Antioxidant Capacity of *Bauhinia championii* Honey from Guangxi

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Abstract [**Objectives**] To investigate the impact of storage duration on the DPPH free radical scavenging activity in *Bauhinia championii* honey and to assess its antioxidant activity. [**Methods**] The free radical scavenging activity of 38 batches of *B. championii* honey (fresh honey stage, aged honey stage I, aged honey stage II), was evaluated using the microplate DPPH assay. Honey sample solutions at varying concentrations (10, 25, 40, 55, and 70 g/L) were prepared, and 100 μL of each was combined with 100 μL of 0.05 g/L DPPH ethanol solution. The reaction mixtures were incubated at room temperature in the dark for 30 min. Subsequently, absorbance was measured at 517 nm, and both the scavenging rate and the half maximal inhibitory concentration (IC_{50}) were calculated. [**Results**] The IC_{50} values for the DPPH free radical scavenging activity in 38 batches of *B. championii* honey at the fresh honey stage ranged from 8.96 to 58.65 mg/mL, with a mean value of 30.48 mg/mL. During the aged honey stage I, the IC_{50} values ranged from 8.32 to 55.08 mg/mL, averaging 28.48 mg/mL. In the aged honey stage II, the IC_{50} values ranged from 16.71 to 68.01 mg/mL, with a mean of 41.91 mg/mL. The methodological evaluation revealed that the relative standard deviations (*RSD*) for precision, repeatability, and stability were 0.02%, 2.21%, and 2.23%, respectively. [**Conclusions**] The DPPH free radical scavenging activity of 38 batches of *B. championii* honey remained robust throughout a 3-year storage period. However, it is advisable to limit the storage duration to within 2 years during production and storage to better preserve its antioxidant properties. The IC_{50} value for DPPH free radical scavenging activity serves as a critical indicator for assessing the storage stability and freshness of *B. championii* honey.

Key words Antioxidant capacity, *Bauhinia championii* honey, DPPH, Radical scavenging activity

1 Introduction

Honey is a natural sweet substance produced by bees through the combination of nectar, plant secretions, or honeydew with their own secretions, followed by a maturation process. It possesses high nutritional value[1] and is abundant in bioactive compounds, including polyphenols and flavonoids[2]. Research has demonstrated a significant positive correlation between the DPPH free radical scavenging activity of honey and its polyphenol and flavonoid content[3]. The DPPH free radical scavenging activity serves as a crucial indicator for assessing the antioxidant activity of honey, directly reflecting its ability to scavenge free radicals[3]. This antioxidant activity effectively mitigates cellular damage induced by oxidative stress and plays a vital role in the prevention of chronic diseases, such as cardiovascular disorders[4].

Bauhinia championii, a woody vine belonging to the genus *Bauhinia* within the Fabaceae family, is predominantly distributed in South China, with abundant resources found in regions such as Yangshuo in Guilin, Guangxi[5]. *B. championii* honey is recognized as a national geographical indication agricultural product of Guangxi[6]. This honey exhibits a deep amber color and a distinctive herbal aroma, characterized by a sweet taste with subtle bitterness, providing a refreshing sensation to the mouth and throat, accompanied by a prolonged aftertaste. It is a natural, pollution-free product reputed for its heat-clearing and detoxifying properties, demonstrating therapeutic efficacy in treating pharyngitis, lung heat cough, and constipation. As a rare food therapy product, it is regarded as "a unique delicacy in southern China and a premium honey variety"[7-9]. Currently, there is a lack of systematic research regarding the DPPH free radical scavenging activity of *B. championii* honey. Therefore, this study aims to investigate the antioxidant capacity of *B. championii* honey sourced from Guangxi.

2 Materials

2.1 Experimental samples The *B. championii* honey samples utilized in this study were collected from various locations in Guangxi, including Guilin, Hezhou, Chongzuo, Liuzhou, Laibin, *etc.*, comprising a total of 38 batches. The collection period spanned from October to December 2021 (Table 1). Following collection, the samples were stored at a temperature of 5 - 7 ℃ for subsequent analysis. The 38 batches were categorized based on storage duration as follows: samples stored for less than 1 year were classified as fresh honey stage; those stored for more than 1 year but less than 2 years were designated as aged honey stage I; and samples stored for more than 2 years were classified as aged honey stage II.

