilization of mulberry tree resources. Leaves of five mulberry varieties,'Yao Sang' (*M. nigra* L.), 'Bai Sang' (*M. alba* L.), 'Hei Sang'(*M. alba* L. var. Tatarica), Nong Sang Number 14 (*M. multicaulis* Perr.), and'Danbai Sang'(*M. alba*), were collected on seven different dates in 2024 and tea was made from each sample following a replicated standardized process. The main chemical components, including free amino acids, flavonoid glycosides, total phenols, total polysaccharides, alkaloids, γ-aminobutyric acid (GABA), and 1-Deoxynojirimycin (DNJ) were analyzed and sensory evaluations were conducted. Two-way ANOVA was conducted, and pairwise comparisons were performed between groups in which the interaction was statistically significant. The optimal harvest date was determined using principal component analysis to comprehensively evaluate tea quality and identify the optimal date for harvesting. Based on the results of the two-way ANOVA, a correlation was found between the repeated measurement data ($p \leq 0.05$) and Tukey's post-hoc test was used to make pairwise comparisons. Among the five species groups, there were significant differences in the mean values for most varieties. At the same time, there were significant differences between the mean values at most of the seven time points. The index values of the mulberry leaves at different harvest dates were used to calculate the primary component score function. The overall quality ranking was determined based on the comprehensive score: Yao Sang and Nong Sang 14 achieved their highest scores on May 27th, Danbai Sang peaked on June 24th, Bai Sang reached its maximum on July 8th, and Hei Sang attained its highest score on April 29th. This analysis method integrated the contents of several main bioactive substances and sensory scores of mulberry leaf tea to objectively and comprehensively determine the optimal harvest date for the five mulberry leaf varieties in Turpan City. These findings provide a theoretical reference for the preparation and utilization of mulberry leaf tea.

Mulberry foliage is the main product of the genus *Morus* spp. in the family Moraceae (Wu et al., 2003). It has been an important herb for the treatment of cold, fever, headache, cough, rheumatism, and other diseases for thousands of years (Arabshahi-Delouee et al., 2007; Gryn-Rynko et al., 2016). It is safe, less expensive than other drugs, and contains various phytochemical compounds (Li et al., 2017). Among them, total flavonoids (Kwon et al., 2019) have anti-aging, hypoglycemic, lipid-lowering, antihypertensive, and antitumor effects. Total polysaccharides have functions, such as lowering blood glucose and blood lipids, increasing antioxidants, and regulating the immune system (Ying et al.,

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2011). Total polyphenols have anti-radiation, anti-aging, anti-oxidation, and therapeutic effects in fatty liver disease, diabetes, and tumors (Khalifa et al., 2018). GABA can lower blood pressure and induce sedation (Kwon et al., 2013). Alkaloids can lower triglycerides and cholesterol in the blood and show good hypoglycemic and antibacterial effects with few side effects in clinical trials (Asano et al., 2001; Chrubasik-Hausmann, 2016; Kim et al., 2013). DNJ is an alkaloid found in mulberry leaves, which possesses a variety of biological activities, particularly showing significant effects in lowering blood sugar, regulating blood lipids, and exhibiting antimicrobial properties (Zhang et al., 2018). Free amino acids are the main evaluation indices for measuring taste and quality (Ruan et al., 2019). These results will be helpful in the selection of quality indices for mulberry green tea.

Harvesting is an important step in the processing and utilization of mulberry leaf tea and is related to the quality and pharmacological effects of mulberry leaf tea products (Lee et al., 2012; Zou et al., 2012). The quality of mulberry leaves plays an important role in the quality of mulberry leaf tea products (Mei et al., 2023). Currently, significant alterations have been observed in the timing of mulberry leaf harvesting as well as in the extended production cycles. Identifying the key components that differ between mulberry leaves in various seasons helps to further enrich the processing theory of mulberry leaf green tea and provides a theoretical reference for understanding the formation mechanism of mulberry leaf green tea quality.

Jia et al. (1999) analyzed the variation in flavonoid content in mulberry leaves during spring and autumn. Lee et al. (2012) measured and analyzed the variation in the levels of six polyphenolic compounds in mulberry leaves to determine the optimal harvesting period for mulberry leaves. Hu et al. (2021) determined the changes in flavonoid levels in mulberry leaves during two growth stages. However, it is not possible to determine the harvest date of mulberry leaves using only these indicators, which need to be comprehensively analyzed and evaluated.

The composition and content of nutritive active substances in mulberry leaves are affected by both the harvest date and traits of the mulberry varieties. Numerous studies have been conducted on the chemical composition and pharmacological activity of mulberry plants, both domestically and internationally. However, there are few studies on mulberry resources in Turpan City. Turpan City has a large area planted with mulberry trees but there are few species. These are mainly ‘Yao Sang’ (*M. nigra* L.), ‘Bai Sang’ (*M. alba* L.), ‘Hei Sang’ (*M. alba* L. var. Tatarica), Nong Sang Number 14 (*M. multicaulis* Perr.), and ‘Danbai Sang’ (*M. alba*). In this study, the contents of free amino acids, total flavonoids, total polyphenols, total polysaccharides, alkaloids and GABA, in the leaf tea of five mulberry varieties in Turpan were determined. In addition, this study compared the variations in sensory quality and the main physicochemical components of mulberry leaf tea across different varieties and seasons using two-way ANOVA. A comprehensive quality evaluation of mulberry green tea during different harvest dates was conducted based on the above indices, and the optimal harvest dates for different mulberry leaves was determined. This study provides a theoretical reference for the preparation of high-quality mulberry leaf green tea in Turpan City.

