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Antioxidant Activity of Some Monofloral Honeys: Different Contributions of the Raw Honey and Phenolic Extract

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Abstract

Ten Chinese monofloral honey samples from different floral sources and different areas were measured considering the different contribution of raw honey samples and phenolic extracts. Total phenolic content (TPC) of Chinese monofloral honey samples were determined by Folin-Ciocalteu method, free radical-scavenging assay by 1,1-dipheyl-2-picrylhydrazyl (DPPH)and Ferric-reducing/antioxidant power assay (FRAP) for reducing capacity. The TPC of the honey samples were varied from 9.57±0.12 to 136.17±5.46 mg GAE/100 g of honey for raw honey samples and 5.64±0.31 to 55.11±0.52 mg GAE/100 g of honey for phenolic extract samples. Comparatively higher antioxidant contents and the lower DPPH values were found in the dark colored honey. This study results shows that TPC, flavonoid contents and antioxidant activity of Chinese monofloral honey samples are closely related to their floral sources and also their color intensity.

Keywords: Honey; Phenolic Extracts; Antioxidant Activity; DPPH; FRAP.

Introduction

In recent years in the world, people are more concern about to the consumption of different honeys, since people are now more conscious of their health-promoting effects. Honey is a natural saturated substance produced by honey bees from the nectar of blossoms or from the secretion of living parts of plants, which honeybees collect transforms and combine with specific substances of their own, store and leave in the honeycomb to ripen and mature[1].

Honey mainly consists of glucose and fructose, and also considered as a traditional medicine which contains about more than 180constituents (a complex mixture of sugars, but also a minor amounts of other constituents such as minerals, proteins, vitamins, organic acids, flavonoids, phenolic acids, enzymes and other phytochemicals)[2]. Many authors has been studied about honey that functions in the treatments of burns, gastrointestinal disorders, asthma, infections, and skin ulcer different inflammatory processes as well as cataracts and other eye ailments[3-6]. Honey has a good antibacterial and antioxidants activities. Honey contains both enzymatic such as catalase, glucose oxidase, and peroxidase and non-enzymatic constituents like ascorbic acid, α-tocopherol, carotenoids, amino acids, proteins, Millard reaction products, flavonoids, and phenolic acid. The type and amount of this antioxidant depends according to the floral basis or area of the cultivation of the honey[7]. The antioxidant activity of honey mostly depends on flavonoids and phenolic acids[8]. Many authors has been reported that the antioxidant activity can influence by the botanical sources of honey, during processing, handling, and storage affect the antioxidant activity of honey only to a minor degree[6].

In previous many methods has been established to determine the antioxidant activity in honey, e.g. determination phenolics, the formation of and following scavenging as in 2,2-diphenyl-1-picrylhydrazyl (DPPH) and superoxide radical scavenging activity measurements the ferric reducing/antioxidant power FRAP assay[9,10].

Although many author studied that honey can be used as a good source of antioxidant and consist of different antioxidant compounds, nothing is reported yet about the different contribution of the entire honeys and the extracted phenolic properties. The main focus of this study, the antioxidant properties of different floral origin of Chinese monofloral raw honey samples, and phenolics extracts were measured through several chemical assays and also the physicochemical properties of different areas honeys in China.

Materials and Methods

Collection of Honey samples: Total ten Chinses monofloral samples of five different floral origins of freshly honeys were obtained from different bee hive. The samples including acacia, linden, jujube, vitex and rape honeys were collected during 2014 and 2015 harvests from different area across China (Table-1). The analysis was carried out within six months after collection. The samples were stored at room temperature until analyzed.