2.2 Instrument The instruments employed in this study comprised the EX225DZH electronic analytical balance [Ohaus Instruments (Changzhou) Co., Ltd.], the CARY60 ultraviolet-visible spectrophotometer [Agilent Technologies (China) Co., Ltd.], and the Infinite F50 microplate reader [Diken (Shanghai) Trading Co., Ltd.].

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Table 1 Sample information of *Bauhinia championii* honey

Sample No.	Collection site	Origin of bee species	Collection month
FM-001	GY-Fuli Town	M	October
FM-002	GY-Yangdi Township	M	October
FM-003	GY-Puyi Township	M	October
FM-004	GY-Putao Town	M	October
FM-005	GY-Baishao Town	M	October
FM-006	GY-Puyi Township	M	November
FM-007	GY-Yangshuo Town	M	November
FM-008	GY-Jinbao Township	M	November
FM-009	GY-Baisha Town	M	November
FM-010	GY-Putao Town	M	November
FM-011	GY-Yangdi Township	M	November
FM-012	GY-Xingping Town	M	November
FM-013	GY-Fuli Town	M	November
FM-014	Guilin City, Pingle County	M	November
FM-015	Guilin City, Gongcheng County	M	November
FM-016	Guilin City, Quanzhou County	M	November
FM-017	Guilin City, Lingchuan County	M	November
FM-018	Guilin City, Xing'an County	M	November
FM-019	Guilin City, Guanyang County	M	November
FM-020	Guilin City, Lingui District	M	November
FM-021	H-Fuchuan Yao Autonomous County	M	November
FM-022	Chongzuo City, Fusui County	M	November
FM-023	Liuzhou City Liujiang County	M	November
FM-024	LB-Xingbin District	M	November
FM-025	GY-Puyi Township	M	December
FM-026	GY-Yangshuo Town	M	December
FM-027	GY-Jinbao Township	M	December
FM-028	GY-Baisha Town	M	December
FM-029	GY-Putao Town	M	December
FM-030	GY-Yangdi Township	M	December
FM-031	GY-Xingping Town	M	December
FM-032	GY-Fuli Town	M	December
FM-033	GY-Yangdi Township	C	November
FM-034	GY-Xingping Town	C	November
FM-035	GY-Xingping Town	C	December
FM-036	Guilin City, Xing'an County	C	November
FM-037	Guilin City, Gongcheng County	C	November
FM-038	Guilin City, Lingui District	C	November

NOTE The data were collected exclusively in the year 2021; GY. Yangshuo County, Guilin City; H. Hezhou City; LB. Laibin City; M. *Apis mellifera*; C. *Apis cerana*.

2.3 Main reagents The primary reagent utilized was DPPH free radicals (batch No.: C13168722, purity ≥95.0%, Sinopharm Group Chemical Reagent Co., Ltd.). Anhydrous ethanol, ethanol, water, and other reagents employed were all of analytical grade.

3 Methods and results

3.1 Experimental methods Based on the method proposed by Yang Jialin *et al.* [10], with certain modifications, 100 μL of honey sample aqueous solutions at varying mass concentrations (10, 25, 40, 55, and 70 g/L) were accurately pipetted and combined with 100 μL of freshly prepared 0.05 g/L DPPH ethanol solution. The mixtures were then transferred to a 96-well plate and incubated in the dark at room temperature (25 ℃) for 30 min. Subsequently, the absorbance was measured at 517 nm and recorded as A_1. The absorbance of 100 μL of DPPH solution mixed with 100 μL of ethanol was used as the sample blank control and recorded as A_2. The absorbance of 100 μL of ethanol mixed with 100 μL of honey water solution served as the solvent blank control and recorded as A_0. Each experimental group was measured in triplicate. The half maximal inhibitory concentration (IC_{50}) was calculated based on the sample mass concentration and scavenging rate. The DPPH scavenging rate was determined using the following formula.

$$\text{DPPH scavenging rate}\ (\%) = [1 - (A_1 - A_0)/A_2] \times 100$$

3.2 Preparation of standard solutions 100 mg sample of the DPPH standard was accurately weighed and transferred into a 10 mL volumetric flask. Anhydrous ethanol was added to dissolve the sample, and the volume was adjusted to the calibration mark. After thorough mixing, a DPPH stock solution (A1) with a concentration of 10 mg/mL was prepared. Subsequently, 50 μL of the A1 solution was precisely pipetted into a 10 mL volumetric flask, and anhydrous ethanol was added to reach the calibration mark. Following thorough mixing, a DPPH working solution (A2) with a concentration of 0.05 mg/mL was obtained.