Materials and Methods

Overview of the Experimental Site

The experimental materials were collected from the germplasm resource garden of the Turpan Institute of Agricultural Sciences, Xinjiang Academy of Agricultural Sciences, located in Turpan City, Xinjiang, China. Turpan has an extremely arid warm temperate inland desert climate, characterized by abundant sunlight, rich heat, and minimal precipitation. Summers are hot, winters are extremely cold, and there is a large diurnal temperature range. The region has a long frost-free period, frequent and strong winds, severe dry hot winds, with gales of over eight levels mostly coming from the northwest. The annual sunshine duration is 3,043.3 hours, with a sunshine rate of 69%, and the average annual temperature is 13.8°C. The average annual precipitation is 6.30 mm, with the maximum being 23.80 mm and the minimum only 0.50 mm. On average, there are 108 days of winds over level eight per year, with the maximum wind force reaching 12 on the Beaufort scale, and wind speeds exceeding 40.00 $m \cdot s^{-1}$. The total water resources in Turpan City amount to 12.26 billion m^3, including 6.32 billion m^3 of utilizable surface water and 5.94 billion m^3 of utilizable groundwater. The climate conditions of Turpan City from April to July 2024 are shown in Table 1.

Plant Material

A randomized complete block design was used, consisting of five varieties and three replications in a total of 15 plots with 100 plants per plot. The mulberry trees are one year old with moderate vigor. Row spacing was set at 150 cm, plant spacing at 35 cm, with protective rows on both sides of each plot. The environmental conditions between the plots, such as soil type and Irrigation and Fertilization Management, were ensured to be consistent. The soil type in the mulberry garden is brown desert soil. The base fertilizer is sheep dung at 10 kg per plant, and top dressing is applied twice a year during the growing season with diammonium phosphate at 10 kg•hm^{-2} each time. Irrigation is performed based on the requirement. Depending on soil moisture conditions, watering is carried out once every seven to ten days during the growing season.

Following to the sampling guidelines as outlined by Ranalli (2006), This study utilized 5 varieties from the resource garden: 'Yao Sang' (*Morus nigra* L.), 'Bai Sang' (*M. alba* L.), 'Hei Sang' (*M. alba* L. var. Tatarica), Nong Sang Number 14 (*M. multicaulis* Perr.), and 'Danbai Sang' (*M. alba*). For each variety, Twenty trees were randomly marked in each plot. The sampling period spanned from April 15, 2024 to July 8, 2024 with collections occurring at 14-day intervals, totaling seven sessions.

During each harvest date, each variety was harvested in 6 kg batches, which were then blended to form a single composite sample. This process was conducted in triplicate, yielding three distinct leaf samples per variety. Branches were selected at random from various directions and different parts of each mulberry tree. The top three to five tender leaves were carefully gathered from each branch, with petioles excluded. Upon completion of each harvest date, a total of fifteen samples—representing the five varieties—were collected and processed.

Sample preparation of mulberry leaf green tea Mulberry leaf samples were each processed separately through the following steps: cutting the leaves, withering, fixing, rolling, stir-firing, drying, and finally, enhancing the aroma.

Within three to four hours of harvest, leaves were placed in a leaf cutter (Shanghai Customized Electric Cutting Machine, Zhejiang Chuangxian Ke Electric Appliance Co., Ltd., Taizhou City, China) and cut twice

Table 1. Climate Conditions of Turpan City from April to July in 2024.

Month	Average Minimum Temperature (°C)	Average Maximum Temperature (°C)	Precipitation (cm)	Average Sunshine (h)
April	11	24	3.6	12.8
May	20	34	4.5	14.3
June	24	38	8.7	15.1
July	25	38	9.3	15.2

into strips of four by two cm. Leaves were evenly spread at a thickness of three to five cm on a stainless-steel tray with holes and left to wither naturally in an indoor environment (24°C, 28% humidity on average). Leaf samples were turned every half hours during this period. Employing a High-precision Halogen Moisture Analyzer (HM-105L, Shanghai HeGong Scientific Instruments Co. Ltd., Shanghai, China), we meticulously monitored the water content in the leaves.

Upon reaching a 70% moisture level, the leaves were meticulously harvested and then transferred to a cylindrical dehumidifying apparatus (6CS-40, Zhejiang Lvfeng Machinery Co., Ltd., Quzhou City, China). The initial cycle was conducted at a temperature of 250°C, followed by a second cycle at 230°C. The leaf-casting rate was 36 kg per hour.Leaves were then rapidly cooled by a net belt conveyor (6CS-50, Zhejiang Lvfeng Machinery Co., Ltd., Quzhou City, China).