Table-1
The sampling regions of monofloral honeys

Categories of honey	Sample ID	Geographical origin	Characteristics
Acacia	H_1	Changping, Guandong	Light amber color, no crystals
	H_2	Provincce	
Linden	H_3	North-east of China	Extra light amber to light
	H_4		amber, white crystals
Jujube	H_5	Changzhi, Shanxi Province	Amber color, no crystals
	H_6		
Vitex	H_7	Miyun in North east Beijing	White color, no crystals
	H_8		
Rape	H_9	Sichuan Province	White color, white crystals
	H_{10}		

Standards and reagents for Analysis: All chemicals and solvents for the analysis were of analytical grade purity. Catechin, gallic acid, 1,1-dipheyl-2-picrylhydrazyl (DPPH), 2,4,6-Tripyridyl-*s*-triazine (TPTZ) were purchased from Sigma-Aldrich (St. Louis, USA). Methanol, ethanol, hexane, acetone, diethyl ether, Amberlite XAD-2, Folin and Ciocalteu's reagent, sodium carbonate, $AlCl_3$, sodium nitride, sodium hydroxide, sodium acetate, galacial acetic acid, hydrochloric acid, potassium per sulphate, ferrous sulfate, ferric cholride were obtained from Merck (Darmstadt, Germany).

Physicochemical Analysis: Moisture content Analysis: Moisture content of Chinese monofloral honey samples were performed by reractometic method by using hand Atagorefractrometer. The readings were triplicates and further corrected for a standard temperature of 20℃ by adding the correlation factor of 0.00023/℃. The corrected corresponding moisture content refractive index values were calculated using Wedmore's Table[11].

Analysis of pH Content: The pH content of the honey samples were measured by using a pH meter for a 10% (w/v) solution of total honey.

Ash content Analysis: To determine the ash content of honey samples, 4-5 g of honey samples were placed in a crucible in a muffle furnace and heated at 640℃ for 6 h. Experiments were done in triplicate and the mean value was expressed in g%[11].

Water activity: Determination of water activity of honey samples were done by using automatic water activity meter (Novasina AG, CH-8853 Lachen, and Switzerland) at 20℃

Color Intensity: ABS 635nm: Color intensity of honey sample was determined by diluting a 50% (w/v), homogenized, and centrifuged at 3200 rpm for five minutes. Absorbance was taken at 635nm using a spectrophotometer and color intensity was determined using the pfund scale[12].

Phenolic extraction of honey: The honey samples of 20g were mixed with 200 mL acidic water (pH 2 adjusted with HCl).Then the mixed solution was filtered by cotton to remove the solid particles and mixed with 30g of Amberlite XAD-2 resin (pore size 9nm and particle size 0.3-1.2 mm). Then the solution was stirred for 10 min. The solution of mixture was then poured into a glass column (25×2.0 cm). The column was washed with 200 mL acidic water. After that a subsequently wash with 200 mL of distilled water to remove the sugars and other polar compounds of honey and the phenolic fraction were retained in the column. The phenolic fraction was eluted with methanol (300 mL) and evaporated under reduced pressure by a rotary evaporator and dried in vacuum dryer. The residue from the extraction was redissolved in methanol to a final known concentration.

Total phenolic content (TPC): The TPC of the honeys were determined by the method described by Singleton et al. and with some modifications[13]. An aliquot of 200 µL of sample (honey/phenolic extract) mixed with 200 µL Folin-Ciocalteu reagents for 3 min and then 600 µL of a 20% sodium carbonate (Na_2CO_3) solution was added and diluted to a final volume 10 mL with water and mixed thoroughly. The sample was kept for 90 min incubation in the dark place. Absorbance of the solution was taken at 765 nm. Gallic acid was used to calibrate the standard curve (0.2-0.8 mg/mL; $Y=0.5x+0.6515$; $R^2=0.99$) and

the results were expressed as mg of gallic acid equivalent (GAE) per 100g of honey.

Total flavonoid content (TFC): The TFC content was determined by the aluminium chloride colorometric assay and with some modification[14]. A sample of 50 µL honey sample was taken in a volumetric flask. At first, 0.3 mL $NaNO_2$ aqueous solution (5g /100 mL) was added the flask. After 5 min, 0.3 mL of $AlCl_3$ aqueous solution (10 g/ 100 mL) was added to mix vigorously. Then, after 6 min 2 mL of 1 M NaOH was added to the mixture. Immediately, the reaction flask was diluted to 10 mL with distilled water and thoroughly mixed. Absorbance of the mixture, pink in color, was measured at 510 nm versus prepared water blank using a spectrophotometer. The reading was used and expressed as mg of catech in equivalents (mg CE/100 g) of honey.

