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An Exploration of Middle Himalayan Range Acacia Honey: its Physical and Antioxidant Capacities

Anamika Dhiman, Jaswinder Kour, Anjali Thakur and Meenakshi Sharma*
Department of Zoology, Sri Sai University, Palampur (Himachal Pradesh) India.

(Corresponding author: Meenakshi Sharma)*
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ABSTRACT: **This research delves into the unique characteristics of Acacia honey sourced from the Middle Himalayan Range (Jammu, Lahaul, Chamba, and Kangra). Employing a comprehensive analysis, we examined the physical properties and antioxidant potential of this distinct honey variety. The results indicate the dominance of Acacia species, as reflected in the physical profile in terms of specific gravity (1.36), pH (3.67), and moisture content (18.69%). These values align with established quality standards. Furthermore, the honey exhibits significant antioxidant activity, as evidenced by its FRAP, ABTS assay and radical scavenging potential. Insights from this exploration contribute to a deeper understanding of Middle Himalayan Range Acacia honey, offering valuable information for consumers, researchers, and the apiculture industry.**

Keywords: Acacia honey, Middle Himalayan Range, physical properties, antioxidant capacities.

INTRODUCTION

Honey, a natural elixir revered for its diverse flavours and medicinal properties, varies remarkably based on its geographical origin. This study embarks on a journey into the pristine landscapes of the Middle Himalayan Range to unravel the mysteries concealed within Acacia honey from this unique region. As global interest in honey's qualities intensifies, understanding the distinct attributes of Middle Himalayan Range Acacia honey holds the promise of not only enriching our knowledge but also contributing to the broader discourse on honey diversity and its potential applications. Acacia honey is distinguished by its pale color, with a sweet flavour, smooth texture, and a gentle floral fragrance. Its unique characteristic is that it resists crystallization for extended periods, and in some cases, it may never crystallize. If it does crystallize, it takes on a white hue. It is considered one of the most well-loved unifloral honeys. (Matkovits *et al.*, 2023; Abashidze *et al.*, 2024; Jangra *et al.*, 2024).

Against the backdrop of the majestic Himalayan peaks, Middle Himalayan Range Acacia honey emerges as a focal point of this investigation. The geographical and climatic factors of the region intertwine to shape the character of the honey produced, offering a unique terroir that influences its composition. As we embark on this exploration, the aim is to shed light on the distinctive features that make Acacia honey from the Middle Himalayan Range a subject of scientific intrigue and potential economic value. This inquiry not only addresses the curiosity surrounding this specific honey but also contributes to the broader understanding of how environmental factors influence the properties of natural products. Honey is an important asset that contributes significantly to foreign exchange earnings (Mongi, 2024).

The physical evaluation of honey, which encompasses diverse traits such as pH, moisture content, ash content, color, and specific gravity, plays a crucial role in determining its quality and certification (Rehman *et al.*, 2008; Attri, 2011). Checking the quality of honey is important to make sure it's good enough for processing and real, so it meets the needs of both local and global buyers (Mongi, 2024; Bergamo *et al.*, 2019). Additionally, honey's antioxidant capability extends its effects to various molecules, including carbohydrates, proteins, lipids, and nucleic acids, exerting a profound influence on the prevention of acute and chronic diseases such as inflammation, allergies, thrombosis, diabetes, cancer, and many more (Erejuwa *et al.*, 2012).

MATERIAL AND METHODS

Honey samples and storage: Honey samples were collected from Beehive Natural Honey Farm at Kachhiari in District Kangra, Himachal Pradesh (July), all eight unifloral Acacia species samples were stored in fresh, clean polyethylene bottles at room temperature (22–24°C) in air-tight plastic containers until analysis. The samples include *Apis cerana indica* from Jammu (A1), *Apis mellifera* from Jammu (A2), *Apis cerana indica* from Lahaul (B1), *Apis mellifera* from Lahaul (B2), *Apis cerana indica* from Chamba (C1), *Apis*

mellifera from Chamba (C2), *Apis cerana indica* from Kangra (D1), and *Apis mellifera* from Kangra (D2).

Preparation of sample: The samples were placed in a closed container in a water bath without submerging and heated for half an hour at 65°C until liquefied, requiring occasional shaking. After thorough mixing, the samples were cooled rapidly upon liquefaction. Subsequently, each honey sample underwent individual analysis for selected physical parameters and antioxidant activities in triplicate.

