

COLOR INTENSITY AND ANTIOXIDATIVE CAPACITY OF FOUR NATURAL MONOFLORAL HONEY FROM MALDA, INDIA

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Received: 29 May 2019, Revised and Accepted: 09 July 2019

ABSTRACT

Objective: The objective of the study was to explore the color intensity and antioxidative capacity of mango honey available from local niches, in and around Malda, West Bengal, India.

Methods: Four mango honey samples: LMH1, LMH2, LMH3, and LMH4, were collected from bee hives, in and around Malda, India, and were subjected to determine the color intensity and antioxidative capacity in 2, 2-diphenyl-1-picrylhydrazyl system.

Results: The honey samples tested had light to dark color variation with color intensity values of 583.59–1842.84 mAU. The total phenolic content (TPC) of the honey samples ranged from 434.52 to 1450.74 µg gallic acid equivalents/ml, while the total flavonoids content (TFC) ranged 39.4–72.67 mg quercetin equivalents/100 g of honey. The honey samples displayed antioxidative activity (AOA) with 9.91–76% inhibition capacity, and the IC₅₀ values of 8.37–19.38 mg/ml. The color intensity of test honeys displayed positive correlation with the TPC (r=0.907) and TFC (r=0.863), while negative correlation between honey color intensity and IC₅₀ values (r=-0.955) was recorded.

Conclusion: The locally available mango honeys were rich in bioactive components displaying excellent AOA, and thus, the native honey might be utilized as the potential source of natural antioxidant in our part of the globe.

Keywords: Mango honey, Color intensity, IC₅₀ value, Antioxidative capacity, Total phenolics content, Total flavonoids content.

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INTRODUCTION

Much attention has been given to consume natural antioxidants, nowadays, as because of the changes in the dietary habit in modern lifestyle [1] and awareness of the unnecessary side effects of the chemical (synthetic) antioxidants. In human body, when the activity of oxidants exceeds the activity of antioxidants and thus antioxidants are favored causing oxidative stress at the cellular levels, which play a role in the pathogenesis and progression of chronic as well as degenerative diseases, such as Alzheimer's disease, atherosclerosis, heart and lung diseases, diabetes, hypertension, and cancer [2,3]. To tackle such a hostile situation, the prime thing is to exogenous administration of antioxidants that comprises micronutrients (Vitamin C and/or Vitamin E, plant phenolics). Other antioxidant forms (butylated hydroxytoluene; butylated hydroxyanisole; tert-butyl hydroquinone; and propyl gallate), which might otherwise compromise the DNA, protein or (essential) lipid functionalities, were found hazardous to human health [1,4]. However, the natural antioxidants, in dietary materials, have better scavenging properties and are safer and healthier than the synthetic ones [1].

The honey constituents, such as, sugars, vitamins, amino acids, proteins, trace elements, and enzymes (glucose oxidase, diastase, invertase, catalase, and peroxidase) positioned this natural sweetener as a nutritionally therapeutic agent, while its (honey) bioactivities and diverse medicinal properties [5-7] which are mainly due the phenolic compounds, flavonoids, carotenoids, and organic acids present in the honey [8,9].

Honey, being a natural medicine, has been in use since ancient times all over the world to combat different infectious diseases as well as non-infectious diseases [10]. The medicinal property and biological activities, including antioxidative capacity, of natural as

well as medicated honey, have been reported from various parts of the world [7,11-13]. It has been reported by earlier authors [14,15] that apart from carbohydrates, honey contains compounds such as phenolic acids, ascorbic acid, flavonoids, tocopherols, enzymes (catalase and superoxide dismutase), reduced glutathione, amino acids, and carotenoids; however, the phenolics and flavonoids in honey play a major role as the antioxidants [12,16]. Ahmed *et al.* [12] studied that the presence of pigments such as carotenoids and flavonoids was related to color of honey, and dark colored honey possesses a large amount of such kind of pigments with higher antioxidation potential. Sohaimy *et al.* [17] reported that the honey with high color intensity might be the indicative of the presence of a high amount of phenolic compounds and flavonoids in honeys. Earlier the phytochemical components of honey bee products as well as wild honey have been explored mentioning different health benefits, including the antioxidative capacity [18,19]. We also have reported the antibacterial and antioxidative property of two natural honey samples [20]. In the current study, mango honeys from naturally grown bee hives, in and around Malda town, India, were tested to record the antioxidative capacity as well as the color intensity since no scientific data are available on mango honeys from our part of the globe.