3.3 Preparation of sample solutions A 3.5 g sample of *B. championii* honey was accurately weighed and transferred into a 50 mL volumetric flask. Ultrapure water was added to dissolve the sample, and the volume was adjusted to the calibration mark. After thorough mixing, a stock solution (B1) with a mass concentration of 70 g/L was obtained. Subsequently, a series of diluted sample solutions were prepared using the stepwise dilution method. 11 mL of the B1 solution was precisely pipetted and combined with 3 mL of ultrapure water, mixed thoroughly to yield a 55 g/L sample solution (B2). Then, 8 mL of B2 solution was accurately pipetted and diluted with 3 mL of ultrapure water, resulting in a 40 g/L sample solution (B3). Next, 5 mL of B3 solution was measured and diluted with 3 mL of ultrapure water to obtain a 25 g/L sample solution (B4). Finally, 2 mL of B4 solution was accurately drawn and diluted with 3 mL of ultrapure water, producing a 10 g/L sample solution (B5).

3.4 Operation process The sample addition was performed in accordance with the experimental procedure described in Section **3.1**. The layout for sample addition in the 96-well plate is presented in Table 2. Wells B1 to B5 contained sample solutions at varying mass concentrations, each with a volume of 100 μL. P represented the DPPH working solution at a concentration of 0.05 mg/mL, with a volume of 100 μL; k denoted anhydrous ethanol, also with a volume of 100 μL. The blank control corresponded to P + k (100 μL of DPPH working solution combined with 100 μL of anhydrous ethanol).

Table 2 Sample loading layout for the determination of DPPH free radical scavenging activity

	1	2	3	4	5	6	7	8	9	10	11	12
A	B1 + P	B2 + P	B3 + P	B4 + P	B5 + P	B1 + k	B2 + k	B3 + k	B4 + k	B5 + k	/	/
B	B1 + P	B2 + P	B3 + P	B4 + P	B5 + P	B1 + k	B2 + k	B3 + k	B4 + k	B5 + k	/	/
C	B1 + P	B2 + P	B3 + P	B4 + P	B5 + P	B1 + k	B2 + k	B3 + k	B4 + k	B5 + k	/	/
D	B1 + P	B2 + P	B3 + P	B4 + P	B5 + P	B1 + k	B2 + k	B3 + k	B4 + k	B5 + k	/	/
E	B1 + P	B2 + P	B3 + P	B4 + P	B5 + P	B1 + k	B2 + k	B3 + k	B4 + k	B5 + k	/	/
F	B1 + P	B2 + P	B3 + P	B4 + P	B5 + P	B1 + k	B2 + k	B3 + k	B4 + k	B5 + k	/	/
G	B1 + P	B2 + P	B3 + P	B4 + P	B5 + P	B1 + k	B2 + k	B3 + k	B4 + k	B5 + k	/	/
H	B1 + P	B2 + P	B3 + P	B4 + P	B5 + P	B1 + k	B2 + k	B3 + k	B4 + k	B5 + k	Blank	Blank

NOTE B1-B5 represent sample solutions with serial concentrations prepared according to the procedure in Section **3.3**, each with a volume of 100 μL; P denotes the DPPH working solution at a concentration of 0.05 mg/mL (100 μL); k refers to anhydrous ethanol (100 μL); the blank control corresponds to P + k.

3.5 Methodological investigation

3.5.1 Precision test. Six honey samples from the same batch (FM-005) were prepared and processed following the procedure in Section **3.1**. Subsequently, 100 μL of 0.05 g/L DPPH ethanol solution was added to each sample. After incubation in the dark at room temperature for 30 min, the absorbance was measured six times consecutively at a wavelength of 517 nm. The results are presented in Table 3. The relative standard deviation (*RSD*) was 0.02%, demonstrating that the instrument exhibits high precision.