Once the samples had cooled, they were subjected to a rolling process (6CR-40, Zhejiang Lvfeng Machinery Co., Ltd., Quzhou City, China), for a duration of 25 minutes under a sequence of varying pressure conditions: 10 minutes of light pressure, followed by 10 minutes of heavy pressure, and concluding with five minutes of light pressure.

After the rolling process, the leaves were meticulously poured into the Double Pot Quhao Dry Frying Machine (6CCGQ-50, Zhejiang Shangyang Machinery Co., Ltd., Quzhou City, Zhejiang, China). The leaves underwent a meticulous drying process, initially at a temperature of 170°C for 40 minutes, and then continued at a slightly lower temperature for an additional 20 minutes. The process was meticulously monitored until the water content of the tea leaves was reduced to 30% (HM-105L, Shanghai HeGong Scientific Instruments Co. Ltd., Shanghai, China), at which point the stir-frying was concluded. After the leaves had been removed from the Double Pot Quhao Dry Frying Machine, they were allowed to cool for a period of 15 minutes. Subsequently, they were subjected to a dual drying regimen using a chain plate dryer, with each cycle consisting of five minutes at a temperature of 100°C. (6CH-3, Zhejiang Lvfeng Machinery Co., Ltd. Quzhou City, Zhejiang, China). When the moisture content of the tea leaves reaches 7%, the processing is completed (HM-105L, Shanghai HeGong Scientific Instruments Co. Ltd. Shanghai, China).

To stimulate the high-boiling aromatic substances, dried leaves were transferred to an aroma lifting machine (6CTH-6.0, Zhejiang Lvfeng Machinery Co., Ltd., Quzhou City, China) and heated at 110°C for 15 min. Once the aromatization process is finished, the tea can be taken out.

Chemical analysis

Free amino acid content was determined via the photometric ninhydrin chromatography method (Moore et al., 1948; Ramanjulu et al. 1997). Total flavonoid content was determined using the aluminum chloride colorimetric method (Iqbal et al., 2006; Kim et al., 2002; Zhishen et al., 1999). The total phenol content was determined using the Folin–Ciocalteu reagent-based spectrophotometric assay (Folin et al., 1927; Iqbal et al., 2006; Singleton et al., 1965). Total polysaccharide content was determined using the anthrone-sulfuric acid colorimetric method (Dubois et al., 1956; Skoog et al.,1992). The alkaloids and DNJ content was measured by HPLC–MS/MS (Zhang et al., 2014; Zhang et al., 2018). The GABA content was determined using paper chromatograph (Shiwang et al., 2011).

Sensory evaluation

In adherence to the rigorous sensory evaluation protocol established by Horie et al. (1992), a panel of seven dedicated individuals was selected and thoroughly trained. The age composition of this panel is as diverse as it is strategic, ranging from 28 to 53 years old, with the specific ages being 28, 29, 43, 45, 48, 52 and 53. Begin by measuring three g of tea into a Tea Tasting Cup. Pour 150 ml of boil-

Table 2. Criteria for Sensory Scoring of Mulberry Teas. Criteria for Sensory Scoring of Mulberry Teas.

	Grade	Evaluation Criteria Score	Coefficient
Shape	90-99 points	light green or dark green color, the particles are tight, the oil is uniform, and the clarity is better	25%
	80-89 points	more tender, dark green or yellow-green color, the particles are tight, still oil smooth uniform, and good clarity	
	70-79 points	low tenderness, color green or dark, more uniform, and clarity is still good.	
Liquid Color	90-99 points	yellow-green and bright, clear	10%
	80-89 points	dark yellow-green, and still bright	
	70-79 points	dark brown, under light or cloudy	
Aroma	90-99 points	high aroma and intense	25%
	80-89 points	obvious fragrance and no grassy smell	
	70-79 points	have fragrance and slightly grassy or dull	
Taste	90-99 points	mellow and fresh	30%
	80-89 points	still mellow and refreshing	
	70-79 points	flat and green	
Leaf Base	90-99 points	tender green, uniform, and bright	10%
	80-89 points	still green, bright, and uniform along	
	70-79 points	yellow-green and uneven	

ing water over the tea and let it steep for four minutes. After the steeping period, gently filter the tea liquid into another cup, leaving the leaf base behind in the original cup. The panel reviewed the appearance, color, aroma, taste, and leaf base of the mulberry leaf tea and assigned points for each criterion (Table 2). These coefficients were 25% for appearance, 10% liquid color, 25% aroma, 30% taste, and 10% leaf base. The maximum and minimum scores were excluded, and the average of the total scores from the remaining five reviewers was used as the sensory score.

Result calculation: The score of a single factor was multiplied by the score coefficient of the factor and the value of each product was added. The sum represents the total score for the tea sample reviews. The formula used is as follows:

$$Y = A\times a + B\times b + ... + E\times e$$

where Y is the total score of tea review A, B……E are evaluation scores of each quality factor and a, b,……e are scoring coefficients for each quality factor.