METHODS**Collection of honey samples**

Four natural monofloral (mango: *Mangifera indica* L.) honey samples: LMH1, LMH2, LMH3, and LMH4, were collected, from four different areas (Kendpukur, Sahapur, Chalandar, and Mahinagar, respectively) of Malda district, West Bengal, India, during the month of February, 2017–May 2017. The honey samples were collected by the help of honey collectors, and the identity of the collected honeys was confirmed by the honey collectors depending on the seasonal as well as vegetation variation around the foraging areas of bee hives [15,20]. All the honey samples were kept in the dark at room temperature for further study.

Determination of honey color intensity

The color intensity of honey samples collected was determined following the protocol of Beretta *et al.* [19]. Briefly, each of the honey samples was diluted to 50% (w/v) with double distilled water (45°C–50°C) and filtered through filter paper (Whatman No.1, Merck), to remove the suspended particles. The absorbance of the honey solution was measured at 450 nm and 720 nm, using double Ultraviolet (UV)-visible spectrophotometer (Lim 332, Labard Instruchem Pvt., Ltd, India), and the difference between the absorbance was considered as the color intensity, and expressed in mAU (milli absorbance units) [21].

Determination of total phenolics content (TPC)

The TPC of honey was determined spectrophotometrically by the Folin-Ciocalteu (FCR) technique, following Alzahrani *et al.* [11]. For this purpose, 200 µl of 10% (v/v; with double distilled water) filtered honey solution was mixed with 500 µl of 10% Folin-Ciocalteu reagent (FCR; with double distilled water) for 5 min, and 1.5 ml of Na₂CO₃ solution (7.5% with double distilled water) was added and mixed. The reaction mixture was left for 30 min at room temperature in darkness, and thereafter, absorbencies were read, against a blank (methanol, Folin reagent, and sodium carbonate), using double beam UV-visible spectrophotometer (Lim 332, Labard Instruchem Pvt., Ltd, India), at 725 nm, using gallic acid (GA) calibration curve (concentration range: 10–100 µg/ml; R²=0.9216). The TPC was expressed as µg of GA equivalents (GAE)/ml of honey (µg GAE/ml).

Determination of total flavonoids content (TFC)

The TFC was determined using AlCl₃, according to the protocol of Arvouet-Grand *et al.* [22] with slight modifications. Briefly, 1.5 ml of 2% AlCl₃ in methanol was mixed with an equal volume of honey solution (1:3, w/v, ratio, in methanol). After incubation at room temperature, for 15 min, in darkness, the absorbance of the mixture was measured at 415 nm against a blank solution (consisting of methanol without AlCl₃). The TFC was determined using the calibration curve of quercetin (concentration range: 25–150 µg/ml; R²=0.952), and the results were expressed as mg of quercetin equivalents (QE)/100 g of honey.

Antioxidative activity (AOA) of honey

The AOA of each of the honey samples was evaluated in 2, 2-diphenyl-1-picrylhydrazyl (DPPH) system, which was based on the protocol of Habib *et al.* [23]. The honey solutions: 3–15 mg/ml for LMH1 and LMH2, and 6–30 mg/ml for LMH3 and LMH4, in methanol were prepared, and 0.1 ml of such honey extract (for all honey types of all concentrations) was mixed with 0.3 ml methanol and 0.4 ml 0.3 mM DPPH in methanol, by shaking, and left for 30 min in darkness at room temperature. The reduction (inhibition) of DPPH radical was measured by checking the absorbencies of the mixture at 517 nm, against the control prepared with DPPH in methanol without honey, and the radical scavenging activity (RSA) was calculated as the percentage of DPPH radical inhibition, using

the formula: % inhibition = $\frac{\text{ControlOD} - \text{SampleOD}}{\text{ControlOD}} \times 100$ [24]. The

IC₅₀ (half-maximal inhibitory concentration) value of individual honey samples was determined as per the protocol mentioned earlier [24].