Antioxidant activity of Honey: DPPH free radical-scavenging assay (%RSA): To determine (%RSA), DPPH assay was used described by Gyamfiet al. and Siliciet al., (2010); and with some modifications [15,16]. Briefly, each sample of 50 µL of honey (1 g honey in 4 mL methanol and phenolic extract) was mixed with 450 µL Tris-HCl and 1000 µL of 6×10^{-5} M DPPH in methanol. The mixture was kept for 2 h at room temperature for incubation in the dark and the absorbance was taken at 517 nm using a spectrophotometer and methanol was used as a blank. RSA% of the sample was calculated according to the formula:

$$\%\text{RSA} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}}$$

Ferric-reducing/antioxidant power assay (FRAP): FRAP assay of honey samples was measured according to Benzie and Strain[17] and with some modifications. The prepared stock solutions contained 300 mM acetate buffer (3.1 g Sodium acetate and 16 mL galacial acetic acid) at pH 3.6, 10 mM TPTZ (2, 4, 6- Tripyridyl-*s*-triazine) solution in 40 mMHCl, and 20 mM $FeCl_3.6H_2O$ solution and then kept in at 37℃ warm before using. The sample of honey (150 µL) was mixed with 2850 µL and then allowed to react with FRAP solution for 30 min in the dark condition at room temperature. Reading of the incubated sample was taken at 593 nm. $FeSO_4.7H_2O$ (100-600 µM) solution was used for the calibration curve and the results were expressed as the FRAP value (µM Fe (II)) of per g honey solution.

Statistical analysis: The data were analyzed by analysis of variance (ANOVA) using in SPSS v.20software (Chicago, IL, USA). Tukey's HSD multiple range test was applied for comparing the differences among obtained triplicate results. The difference was considered to be significant at $P< 0.05$. Correlations of the analyzed data were obtained by Pearson's correlation coefficient (r) in bivariate linear correlations

Results and Discussion

Physicochemical Characteristics: The physicochemical characteristics of ten monofloral honey were determined shown in Table-2. pH is an indicator of acidic or alkaline of samples. Honey samples were analyzed as an acidic nature, with pH values ranging from 3.94±0.01-4.93±0.06 where the sample H_2 showed highest pH and sample H_5 showed lowest. The results were similar to 3.50–4.43 for Algerian honeys[18].

The moisture content of investigated monofloral honey samples ranged 15.40±0.38% to 21.53±0.14% (Table-2). Except the H_1, H_3 and H_9 samples all other honeys had moisture contents below 20%.The previous study about Chines honey showed the moisture content ranged from 19.8% to 29%[19]. According to Kayacier and Karaman; honey moisture of different origins showed varietal differences and it might range from 13% to 29%[20].

The ash content of Chinese monofloral honey samples measured 0.04±0.01% - 0.47±0.02% (Table-2). The similar results reported by some Romanian honeys ranged from 0.03 to 0.4[7].

The water activity of Chinese honey samples were ranged 0.502-0.605 presented in Table-2. This results were comparable to those honeys from arid region (0.52 to 0.64) reported by Habib et al.[21].

Color intensity of Chinese honey samples were varied 20.35±0.37 to 94.75±0.93 mm pfund. The color intensity of honey depends on its chemical composition especially pigments like chlorophylls, carotenoids, flavonoids, tannin derivatives and polyphenols. In this study sample H_5 had the highest intensity and sample H_{10} showed lowest lightness (Table-2).

Total phenolic content (TPC): According to analyzed data raw honey samples showed significantly higher phenolic content when compared with their phenolic extract. The TPC ranged from 5.64±0.31 to 55.12±0.51 (mg GAE/100g of honey) for phenolic extract samples and 8.70±1.20-136.17±5.46 (mg GAE/100 g of honey) for entire honey samples of different floral Chinese honeys presented in Table-3. It was observed that the TPC showed significant differences among the different floral sources sample. The average value of TPC was found in Chinese honeys were in between to the value ranged from 0.2 to 141.83 mg/100g gallic acid equivalent of Rhododendrom honeys[16].