Data processing methods

Excel 2007 software was used for data sorting, formula calculation. All collected data were analyzed using a five by seven factorial arrangement of treatments with the GLM procedure in SAS software (version 9.4). The main effects of harvest date and variety, as well as their interaction, were assessed. Tukey's post-hoc test was utilized to separate means among treatments. Variability in the data was expressed as the standard error of means (SEM). Results were considered significant at $p < 0.05$. SPSS 26 (IBM Corp., Aramonk, NY) was used for principal component analysis.

Results

Analysis of physicochemical indices of different varieties and harvesting dates of mulberry green tea

Table 3 presents the measurement results of the average values of the main evaluation indices of mulberry leaf green tea prepared using different varieties and at different times. With variety and harvest date as independent variables and active ingredient content and sensory evaluation as dependent variables, a five by seven two-way ANOVA analysis of variance was performed to determine whether the two variables had a main effect on the dependent variable and whether there was an interaction effect between the two factors.

The mulberry variety and harvest date both had a significant influence on the content of the main chemical components of mulberry green tea, including flavonoids, free amino acids, total phenols, total polysaccharides, alkaloids, GABA, and DNJ, as well as on sensory evaluations ($p < 0.001$) (Table 4). At the same time, the interaction effect of variety and harvest date had a significant influence on physicochemical content. This shows that the physicochemical contents of the different varieties differed significantly, as did the physicochemical contents across different harvest dates. Therefore, pairwise comparison was necessary.

A pairwise comparison of the average contents of each active substance in the five varieties is presented in Table 5. The flavonoids content of the five varieties, from highest to lowest, was Yao Sang, Danbai Sang, Hei Sang, Nong Sang Number 14, and Bai Sang. The total flavonoid content of Danbai Sang and Hei Sang is at the same level, markedly surpassing that found in Nong Sang Number 14. Notably, substantial variations were observed among the other species.

The free amino acid content of the five varieties, arranged in the descending order from the highest to lowest was Yao Sang, Bai Sang, Danbai Sang, Nong Sang Number 14 and Hei Sang. The free amino acid content in Danbai Sang and Bai Sang is strikingly similar, both significantly exceeding the levels present in Nong Sang Number 14. Furthermore, it is observed that there are considerable variations among the amino acid profiles of the other species.

The total phenol content of the five varieties, arranged in order from high to low was Yao Sang, Danbai Sang, Hei Sang, Nong Sang Number 14 and Bai Sang. There were significant differences among the species.

The total polysaccharide content of the five varieties, arranged in descending order from highest to lowest, was Yao Sang, Bai Sang, Nong Sang Number 14, Hei Sang and Danbai Sang. Significant disparities were evident across the species.

The alkaloid content of the five varieties, arranged from high to low was Yao Sang, Nong Sang Number 14, Danbai Sang, Hei Sang and Bai Sang. There were significant differences among the species.

The GABA content in Hai Sang did not significantly outperform that of Nong Sang Number 14, yet it was notably elevated when compared to the other species. However, the GABA content levels among Nong Sang Number 14 and the other species did not present any significant distinctions.

The content of DNJ in the five varieties, from highest to lowest, is as follows: Yao Sang, Bai Sang, Nong Sang Number 14, Hei Sang, and Dan Bai Sang. Except for Nong Sang Number 14 and Hei Sang, which are at the same level, there are significant differences among the other species.

The sensory scores of five tea varieties are as follows: Yao Sang, Nong Sang Number 14, Danbai Sang, Bai Sang, and Hei Sang from high to low. Pronounced variations were observed among the various species.

Table 6 illustrates a detailed pairwise comparison of the average content levels for each bioactive substance during the seven unique harvest dates. A discernible trend is the steady decrease in flavonoid content, commencing in mid-April and continuing through to early July. While the batches harvested on April 15th and April 29th showed no significant

Table 3. Physicochemical Indicators of Mulberry Green Tea of Different Varieties and Harvesting Dates.