PHYSICAL PROFILE:

Determination of moisture content: To calculate the moisture content from the honey samples, oven drying method (60°-70°C) was used.

Determination of ash content: Ash content analysis done by burning the samples at 600°C in muffle furnace. The ash content was calculated according to the following equation:

Ash content (%) = (C-A)/(B-A) *100

Where: A= Weight of crucible, B= Weight of crucible and sample after evaporation, C= Weight of crucible and sample after ashing.

Determination of acidity: Acidity is also one of the important parameters which may influence the quality of honey. According to the study of Amir *et al.* (2013), the acidity can influence the stability and self-life of honey. Acidity analysis done with the formula:

Acidity as formic acid (%) by weight = 0.23*V / M

Where, V = corrected volume of 0.05 N Sodium Hydroxide used, M = weight in gm. of the sample taken for test

Determination of electrical conductivity: The electrical conductance of the solution was gauged with a calibrated digital conductivity meter. To prepare the solution, 5 g of the honey sample was dissolved in 20 ml of distilled water, and the final volume was adjusted to 50 ml by adding distilled water. The outcomes were reported in milli Siemens per centimetre (mS/cm).

Determination of pH: The pH of honey was assessed following the protocol outlined by the IHC (2002). Each honey sample (5 grams) was diluted with 50 ml of distilled water to create a 10% solution. A digital pH meter, calibrated with pH 4 and 7 buffer solutions at room temperature, was employed for pH measurement. Calibration was performed before each use and recalibrated every two to three hours to account for potential sensitivity loss.

Determination of color analysis: The color assessment of honey samples was conducted using a Lovibond tintometer, employing color standards from the United States Department of Agriculture (USDA, 1985). About 3 g of each honey sample was placed in the sample holder and heated up to 65°C to dissolve sugar crystals. The color of the dissolved sample was visually compared in the Lovibond tintometer against Pfund graded coloured glass filters, with the results expressed in millimetres (mm).

Determination of specific gravity: Specific gravity is the ratio of the density of a substance to the density of a reference substance. This analysis was done using pycnometer at 27˚C.

ANTIOXIDANT PROFILE:

Free Radical Scavenging Activity (DPPH): The free radical scavenging activity of honey samples was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, following the procedure outlined by Isla *et al.* (2011) with minor modifications. A solution (20 mg/L) was prepared by dissolving 2 mg in methanol (100 mL). Subsequently, 0.75 mL of the methanolic honey solution, with concentrations ranging from 20 to 40 mg/mL, was added to 1.5 mL of the DPPH solution. After 15 minutes of incubation at 25°C, the absorbance was measured at 517 nm. Ascorbic acid served as the positive control, and the concentration of honey sample needed to scavenge 50% of DPPH was determined based on the ascorbic acid calibration curve (0–10 mg/L).

ABTS assay: This assay, adapted from the procedure by Re *et al.* (1999) with a reduced total volume to 1 ml, involved preparing a stock solution by incubating a 1:1 mixture of ABTS (7 mmol/l) and potassium persulphate (4.95 mmol/l) for 12 hours at room temperature in the dark to form the radical-cation ABTS•+. This stable solution was stored in the dark at 4°C for at least one week. Absorbance values between 1.0 and 1.5 AU at 734 nm were considered optimal, and if exceeded, the stock solution was diluted with phosphate buffer solution. After 30 minutes of incubation, the reduction in absorbance at 734 nm was measured. Radical scavenging activity, expressed as SC50 (mg sample per mL), indicating the concentration causing 50% scavenging of the ABTS radical, was assessed using Trolox and BHT as standards.

Ferric Reducing/Antioxidant Power Assay (FRAP): It was utilized to evaluate the reducing power of honey samples, adapting the method by Benzie and Strain (1996) with slight modifications. The FRAP reagent, freshly prepared by combining 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM FeCl3, and 25 mL of 0.3 M acetate buffer at pH 3.6, was warmed to 37°C before application. One gram of honey dissolved in a 10 mL n-hexane-acetone mixture (6:4) underwent filtration through Whatman no. 4 filter paper. Subsequently, 200 µL of the honey solution was mixed with 1.8 mL of the FRAP reagent, and the resulting mixture's absorbance was measured spectrophotometrically at 593 nm following a 10-minute incubation. The calibration curve was established using Trolox, and the results were expressed as milligrams of Trolox equivalent per 100 grams of honey.