Total flavonoid content (TFC): Using a standard curve obtained from standard catech in (R^2=0.9942), the total flavonoid content of honey samples (mgCE/100 g) varied from 2.7±0.05-7.96±0.07shown in Figure-1. The highest amount of flavonoids was found in sample H_5 while the lowest value was found in sample H_{10}. Therefore, the results showed that TPC and TFC was affected by the honey color. The Similar results have been reported by Meda et al.[10].

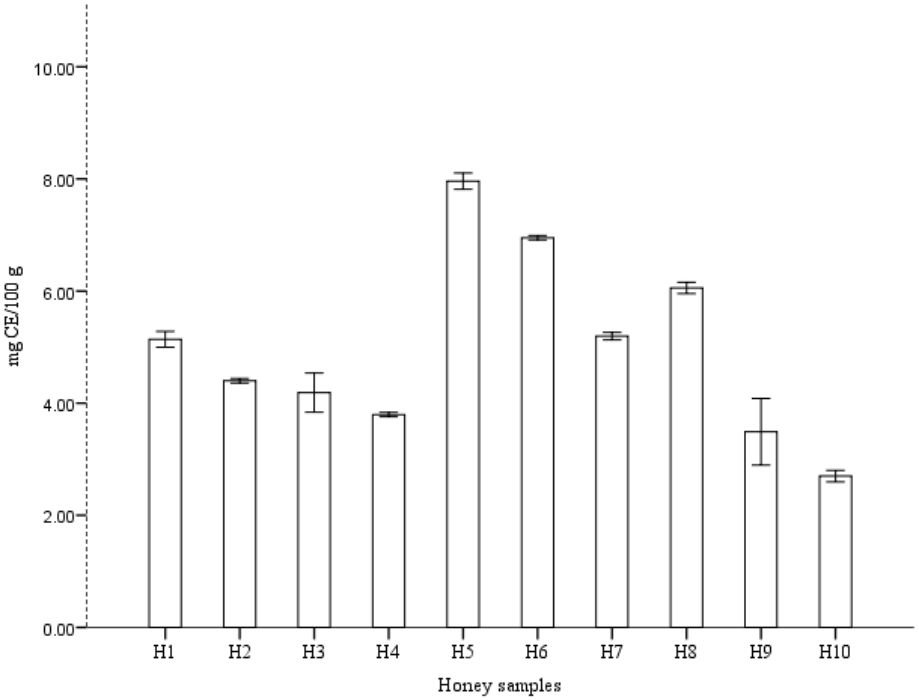

Figure-1
Total flavonoid contents (TFC) of honey samples. The data are displayed with mean ± standard deviation (bars) of three replications. TFC values of monofloral honeys were significantly different at *P*<0.05

Antioxidant activity: DPPH free radical-scavenging assay (%RSA): The analysis of free radical scavenging activity (%RSA) of Chinese honey samples determined by DPPH is presented in Table-3. The free radical scavenging assay ranged from 22.40±2.49% to 92.50±0.20%. In this study most of the Chinese honey samples of phenolic extracted showed >50% RSA (%) which was similar results of 58 Pakistani honeys was 30.50±0.31%–77.43±0.77%[22].

Ferric-reducing/antioxidant power assay (FRAP): In general, higher TPC of honey samples indicated higher antioxidant capacity as well as FRAP values[23,24]. However, in overall honey samples H_5(20.72±0.44 and 8.93±0.12 µmol $FeSO_4.7H_2O$/g honey)showed highest FRAP value while the sample H_9(9.30±0.26 and 6.01±0.04 µmol $FeSO_4.7H_2O$/g honey) had lowest FRAP value presented in Table-3. In this study the FRAP values of honey were found higher in entire samples.