Variety	Harvest Date	Flavonoid (mg/g)	Amino acid (mg/g)	Phenols (mg/g)	Polysaccharides (mg/g)	Alkaloids (mg/g)	GABA (µg/g)	DNJ (mg/g)	Sensory Score (points)
Yao Sang	15-Apr	9.75	5.85	17.41	14.95	9.55	0.86	0.88	92.78
	29-Apr	9.35	5.73	17.02	15.77	9.27	0.74	0.73	91.31
	13-May	9.72	5.59	18.2	15.58	10.45	0.68	1.05	90.18
	27-May	9.16	4.52	19.53	16.12	11.35	0.52	1.62	85.92
	10-Jun	8.96	4.58	20.29	17.61	10.23	0.64	1.43	82.89
	24-Jun	7.71	4.07	18.7	18.42	11.56	0.76	1.85	78.77
	8-Jul	7.52	3.74	19.31	17.32	12.19	0.54	1.92	76.32
NongSang Number 14	15-Apr	7.82	2.88	12.42	13.65	8.02	0.68	0.79	91.23
	29-Apr	8.25	4.57	13.51	14.88	7.92	0.81	0.68	90.2
	13-May	7.33	4.63	13.17	14.58	7.86	0.75	0.93	89.89
	27-May	6.54	4.37	13.54	15.17	7.95	0.84	1.45	83.83
	10-Jun	6.03	4.41	16.65	16.38	10.26	0.66	1.32	81.07
	24-Jun	5.53	4.44	15.62	16.35	10.53	0.74	1.22	76.41
	8-Jul	5.65	4.01	16.63	17.33	11.21	0.62	1.41	74.92
Danbai Sang	15-Apr	7.66	4.85	15.63	13.59	7.66	0.79	0.64	90.03
	29-Apr	7.72	4.73	15.67	14.57	7.72	0.74	0.75	88.51
	13-May	7.34	4.23	15.35	14.9	7.98	0.75	0.66	87.27
	27-May	7.11	3.96	16.62	15.38	8.46	0.65	0.89	84.06
	10-Jun	6.76	3.97	16.43	16.02	8.35	0.72	1.24	81.05
	24-Jun	6.82	4.02	17.75	16.27	10.51	0.54	1.62	76.67
	8-Jul	6.58	3.95	17.56	15.84	10.14	0.61	1.53	74.32
Bai Sang	15-Apr	6.64	4.24	9.25	13.75	7.85	0.83	0.69	89.11
	29-Apr	6.77	4.32	9.02	15.62	7.66	0.81	0.88	87.73
	13-May	6.42	4.26	8.61	14.73	7.8	0.72	1.13	86.14
	27-May	5.83	4.31	8.93	16.69	8.19	0.74	1.63	82.53
	10-Jun	5.75	4.16	10.41	17.83	8.03	0.59	1.52	80.2
	24-Jun	6.06	4.24	10.54	17.89	8.56	0.51	1.45	76.77
	8-Jul	6.18	4.37	10.59	15.94	9.56	0.67	1.61	73.81
Hei Sang	15-Apr	7.88	3.78	14.75	12.71	7.59	0.89	0.52	88.17
	29-Apr	7.7	3.66	15.87	13.87	7.56	0.71	0.71	86.66
	13-May	7.15	3.58	15.37	15.8	8.16	0.83	1.12	85.77
	27-May	6.76	3.51	16.12	14.92	8.25	0.74	1.05	81.71
	10-Jun	6.85	3.44	15.76	15.79	8.62	0.82	1.25	79.22
	24-Jun	6.87	3.2	15.92	16.86	9.37	0.64	1.53	74.97
	8-Jul	6.63	3.33	14.28	17.03	9.32	0.69	1.46	72.86

Table 4. Variance Analysis of Two-factor Experiment Results.

Variety	Harvest Date	Flavonoid	Amino acid	Phenols	Polysaccharides	Alkaloids	GABA	DNJ	Sensory Score
P-value	Variety	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
	Harvest date	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
	Interaction	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

variance in their flavonoid levels, a stark contrast was observed when comparing the content across the remaining harvest dates.

The free amino acid content initially increased and then decreased from mid-April to early July. No significant differences were observed between the batches harvested on May 27th and June 10th. However, there were significant differences among the other harvest dates.

The total phenol content in mulberry leaf green tea experienced a gradual increase from April to July. No significant differences were identified between the batches harvested on June 24th and July 8th, as well as between those on April 29th and May 13th. In contrast, pronounced differences were noted among the other harvest dates.

The total polysaccharide content of the five varieties of mulberry leaf green tea saw a steady rise from April to June, followed by a slight decrease in early July. No significant differences were detected between the batches harvested on June 10th and July 8th. However, there were substantial differences observed among the other harvest dates.

The majority of mulberry leaf green tea varieties exhibited a decline in alkaloid levels from April 15th to April 29th, followed by a steady rise from May through to July. Notably, significant variations were observed across all harvest dates.

The GABA content across the five varieties of mulberry leaf green tea showed a consistent upward trend from April to July. Notably, the GABA levels in the batches harvested on April 29th and May 13th, as well as those on May 27th and June 10th, and June 24th and July 8th, did not differ significantly from each other. However, a discernible divergence was observed in the GABA content when comparing the other harvest dates.

The DNJ content in the five varieties of mulberry leaf green tea demonstrated a steady upward trajectory from April to July. There were no significant differences between the batches harvested on May 27th and June 10th. Nonetheless, significant variations were

Table 5. Pairwise Comparisons of Different Mulberry Varieties.

Variety	Harvest Date	Flavonoid	Amino acid	Phenols	Polysaccharides	Alkaloids	GABA	DNJ	Sensory Score
P-value	Yao Sang	8.88a	4.87a	18.63a	16.54a	10.66a	0.68b	1.36a	85.45a
	Nong Sang Number 14	6.74c	4.19c	14.51d	15.48c	9.11b	0.73ab	1.12c	83.94b
	Danbai Sang	7.14b	4.24b	16.43b	15.23e	8.69c	0.68b	1.05d	83.13c
	Bai Sang	6.24d	4.27b	9.62e	16.06b	8.24e	0.70b	1.27b	82.33d
	Hei Sang	7.12b	3.50d	15.44c	15.28d	8.41d	0.76a	1.09c	81.34e

Table 6. Pairwise Comparisons of Harvest Date.