Statistical Analysis: The results of all experiments were expressed as mean ± SD of triplicate measurements. The significant differences were represented by ANNOVA and were calculated using Microsoft office excel 2010 and SPSS variants 22 (TBM, corporation New York, USA).

RESULTS AND DISCUSSION

PHYSICAL PROPERTIES

Moisture content: The moisture content of all honey samples, as mentioned by AOAC (2012), was within the reference range of (16%- 25%) and exhibited a significant level of $p<0.01$. The B1 sample had the lowest moisture content (18.38±0.01) among all the samples. The moisture content of all samples fell below 20 g/100 g, meeting the standards of Ethiopia, Codex, and the EU (<20 g/100 g). The low moisture content of honey is crucial of the preservation and stability of honey, preventing fermentation and spoilage. Honey with high moisture content is more prone to fermentation, which can affect its taste, quality and shelf life. Similar studies were reprted by Sharma *et al.* (2021); Rana *et al.* (2023) where low moisture content was reported.

Total solid content: B1 showed the highest value of total solid content 81.60±0.003%. all other honey samples were also in reference range of 80%-82% which was found to be concordant to the observations of Rana *et al.* (2023) where it has been shown that total solid content above or equal to 81% were classified as of higher grade (A and B) (USDA, 1985; Nyau *et al.*, 2013).

Refractive index: The refractive index values of honey, measured at 20°C, were statistically insignificant. The refractive index of honey is commonly around 1.5 and can vary depending on moisture content and floral source, measured with a refractometer. It is often used as a parameter for assessing the honey quality.

Ash content: The ash content in honey is generally small and depends on nectar composition of predominant plants in their formation. The results obtained from the analysis of all samples were proved to be significant ($p<0.05$) and within standard range. The results were comparable to the findings of Prica *et al.* (2015) ; Sharma *et al.* (2021).

Acidity and pH: Acidity represents the amount of acidic character in a honey sample. The maximum acidity was found in sample B1, but the difference was deemed insignificant and fell within the acceptable maximum value of free acidity in honey (*i.e.* limit 40±15 $mEqKg^{-1}$ as per IS standard, IS: 4941-1974).

The pH of honey samples was analysed by electronic pH meter. The result obtained were statistically insignificant but aligned with the Codex Alimentarius Commission (2001), which predetermined an acceptable pH range of honey between 3.2 and 4.5. In our study, all the investigated middle Himalayan range acacia honey samples were acidic (3.4-4.0) and fell within the accepted limit (pH 3.4 to 6.1), indicating freshness. According to the study of Irshad (2024); Sulaiman *et al.* (2022). Honey's acidic pH, ranging from 3.2 to 4.6, helps restrict bacterial growth and aids in wound healing by facilitating epithelialization.

Electrical conductivity: Electrical conductivity of different honey samples was measured by electrical conductivity meter where highest conductivity was reported in A2 and lowest was in C1 and results obtained were significant ($p<0.05$) to each other. The observations were similar to study of Santos *et al.* (2018); Ratiu *et al.* (2020).

Color analysis: The analysis of honey samples' color was conducted using the Lovibond Tintometer, with absorbance values being converted. Honey color, a crucial physiochemical parameter, serves as an indicator of quality (Muruke, 2014). The color is influenced by factors such as ash content, temperature, and storage time (De Silva *et al.*, 2016; Szabó *et al.*, 2016). While the Pfund scale is widely recognized for honey color assessment, alternatives like the Lovibond scale and spectral analysis were also employed.

Specific gravity: Honey's specific gravity is inversely related to its moisture content; denser honey indicates lower humidity (Attri *et al.*, 2011). This parameter can help detect honey adulteration and track stored honey amounts (Crane, 1976). Observations aligned with IS: 4941-1974, indicating acceptable ranges of species-specific gravity for honey. The sample results were statistically insignificant.

Table 1: Physical properties of honey: Moisture (%), Total solid content, Refractive index, Ash content (%), Acidity (mEq), Electrical Conductivity (mS/cm), pH, Color analysis (mAU), Specific gravity (gm^{-1}).