Statistical analysis

The tests were performed in triplicates, and the results have been represented as mean ± standard deviation. The statistical analyses were done using Microsoft Excel 2016. One-way analysis of variance (ANOVA) was used to determine significant differences (p<0.05) among utilized parameters of honey samples. The degree of association between color intensity and other variables (TPC, TFC, and IC₅₀) was assessed by Pearson's correlation coefficient (r).

RESULTS AND DISCUSSION

Color intensity of honey

Among the collected four mango honeys, two (LMH1 and LMH2) were dark amber in color, while the remaining honey samples (LMH3 and LMH4) were light amber in color (Fig. 1). The color intensity test results for honey samples are represented in Table 1; the color intensity ranged



Fig. 1: Color pattern of four locally available monofloral (mango) honey samples procured from different parts of Malda; LMH1: Kendpukur, LMH2: Sahapur, LMH3: Chalandar, LMH4: Mahinagar

Table 1: Color intensity of mango honey collected from Malda, India

Honey sample	Visual color	Color intensity (mAU)
LMH1	Dark amber	1842.84±21.17
LMH2	Dark amber	1021.44±18.56
LMH3	Light amber	633.32±3.51
LMH4	Light amber	583.59±8.08

from 583.59±8.08 mAU (for LMH4 honey) to 1842.84±21.17 mAU (for LMH1 honey).

The honey color intensity is an important parameter for the determination of antioxidant properties of honeys. The color intensity has been demonstrated earlier by several authors around the globe. As has been assessed through visual observation, by Bertonecjl *et al.* [25], the Slovenian honey color ranged from colorless to dark brown, the acacia (HCl: 70 mAU) and lime honeys being the brightest to pale yellow, while the chestnut (HCl: 495 mAU) and fir (HCl: 405 mAU) honeys being the amber less dark to grey-brown. Islam *et al.* [26] assessed different monofloral and multifloral, amber to dark amber color, Bangladeshi honeys, and recorded their color intensity ranged from 254 mAU to 2034 mAU. Moniruzzaman *et al.* [27] reported four Malaysian honey samples with ABS₄₅₀ values 312–544 mAU, among which, the amber color Tualang honey had the highest color intensity (544.33±11.68 mAU). The Polish goldenrod (*Solidago virgaurea* L.) honey had a low range of color intensity (138–205 mAU), as has been reported by Jasicka-Misiak *et al.* [28]. Ahmed *et al.* [12] analyzed 16 honey samples for which color intensity values ranged 386.23–748.83 mAU, and 630–1006.33 mAU for nine honey samples as reported by Nweze *et al.* [29]. Moloudian *et al.* [30] investigated on 48 Iranian honeys with color intensity of 25–743 mAU, while Nayik and Nanda [31] recorded the values 18–795 mAU for 37 honeys of varied floral origin. In the current study, color intensity ranged 1021.44±18.56–1842.84±21.17 mAU for dark variety honey, and 583.59±8.08–633.32±3.51 mAU for light variety honey. Such honey color grades with different intensity might be due to variation in floral and geographical origin as well as dissimilarity in the chemical composition of honeys harvested in different seasons.

TPC of honey

The TPC of the test honey samples is represented in Table 2. The TPC values ranged from 1450.74±5.98 µg GAE/ml to 434.52±5.96 µg GAE/ml; highest TPC content was found in LMH1 (dark variety honey) while the lowest value was seen in LMH4 (light variety honey).