Variety	Harvest Date	Flavonoid	Amino acid	Phenols	Polysaccharides	Alkaloids	GABA	DNJ	Sensory Score
P-value	15-Apr	7.95a	4.32c	13.89e	13.73f	8.13f	0.81a	0.70f	90.26a
	29-Apr	7.96a	4.60a	14.22d	14.94e	8.03g	0.76b	0.75e	88.88b
	13-May	7.59b	4.46b	14.13d	15.12d	8.45e	0.75b	0.98d	87.85c
	27-May	7.08c	4.14d	14.95c	15.66c	8.84d	0.70c	1.33c	83.61d
	10-Jun	6.87d	4.11d	15.91a	16.73b	9.10c	0.69c	1.35c	80.89e
	24-Jun	6.60e	3.99e	15.71b	17.16a	10.11b	0.63d	1.54b	76.72f
	8-Jul	6.51f	3.88f	15.67b	16.69b	10.48a	0.63d	1.59a	74.45g

Abbreviation: $^{a-m}$ Different superscripts within a column indicate a significant difference ($p < 0.05$).

detected in the levels of DNJ when comparing the DNJ content across the remaining harvesting periods.

In April, the sensory score of the leaf green tea was the highest. The processed mulberry leaf green tea had a bright green color, round and plump grains, and a fragrant smell. Its liquid color, leaf base, and taste were better than those in other months, followed by May mulberry leaf green tea. With a delay in the harvest date, the sensory quality of the prepared mulberry leaf green tea worsened. Marked variations were identified when comparing the different harvest dates.

Principal component analysis (PCA) of comprehensive quality of mulberry green tea of different mulberry varieties and harvest dates

Mulberry green tea contains high levels of nutrients and active substances. PCA is used to condense the information from multiple measurements of nutrients and active substances into a smaller number of significant components that can be used to assess the overall quality of the mulberry green tea. The measured results were subjected to principal component analysis. As shown in Table 7 and Table 8, two principal components were extracted using PCA. From the dataset of nutrient and active substance levels in mulberry green tea, two principal components (PC1 and PC2) are extracted. These components are linear combinations of the original variables (X1 to X7) that capture the maximum variance in the data. And the cumulative contribution rate of 91.189% indicates that these two components explain most of the variability in the dataset. The cumulative contribution rate of PC1 and PC2 was 91.189%. Therefore, PC1 and PC2 represented the vast

Table 7. Analysis of principal component variance of the quality indices of mulberry green tea at different harvest dates.

Principal component	Eigen value	Variance contribution (%)	Cumulative variance proportion (%)
1	6.289	78.618	78.618
2	1.006	12.571	91.189

majority of the information.

Each feature vector was calculated based on the contribution rate and eigenvalue of each index. Using the first two principal components, we established a comprehensive evaluation model for mulberry leaf quality and expressed it as follows:

$$F_1 = -0.141X_1 - 0.158X_2 + 0.124X_3 + 0.136X_4 + 0.145X_5 - 0.103X_6 - 0.156X_7$$

$$F_2 = 0.397X_1 + 0.032X_2 + 0.437X_3 - 0.345X_4 + 0.072X_5 - 0.700X_6 - 0.009X_7$$

$$F = 0.786F_1 + 0.126F_2$$

The index values for mulberry leaf green tea at different harvest dates were substituted into this function to obtain a comprehensive quality score of F for mulberry leaf green tea at different harvest dates.

The higher the F value, the greater the comprehensive quality of mulberry leaf green tea. As shown in Table 9, the study demonstrated that the varieties Yao Sang and Nongsang 14 achieved the highest scores on May 27th, Danbai Sang on June 24th, Bai Sang on July 8th, and Hei Sang on April 29th. Consequently, we have determined the optimal harvest dates for each variety of mulberry leaf green tea.

Discussion

There is good potential for the development and utilization of mulberry leaves as a valuable medicinal and edible plant resource. This study provides new information to guide the selection of mulberry varieties and their harvest dates to produce high-quality leaf tea. In this study, mulberry leaves were harvested from five mulberry varieties on seven dates from April to July in Turpan, and tea was prepared. The levels of the main active substances were determined and sensory evaluations were performed.

Two-way ANOVA indicated an interaction between variety and harvest date ($p < 0.001$), suggesting the need for a separate analysis of the effects of variety and harvest date. These findings revealed that in most cases, different harvest dates also revealed significant differences between each harvest date in most cases.

During the various harvest dates, the annual changes in each index were monitored, revealing that the mean values of total flavonoids exhibited an overall downward trend from mid-April to early July. Initially, the decline was gradual, but it progressively accelerated until late June to early July, at which point the rate of decrease began to subside. The research by Lei Hu et al. (2021) observed the fluctuations in flavonoid levels in mulberry leaves throughout their growth period, specifically from three to six months. The results showed that mulberry leaves grown for 6 months had higher levels of flavonoids. Our study's findings are consistent with his research results. Notably, total flavonoids, particularly flavonols such as quercetin, are pivotal for tea's taste profile, contributing to its astringency and bitterness. These compounds are not only key to the sensory experience of tea but also renowned for their

Table 8. Characteristic vectors of the main components selected for mulberry green tea indices at different harvest date.