Table-2
Physicochemical analysis of ten Chinese honeys (means ± standard deviation)

Sample	pH	Moisture (%)	Ash (%)	Water activity	Color (mm pfund)
H_1	4.14±0.01	21.53±0.14	0.05±0.00	0.555	71.11±0.57
H_2	4.93±0.06	15.40±0.38	0.28±0.01	0.605	45.61±0.37
H_3	4.46±0.01	21.00±0.78	0.08±0.06	0.502	38.30±0.21
H_4	4.06±0.02	19.40±0.55	0.04±0.01	0.597	28.89±0.37
H_5	3.94±0.01	17.87±0.15	0.06±0.02	0.534	94.75±0.93
H_6	4.01±0.01	17.46±0.15	0.15±0.00	0.573	89.92±0.56
H_7	4.38±0.01	18.93±0.15	0.33±0.07	0.581	84.23±1.33
H_8	4.32±0.01	18.10±0.10	0.38 0.01	0.546	87.32±0.57
H_9	4.14±0.01	20.43±0.12	0.47±0.02	0.550	25.80±0.21
H_{10}	3.97±0.01	16.46±0.15	0.13±0.08	0.545	20.35±0.37

Table-3
Total phenolic content (TPC), DDPH free radical-scavenging assay (%RSA), and Ferric-reducing/antioxidant power assay (FRAP) of Chinese honey samples

Samples		TPC (mg GAE/100g)	% RSA	FRAP (µmol $FeSO_4.7H_2O$/g)
H_1	Entire	52.70±0.40^{d}	28.59±0.24^{b}	13.79±0.14^{d}
	Phenolic extract	16.22±0.66^{c}	56.18±0.98^{e}	6.63±0.03^{c}
H_2	Entire	36.57±0.81^{c}	33.53±0.12^{c}	13.71±0.26^{d}
	Phenolic extract	14.24±0.43^{bc}	72.71±0.18^{f}	6.34±0.01b^{c}
H_3	Entire	15.03±0.23^{b}	37.64±0.42^{d}	13.05±0.14^{c}
	Phenolic extract	13.63±2.17^{bc}	81.02±0.58^{g}	6.36±0.01b^{c}
H_4	Entire	12.30±0.53^{ab}	50.35±2.25^{e}	11.18±0.15^{b}
	Phenolic extract	12.74±0.47^{b}	83.29±0.53^{g}	6.20±0.02^{ab}
H_5	Entire	136.17±5.46^{g}	22.40±2.49^{a}	20.72±0.44^{h}
	Phenolic extract	55.11±0.52^{g}	32.78±0.11a	8.93±0.12^{f}
H_6	Entire	114.63±0.70^{f}	25.36±0.26^{ab}	19.02±0.12^{g}
	Phenolic extract	36.82±1.08^{f}	38.85±0.69^{b}	8.69±0.15^{f}
H_7	Entire	67.30±1.40^{e}	26.30±0.19^{b}	16.92±0.10^{e}
	Phenolic extract	23.45±1.36^{d}	51.04±0.52^{d}	7.82±0.22^{d}
H_8	Entire	68.77±0.50^{e}	25.39±0.95^{ab}	18.24±0.23^{f}
	Phenolic extract	29.05±0.74^{e}	48.08±0.83^{c}	8.16±0.10^{e}
H_9	Entire	8.70±1.20^{a}	62.96±0.37^{f}	9.30±0.26^{a}
	Phenolic extract	8.09±0.43^{a}	92.50±0.20^{i}	6.01±0.04^{a}
H_{10}	Entire	9.57±0.12^{a}	52.89±2.44^{e}	11.77±0.21^{b}
	Phenolic extract	5.64±0.31^{a}	88.14±1.92^{h}	6.02±0.05^{a}

Data are presented as mean ± SD. Significantly different values are represented by different letters. GAE indicates gallic acid equivalents; CE, Catechin equivalents

Conclusion

In conclusion, the entire honey showed higher amount of antioxidant activity compared to the phenolic extract of honey samples. In the analysis process phenolic extracted honey samples were reliable and more accurate activity results. This study showed that the different floral sources and different regional honeys in China contained phenolic compounds, flavonoids and as well as good quality of antioxidant activity, which might have health protection potential. The antioxidant contents and physicochemical properties of different floral sources honey sample might be depend on the botanical and geographical origin and climatic characteristics of different areas.

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