As per the report of Dezmierean *et al.* [32], the light colored raspberry honey had the lowest amount of total phenolics (148.47 mg GAE/kg), while in the dark colored heather honey the concentration of total phenolics (435.99 mg GAE/kg) was ≈ 3-fold higher than that contained

in raspberry honey. The TPC for Indian honeys has been reported to be ranged from 47 mg GAE/100 g to 98 mg GAE/100 g [33], while the sourwood honey contained TPC of 580.03±0.38 mg GAE/kg [27]. The TPC for four Malayan monofloral and multifloral honey samples ranged 186.70–352.73 mg GAE/kg, wherein the Tualang honey had the highest TPC having the highest color intensity (544.33±11.68 mAU) [27]. Mahnot *et al.* [34] from Northeastern India reported the TPC of test honey as 74.42–290.68 mg GAE/100 g. Ávila *et al.* [35] reported the TPC content in Brazilian stingless bee honeys as 220.4–708.1 mg of GAE/kg. In the current study, highest TPC value (1450.74±5.98 µg GAE/ml) was measured in the dark variety honey (LMH1 sample), and the lowest TPC (434.52±5.96 µg GAE/ml) was found in the light variety honey (LMH4 sample).

TFC of honey

The quercetin calibration curve in determining TFC is represented in Fig. 2. The TFC of the mango honey tested is shown in Table 2, with the highest value of 72.67±0.15 mg QE/100 g, for LMH1 (dark variety) honey, and the lowest value of 39.4±0.2 mg QE/100 g, for LMH4 honey (light variety). As has been reported by Moniruzzaman *et al.* [36], the TFC in ten monofloral honey samples from Bangladesh ranged from 11.46 to 116.67 mg catechin/kg. The TFC values in eight commercial Moroccan carob honeys ranged from 2.26 mg QE/100 g to 4.79 mg QE/100g [37]. Mohamed Al-Farsi [38] from Oman investigated 26 natural honeys and reported highest TFC in Sumer samples in a range between 1613 mg/kg and 2890 mg/kg, while the TFC in Sidr and multiflora samples ranged 635–1034 mg/kg and 521– 1354 mg/kg, respectively. The TFC of five honey samples collected from Bangladesh ranged from 4.67 to 6.25 mg CE/100 g [39]. It has been reported that the high TFC (43.17 mg QE/kg) was found in the dark colored heather honey, followed by raspberry honey (15.94– 43.17 mg QE/kg) and multifloral honey (11.95 mg QE/kg), while the lower TFC were recorded in black locust honey (4.61 mg QE/kg) and linden honey (8.90 mg QE/kg) honey [32]. Herein, the dark variety of honeys had TFC values of 68.83±0.21–72.67±0.15 µg GAE/ml, while the values ranged 39.4±0.2–45.67±0.06 µg GAE/ml for light variety of honeys.

Antioxidative capacity of honey

The antioxidant property of mango honey samples has been determined in the form of antiradical activity in DPPH scavenging system. The percent inhibition (DPPH reduction/radical scavenging) activities for light color honeys (LMH1 and LMH2) are represented in Fig. 3, and for

dark color honeys (LMH3 and LMH4) are represented in Fig. 4, while the IC₅₀ values are shown in Table 2. In DPPH system, the scavenging activity of Bangladeshi honeys ranged from 33.6% to 97.5% [26], while the values ranged from 44% to 71%, for Indian honeys [9]. In the current study, the IC₅₀ values of four mango honey samples: LMH1, LMH2, LMH3, and LMH4, have been assessed as 8.37, 12.46, 17.08, and 19.38 mg/ml, respectively. In the instant study, dark colored honey (LMH3 and LMH4) displayed IC₅₀ values of 8.37–12.46 mg/ml, while the IC₅₀ values of light colored honeys were in the range of 17.08–19.38 mg/ml.

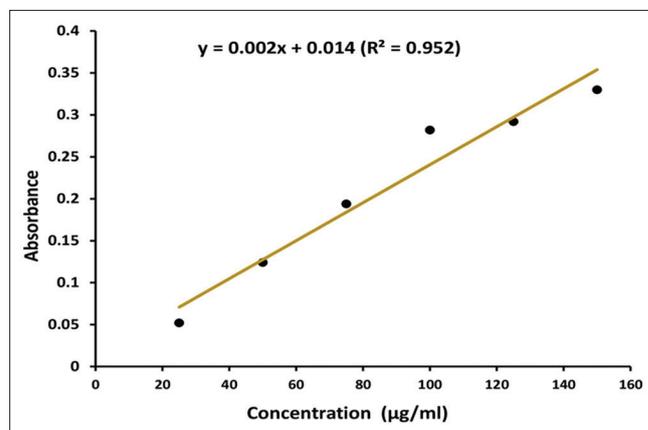


Fig. 2: Quercetin calibration curve for the determination of total flavonoids content in honey samples