Principal component	X_1	X_2	X_3	X_4	X_5	X_6	X_7
1	-1.25545	-1.07294	-0.65883	0.37245	0.39206	0.91647	1.30623
2	-0.5192	-0.43445	0.59785	1.51061	0.45706	-1.6409	0.02904

Table 9. Main composition scores of mulberry green tea at different harvest date.

Mulberry varieties	Sampling date	Score F value	Ranking
Yao Sang	15-Apr	-0.16410	7
	29-Apr	-0.13907	6
	13-May	0.023545	4
	27-May	0.219611	1
	10-Jun	0.088405	3
	24-Jun	-0.13472	5
	8-Jul	0.106329	2
Nongsang 14	15-Apr	-0.42260	7
	29-Apr	0.059418	3
	13-May	0.041692	4
	27-May	0.201405	1
	10-Jun	0.032276	5
	24-Jun	0.129275	2
	8-Jul	-0.04147	6
Danbai Sang	15-Apr	-0.08791	7
	29-Apr	-0.06585	6
	13-May	-0.04595	5
	27-May	0.006986	4
	10-Jun	0.023333	3
	24-Jun	0.088734	1
	8-Jul	0.080658	2
Bai Sang	15-Apr	-0.10152	6
	29-Apr	-0.00778	3
	13-May	-0.09610	5
	27-May	0.032956	2
	10-Jun	-0.26395	7
	24-Jun	-0.02287	4
	8-Jul	0.459168	1
Hei Sang	15-Apr	-0.03504	6
	29-Apr	0.20766	1
	13-May	-0.01509	5
	27-May	0.13803	2
	10-Jun	-0.0065	4
	24-Jun	0.018371	3
	8-Jul	-0.30744	7

antioxidant and anti-inflammatory properties, which significantly promote heart health, support cancer prevention, and strengthen the immune system. Recognizing the health benefits linked to an elevated flavonoid content, it becomes clear that screening for these compounds in tea could be highly beneficial.

The amino acid content shows a trend of an initial significant increase followed by a gradual decrease over the harvest dates. The highest amino acid content is observed in late April, and the lowest is observed in early July. The decrease is not linear, with some fluctuations, but the overall trend is downward. Amino acids, as the fundamental building blocks of proteins, play a significant role in tea by contributing to its umami taste, a savory flavor that enhances the overall drinking experience. Essential amino acids, in particular, are vital for muscle repair and growth, as well as for maintaining overall health. Therefore, profiling the amino acid content in tea can be instrumental in selecting varieties that not only offer a pleasing taste but also provide a rich nutritional profile, ensuring that consumers enjoy a beverage that is both delicious and healthful.

The phenol content shows an overall increasing trend with some fluctuations. The lowest phenol content is observed in mid-April, and the highest phenol content is observed in early June. After reaching the peak in early June, there is a slight downward trend in the following weeks. This indicates that the phenol content generally increases up to a certain point and then starts to decline. The trend contrasts with the data trend reported by Zou Y, et al. (2012), who observed that the polyphenol content in mulberry leaves diminishes as the harvest date advances, with higher polyphenol levels in spring compared to autumn. Phenols, which contribute to the astringency and bitterness of tea, are compounds that not only shape the taste profile but also possess antioxidant properties. They are associated with a range of health benefits, including anti-cancer and anti-inflammatory effects, highlighting their importance in promoting well-being. However, it's important to note that while phenols offer these health advantages, extremely high levels might not necessarily align with higher sensory scores. This suggests that a balanced approach is needed to ensure that the tea delivers both the desired sensory experience and the full spectrum of health benefits that phenols can provide.

Polysaccharides have a large molecular weight in food, and this structure endows them with good hydrophilicity and certain emulsifying capabilities. They can increase the viscosity and stability of tea, while also imparting a fresh taste. Plant polysaccharides can regulate the body's immune function. They also have anti-tumor effects, blood glucose-lowering, antioxidant, and anti-fatigue properties. The polysaccharide content shows a clear increasing trend with some minor fluctuations. The lowest polysaccharide content is observed in mid-April, and the highest polysaccharide content is observed in late June. After reaching the peak in late June, there is a slight downward trend in early July. Therefore, this can be used to optimize the variety and harvest date to enhance the health attributes and market value of tea.

The primary alkaloid in tea is caffeine, which, together with tea polyphenols and amino acids, constitutes one of the three pivotal components that shape the flavor profile of tea. Caffeine imparts a bitter flavor and contributes to the tea's smooth, refreshing character by interacting with polyphenols and their oxidative byproducts to form complexes. Caffeine is known for its stimulating effects, as well as its diuretic and cardiotonic properties. The alkaloid content exhibits an overall upward trend with occasional minor variations. The minimum alkaloid levels are detected in late April, while the maximum levels are found in early July. After a slight dip in late April, the alkaloid content steadily rises, peaking in early July. Selecting the right variety and harvest date can optimize caffeine levels, which in turn can influence both the taste and the health benefits of tea.