Table 3 Precision test results of DPPH free radical scavenging activity (n = 6)

No.	Abs	Mean Abs	*RSD*//%
1	0.523 0	0.523 1	0.02
2	0.523 0		
3	0.523 1		
4	0.523 0		
5	0.523 3		
6	0.523 1		

3.5.2 Repeatability test. Six samples of honey from the same batch (FM-005) were accurately weighed. The DPPH free radical scavenging activity was independently assessed following the procedure in Section **3.1**, and both the IC_{50} values and *RSD* were calculated. The results are presented in Table 4. The *RSD* of the IC_{50} value was 2.21%, demonstrating that the method exhibits good repeatability.

Table 4 Repeatability test results of DPPH free radical scavenging activity (n = 6)

No.	Abs	IC_{50}//mg/mL	Mean IC_{50}//mg/mL	*RSD*//%
1	0.533 0	46.50	45.41	2.21
2	0.523 0	44.45		
3	0.523 1	45.56		
4	0.523 1	44.24		
5	0.522 7	45.10		
6	0.523 0	46.60		

3.5.3 Stability test. The FM-005 sample solution prepared in Section **3.5.2** was utilized to assess the free radical scavenging activity of DPPH, following the procedure in Section **3.1** at time intervals of 0, 1, 2, 3, 6, and 12 h. The IC_{50} values and *RSD* were calculated. The results are presented in Table 5. The *RSD* of the IC_{50} value was 2.23%, indicating that the test solution maintained good stability within 12 h.

Table 5 Stability test results of DPPH free radical scavenging activity (n = 6)

Determination time//h	IC_{50}//mg/mL	Mean IC_{50}//mg/mL	*RSD*//%
0	44.20	45.52	2.23
1	44.25		
2	46.18		
3	45.96		
6	46.02		
12	46.48		

3.6 Experimental results

The determination results of the DPPH free radical scavenging activity for *B. championii* honey at various storage durations are presented in Table 6. The IC_{50} values for the DPPH free radical scavenging rate in fresh honey stage ranged from 8.96 to 58.65 mg/mL, with a mean value of 30.48 mg/mL. In aged honey stage I, the IC_{50} values ranged from 8.32 to 55.08 mg/mL, averaging 28.48 mg/mL. In aged honey stage II, the IC_{50} values varied between 16.71 and 68.01 mg/mL, with a mean of 41.91 mg/mL.

Table 6 Determination results of DPPH free radical scavenging activity of *Bauhinia championii* honey at various storage durations (IC_{50}, mg/mL)

Sample No.	Period		
	Fresh honey stage	Aged honey stage I	Aged honey stage II
FM-001	50.12 ± 0.34	48.26 ± 0.10	60.23 ± 0.26
FM-002	46.14 ± 0.04	46.15 ± 0.13	55.96 ± 0.05
FM-003	50.58 ± 0.07	50.28 ± 0.25	58.46 ± 0.19
FM-004	58.65 ± 0.05	51.08 ± 0.08	66.68 ± 0.07
FM-005	46.46 ± 0.04	43.25 ± 0.22	56.90 ± 0.09
FM-006	18.06 ± 0.41	25.06 ± 0.18	22.29 ± 0.19
FM-007	19.18 ± 0.28	28.17 ± 0.09	19.90 ± 0.23
FM-008	26.35 ± 0.09	25.05 ± 0.18	34.73 ± 0.02
FM-009	26.45 ± 0.43	26.55 ± 0.44	30.50 ± 0.87
FM-010	28.05 ± 0.05	26.32 ± 0.17	30.56 ± 0.07
FM-011	20.03 ± 0.11	28.80 ± 1.03	20.91 ± 0.12
FM-012	15.73 ± 0.11	25.13 ± 0.11	16.71 ± 0.14
FM-013	32.49 ± 0.09	28.75 ± 0.23	35.28 ± 0.23

(To be continued)

(Continued)