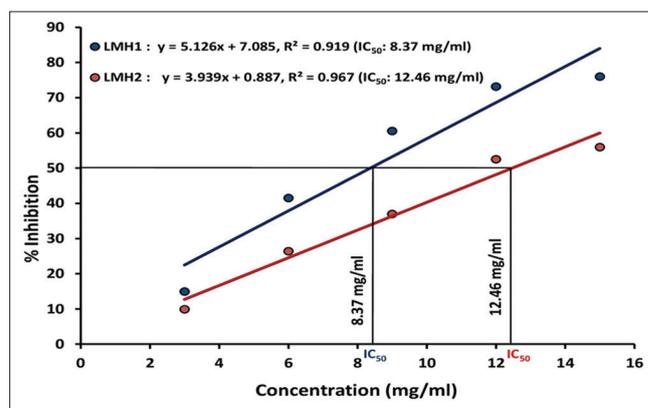


Fig. 3: Antioxidative activity of dark amber color honey (LMH1 and LMH2) in DPPH system; IC₅₀: Half-maximal inhibitory concentration

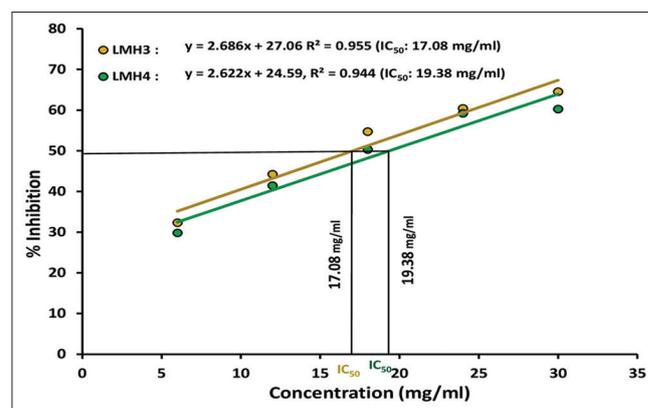


Fig. 4: Antioxidative activity of light amber color honey (LMH3 and LMH4) in DPPH system; IC₅₀: Half-maximal inhibitory concentration

Table 2: Antioxidative substances and antioxidant activity of mango honey samples (n=4)

Honey sample	TPC (µg GAE/ml)	TFC (mg QeE/100 gm)	IC ₅₀ (mg/ml)
LMH1	1450.74±5.98	72.67±0.15	8.37
LMH2	1224.67±5.96	68.83±0.21	12.46
LMH3	468.15±11.91	45.67±0.06	17.08
LMH4	434.52±5.96	39.4±0.2	19.38

TPC: Total phenolic content; GAE: Gallic acid equivalents; TFC: Total flavonoid content; QeE: Quercetin equivalents; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; IC₅₀: half maximal inhibitory concentration. Values are mean±standard deviation

Table 3: Correlation matrix (Pearson correlation coefficients) among different parameters of mango honey samples (n=4)

Pearson correlation	Color intensity ^α	TPC ^β	TFC ^γ	IC ₅₀ ^δ
Color intensity ^α	1			
TPC ^β	0.907	1		
TFC ^γ	0.863	0.988	1	
IC ₅₀ ^δ	-0.955	-0.971	-0.967	1

p value (α, β): 0.047; p value (α, γ): 0.068; p value (α, δ): 0.023; p value (β, γ): 0.006; p value (β, δ): 0.014; p (γ, δ): 0.016. TPC: Total phenolic content; TFC: Total flavonoid content; IC₅₀: Half-maximal inhibitory concentration