GABA is a non-protein amino acid present in tea, known for its ability to mitigate bitterness, particularly that of caffeine. GABA is recognized for its multifaceted health benefits, including anti-depressant effects, blood pressure reduction, memory enhancement, and anti-cancer properties. The GABA content in tea exhibits a distinct downward trend, with occasional minor fluctuations. The peak GABA levels are noted in mid-

April, while the lowest concentrations are detected in late June and early July. Following a marginal decline in mid-April, GABA content experiences a consistent decrease, hitting its nadir in late June and early July. These variations are significant for the development of GABA-enriched tea products tailored to specific health attributes.

DNJ is a polyhydroxy alkaloid that is believed to have blood glucose-lowering effects and plays a role in the prevention and treatment of diabetes. The content of DNJ shows an overall increasing trend with some minor fluctuations. The lowest DNJ content is observed in mid-April, and the highest DNJ content is observed in early July. After a slight increase in mid-April, there is a steady increase in DNJ content, reaching its peak in early July. The study conducted by Hu XQ et al. (2013) underscores the significant influence of mulberry variety on DNJ content, a finding consistent with their research conclusions. Selecting appropriate varieties and harvest dates can optimize DNJ content, thereby enhancing the health attributes of tea.

The biochemical components and volatile aroma compounds of mulberry leaf tea jointly influence the sensory score. The sensory score shows a clear decreasing trend with some minor fluctuations. The highest sensory score is observed in mid-April, and the lowest sensory score is observed in early July. After a slight decrease in mid-April, there is a steady decrease in sensory scores, reaching its lowest point in early July. This is partly because early spring tea typically contains higher levels of amino acids and lower levels of polyphenols, which may result in a fresher taste and less astringency. Therefore, the optimal harvest date and variety selection can be more precisely determined, thereby enhancing the overall quality of the tea and consumer acceptance.

In summary, the dynamic changes of the five antioxidant active substances in date palm leaves at different harvest dates are not consistent. Considering the content of flavonoids, phenolic acids, polysaccharides and GABA, the optimal harvest date for date mulberry leaves is on June 24th. Whereas, if considering the content of Alkaloids and DNJ, the best date for harvest is on July 8th. For the best sensory experience, the harvest date is April 15th, as indicated by the highest sensory score. For the highest amino acid content, the harvest date is April 29th, with the highest value. Exploring the reasons, the formation, transformation, and accumulation of plant active components are influenced by environmental conditions. The environment serves as the material and energy foundation for the growth and development of medicinal plants, as well as the formation of product quality.

Conclusion

In this study, the contents of the main active substances and sensory scores of different varieties of mulberry leaf green tea harvested at different dates in Turpan were quantitatively analyzed. The results of the two-way ANOVA revealed that both the main effects and interactions of harvest date and variety significantly affected on content of active substances and the sensory scores of mulberry green tea. The principal component analysis was used to evaluate each index comprehensively. According to the comprehensive score, the best harvest date for Yao Sang, Nongsang 14, Danbai Sang, Bai Sang, and Hei Sang in this area were approximately 27-May, 27-May, 24-Jun, 8-Jul, and 29-Apr, respectively.

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Ethics Statement

This study on the sensory evaluation of mulberry tea was conducted in strict accordance with ethical guidelines. Sensory testing, as a scientific experimental method, is governed in China by a dedicated organization known as the Standardization Administration of China, which is responsible for establishing and amending relevant national standards. This paper complies with the "GB/T 23776-2018 Method for Sensory Evaluation of Tea" [S]. Beijing: Standardization Press of China, 2018. This ensures the ethical and legal conduct of the sensory testing. Additionally, the study was reviewed and granted approval by the Institutional Review Board of the Turpan Institute of Agricultural Sciences under the authorization of Tudi Nongzi [2024] No. 7, and written informed consent was obtained from each subject prior to their participation in the study.

Participants

The sensory assessment of mulberry tea was conducted with meticulous precision, employing a panel of seven carefully curated individuals. This diverse group was chosen to encapsulate a spectrum of perspectives, ensuring a representative sample. Each participant was subjected to an intensive training regimen, designed to calibrate their sensory evaluation techniques and guarantee uniformity in the evaluation process.

Guided by the esteemed sensory evaluation methodology established by Horie et al. (1992), the panelists meticulously assessed the mulberry tea across a range of criteria: appearance, aroma, taste, and leaf base. The training curriculum was not only focused on honing their sensory discernment but also underscored the ethical imperative of maintaining an impartial stance and respecting the confidentiality of the study's outcomes.

Through strict adherence to these ethical benchmarks and by fostering an environment of diversity and expertise among our panelists, this study is poised to deliver a robust and exhaustive sensory evaluation of mulberry tea. This endeavor aspires to yield insightful contributions to the domain of sensory science and to propel advancements in tea product development.

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