Sr. No.	Parameters	A1	B1	C1	D1	A2	B2	C2	D2
1.	Moisture content	19.20±0.03	18.38±0.01	19.12±0.07	18.54±0.013	18.39±0.015	18.52±0.011	18.98±0.017	18.42±0.10
2.	Total solid content	80.76±0.003	81.60±0.003	80.88±0.10	81.03±0.011	81.6±0.005	81.45±0.003	80.99±0.013	81016±0.028
3.	Refractive index	1.488±0.0001	1.4903±0.00001	1.4902±0.0001	1.487±0.0013	1.4906±0.0001	1.4905±0.002	1.4905±0.003	1.491±0.0005
4.	Ash content	0.416±0.003	0.706±0.003	0.686±0.003	0.67±0.011	0.01±0.005	0.303±0.003	0.7±0.01	0.71±0.005
5.	Acidity	0.116±0.003	0.156±0.003	0.136±0.013	0.12±0.005	0.053±0.003	0.056±0.003	0.046±0.003	0.054±0.003
6.	Conductivity	0.11±0.003	0.18±0.005	0.0186±0.003	0.183±0.003	0.196±0.003	0.163±0.003	0.146±0.003	0.156±0.003
7.	PH	3.403±0.003	3.516±0.03	3.936±0.024	3.93±0.029	3.45±0.003	3.50±0.003	3.63±0.008	4.01±0.003
8.	Color analysis	118.8±0.166	120.16±0.166	120.01±0.020	120.04±0.030	25.03±0.03	24.88±0.01	28.44±0.096	30.67±0.32
9.	Specific gravity	1.35±0.003	1.32±0.005	1.31±0.005	1.38±0.005	1.41±0.003	1.36±0.003	1.37±0.011	1.39±0.003

ANTIOXIDANT PROPERTIES:

DPPH: The DPPH test is based on the ability of the stable 2, 2-diphenyl-1-picrylhydrazyl free radical to react with hydrogen donors (Inoue *et al.*, 2005). In the current study, *Apis mellifera* exhibits highest DPPH activity and *Apis cerana indica* exhibits lowest DPPH activity as shown in Table 2. The difference in radical scavenging activity might be due to difference in coloration where dark coloured honey samples tended to be highly active in the reaction with DPPH (Bertoncelj *et al.*, 2007; Blasa *et al.*, 2006) which justifies our study.

ABTS: The ABTS scavenging potential of honey was much higher than antioxidant activity on DPPH radicals (Bueno-costa *et al.*, 2016) which is also found in our study. According to our study, honey samples which were more effective in DPPH reaction system, showed higher inhibition in the ABTS system too.

FRAP: FRAP method is another method that can be employed for the determination of total antioxidant activities of honey. FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess (Moniruzzaman *et al.*, 2012). FRAP assay is based on the ability of the analyte to reduce the fe^{3+}/ fe^{2+} couple (Lim and Tee 2007). All the honey samples from different sources shows the reducing power. The range of FRAP activity in samples of *Apis mellifera* and *Apis cerana indica* justifies the above study.

Table 2: Antioxidant properties of honey.

Sr. No.	Parameter	A1	B1	C1	D1	A2	B2	C2	D2
1.	DPPH	18.64±0.003	17.81±0.003	17.31±0.153	18.18±0.084	18.73±0.003	18.40±0.003	17.48±0.11	18.23±0.16
2.	ABTS	0.21±0.01	0.22±0.003	0.25±0.020	0.277±0.006	0.25±0.013	0.31±0.016	0.31±0.017	0.29±0.007
3.	FRAP	2.75±0.016	3.02±0.013	2.77±0.107	2.85±0.028	2.36±0.006	2.87±0.003	3.02±0.040	2.85±0.15

CONCLUSIONS

This study comprehensively analysed unifloral honey from Beehive Natural Honey Farm, Kangra, Himachal Pradesh, to evaluate its nutritional quality and storage characteristics. The findings revealed that all honey samples exhibited high-quality attributes, favourable storage capacity, and significant antioxidant potential, underscoring their medicinal value. The studies positioned the honey as a potent source of natural remedies with enhanced value in the market.

FUTURE SCOPE

The study emphasized the intersection of nutritional science and apiculture, providing insights for consumers seeking health-promoting products and producers aiming to market premium-quality honey.

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