Correlation between honey color intensity and antioxidative profiles

The nature and direction of the correlation between honey color and antioxidative profiles (TPC, TFC, and IC₅₀) are represented in Table 3. An immense association exists between the bio-physicochemical agents (color pattern and color intensity, TPC and TFC) and antioxidative capacity (DPPH RSA) of honey, and the dark color honeys (with high color intensity) possessing higher TFC and TPC demonstrated strong AOA, in the instant study. Dezmirean *et al.* [32] established a positive correlation between the AOA of honeys and the total phenolics and flavonoids contained in the honeys and showed that the phenolics and flavonoids are responsible for the AOA of honey. As has been reported by Khalil *et al.* [40], there was a positive correlation between the honey color intensity and various antioxidant parameters of Algerian honeys including TFC and DPPH (0.968 and 0.964, respectively) and between TFC and DPPH RSA ($r=0.888$). As has been reported by Islam *et al.* [26], there were considerable correlations between biochemical and antioxidant factors traced in Bangladeshi honeys; the author demonstrated correlation of honey color pattern/color intensity with TPC ($r=0.820$), TFC ($r=0.727$), and DPPH activity ($r=0.948$), and between TPC and DPPH activity ($r=0.869$) and TFC and DPPH activity ($r=0.786$). A positive correlation between the presence of phenolic compounds and honey color ($r=0.844$), the honey color and the antioxidant capacity ($r=0.675-0.862$), and phenolics and antioxidant capacity ($r=0.615-0.782$) have been observed, when analyzed *in vitro* the Brazilian Pampa biome honey, by Cruz *et al.* [41]. Among six Romanian honeys (black locust, linden, heather, raspberry, canola, and multiflora) tested, the dark colored heather honey contained highest TPC (435.99 mg GAE/kg) as well as TFC (43.17 mg QE/kg) values, while the light colored raspberry honey had TPC and TFC values of 148.47 mg GAE/kg–15.94 mg QE/kg, respectively, with respective AOA (in DPPH system) of 35.77% and 28.9%, for heather honey and raspberry honey [32]. In view of the fact that the honey color reflects, to a certain extent, the content of pigments with antioxidative capacity [42], the current study, for the 1st time, demonstrated a possible correlation between two such factors: Antioxidative capacity (TFC and TPC) and the color intensity of mango honeys available from local niches.

Moniruzzaman *et al.* [26] reported the tualang honey among four Malaysian honeys, which had the highest color intensity, highest TPC and TFC value had the highest scavenging activity in DPPH system (59.89%) and concluded that the higher concentrations of phenolic compounds and flavonoids may have been responsible for the higher percentage of radical-scavenging activity. Neupane *et al.* [43] found a significant correlation between AOA (DPPH RSA) and TPC ($r=0.992$) of honey from different altitudes of Nepal Himalayas. The TPC in multiflora Poland honeys ranged 611–990 mg/kg, and in Slovak honeys ranged 703.7–1257.12 mg/kg, while in Serbian forest honeys the TPC ranged 769–1052 mg/kg, and the highest TPC (2962.24 mg/kg) was recorded in buckwheat honey, which had highest AOA, by ABTS (4.63 mmol/kg) [44], as well as by DPPH [45]. The honey color, in the current study, showed a high positive correlation with TPC ($r=0.907$) and TFC ($r=0.863$) values, and also, the high positive correlations ($r=0.988$) were achieved between TPC and TFC values of the test honeys, while negative correlations were established between AOA (IC₅₀ values) and honey color intensity (-0.955), TPC (-0.971), and TFC (-0.967). Thus, the higher the color intensity, the higher the TPC and TFC values of honey and the lower the IC₅₀ values, the higher the antioxidative activities of the honey.

CONCLUSION

The mango honeys available in local niches are rich in antioxidative components (flavonoids and phenolics), and thus possess excellent antioxidative capacity, wherein the dark colored honey is superior to the light colored honey. The mango honey might be useful as natural antioxidants, and the people in our part of the globe might get beneficial effects on consuming such natural honeys.

AUTHOR'S CONTRIBUTIONS

Surajit Roy performed experimental work and wrote the paper; Shyamapada Mandal designed the study, standardized the protocols, wrote and discussed the paper.

CONFLICTS OF INTEREST

Authors declared that they have no conflicts of interest.

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