Sample No.	Period		
	Fresh honey stage	Aged honey stage I	Aged honey stage II
FM-014	33.68 ±0.07	36.48 ±0.08	52.13 ±0.03
FM-015	46.05 ±0.05	40.84 ±0.21	42.79 ±0.21
FM-016	54.89 ±0.12	48.06 ±0.17	60.79 ±0.04
FM-017	38.76 ±0.16	25.16 ±0.10	40.56 ±0.05
FM-018	40.16 ±0.04	36.79 ±0.22	56.12 ±0.37
FM-019	56.78 ±0.25	55.08 ±0.08	68.01 ±0.49
FM-020	33.25 ±0.25	33.10 ±0.36	55.64 ±0.16
FM-021	42.56 ±0.05	40.86 ±0.14	65.46 ±0.04
FM-022	33.47 ±0.36	23.04 ±0.14	46.78 ±0.19
FM-023	28.29 ±0.04	19.07 ±0.13	28.62 ±0.11
FM-024	31.58 ±0.07	23.84 ±0.15	46.59 ±0.09
FM-025	29.06 ±0.05	25.79 ±0.11	35.56 ±0.09
FM-026	26.46 ±0.18	19.08 ±0.07	40.28 ±0.23
FM-027	15.63 ±0.19	15.02 ±0.21	35.73 ±0.16
FM-028	21.05 ±0.05	21.00 ±0.23	45.08 ±0.07
FM-029	30.10 ±0.22	31.45 ±0.13	57.64 ±0.04
FM-030	25.23 ±0.31	23.49 ±0.12	48.05 ±0.05
FM-031	22.46 ±0.04	19.06 ±0.17	36.42 ±0.08
FM-032	21.32 ±0.28	15.75 ±0.3	33.25 ±0.06
FM-033	15.09 ±0.02	11.04 ±0.16	35.06 ±0.41
FM-034	8.96 ±0.07	8.32 ±0.18	26.38 ±0.13
FM-035	12.14 ±0.31	9.08 ±0.08	19.07 ±0.11
FM-036	16.71 ±0.19	14.76 ±0.30	43.07 ±0.45
FM-037	19.9 ±0.10	19.90 ±0.18	35.64 ±1.09
FM-038	16.29 ±0.04	13.35 ±0.15	28.58 ±0.31

4 Conclusions

This study systematically evaluated the DPPH free radical scavenging activity of Guangxi *B. championii* honey across different storage stages and investigated the variation pattern of its antioxidant activity over time. By measuring and analyzing the IC_{50} values of 38 batches of *B. championii* honey samples at three distinct storage stages (fresh honey stage, aged honey stage I, and aged honey stage II), the following conclusions were derived.

(i) The antioxidant activity of *B. championii* honey remains stable throughout its storage period. Over the storage period of 3 years, *B. championii* honey consistently exhibited strong DPPH free radical scavenging activity, indicating that its antioxidant capacities are well preserved over time. Storage duration exerts a significant influence on antioxidant capacity. The mean IC_{50} values for fresh honey stage and aged honey stage I were 30.48 and 28.48 mg/mL, respectively, with no statistically significant difference observed between these two stages. This finding indicates that antioxidant capacity remains relatively stable within 1 year of storage. However, the mean IC_{50} value for aged honey stage II increased markedly to 41.91 mg/mL, which was significantly higher than those of the earlier stages ($P < 0.05$). This suggests that prolonged storage may lead to the degradation of certain antioxidant components, resulting in a diminished free radical scavenging activity.

(ii) The methodological validation results of this study are satisfactory. The DPPH method employed exhibited relatively low *RSD* values for precision, repeatability, and stability (0.02%, 2.21%, and 2.23%, respectively), indicating that this method is appropriate for the systematic evaluation of the antioxidant capacity of *B. championii* honey.

(iii) The IC_{50} value serves as an evaluative index for the storage stability and freshness of *B. championii* honey. Research indicates that variations in the IC_{50} value of DPPH free radical scavenging activity are closely associated with storage duration, thereby providing a potential indicator for assessing the storage stability and freshness of *B. championii* honey.

In summary, *B. championii* honey retains substantial antioxidant activity for up to 3 years of storage, but prolonged storage exceeding 2 years may result in a decline in its antioxidant capacity. Therefore, it is advisable to limit the storage duration to within 2 years during production and storage processes to better preserve its antioxidant properties. This study offers a scientific basis for the quality control, rational storage, and functional evaluation of *B. championii* honey, and establishes a foundation for future research investigating the relationship between antioxidant components and their activity.

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