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Research Article

PHYSICO-BIOCHEMICAL AND BIOACTIVE PROPERTIES OF HONEY SAMPLES FROM SOUTHERN INDIA

Mary Stella Bhupanapadu Sunkesula*, M. S. Reddy

Centre for Apiculture Studies, Department of Zoology, Bangalore University, Jnana Bharathi, Bengaluru, Karnataka, India

**Corresponding author: stellavictor.ch@gmail.com*

ABSTRACT

Honey is a natural sweet material produced by honey bees, from the nectars of plant flowers and honey dew. The present study was designed to evaluate physico-biochemical and bioactive properties of honey from southern India. In this study, 40 different honey samples were obtained from southern India such as Andhra Pradesh, Karnataka, Kerala and Tamil Nadu, ten samples from each region were collected respectively. The study showed that the color of the honeys is exceedingly variable such as amber, dark amber, light amber and white. The physical properties density, moisture, total solids, water insoluble solids, pH, free acidity and electrical conductivity significantly vary ($p < 0.05$). The highest biochemical properties values were found in Kerala honey, which has minimum level of reducing sugar. The antioxidant property of hydroxyl radical scavenging activity was detected highest in Kerala honey sample than the other analyzed honey samples. Yet, the results suggest that Southern India honeys could be beneficially used as a functional or nutraceutical substance as they prevent or moderate oxidative stress-related diseases.

Keywords: Physico-biochemical, Bioactive, Antioxidant, Honey.

1. INTRODUCTION

Honey is defined as a sweet, sticky yellowish-brown fluid made by bees and collected from flowers. Honey contains more than 200 compounds comprising approximately 38% fructose, 31% glucose, 10% other sugar types, 18% water and 3% of other compounds. However, precisely the great mixture of compounds in these 3% is the product's greatest feature, with special reference to phenolic and carotenoids compounds [1]. Carotenoids were found in small concentrations in the dark honey but they were not found in light colored honey [2]. Honey is one of the most complete food for humans, due to its therapeutic, antioxidant [3], antimicrobial [4], antitumoral [5], anti-inflammatory [6], antiviral [7] and antiulcer [8] activities. In the past three decades, the large number of published studies concerning the physicochemical characteristics of honeys of different botanical and geographical origins illustrates the importance of determining honey's quality [9, 10]. Very few studies, however, have analyzed honey's physicochemical properties, and none of them has determined the physicochemical characteristics of any southern India variety [11]. Southern India has five states on the mainland such as Andhra Pradesh, Karnataka, Kerala, Tamil Nadu and Telangana. It is one of the major economic powerhouses of the nation because of tropical region. The present study was undertaken to determine the physico-biochemical and bioactive properties of southern India honey samples.

2. MATERIAL AND METHODS

2.1. Samples collection

Honeys from entirely randomized different botanic origins were analyzed (Table 1). Honeys were provided by beekeepers and collected from several regions in the state of Andhra Pradesh, Karnataka, Kerala and Tamil Nadu from southern India. The samples were from the following state: Andhra Pradesh (AP1, AP2, AP3, AP4, AP5, AP6, AP7, AP8, AP9, AP10), Karnataka (KA11, KA12, KA13, KA14, KA15, KA16, KA17, KA18, KA19, KA20), Kerala (KL21, KL22, KL23, KL24, KL25, KL26, KL27, KL28, KL29, KL30) and Tamil Nadu (TN31, TN32, TN33, TN34, TN35, TN36, TN37, TN38, TN39, TN40). Samples were acquired between January and December 2019 and kept in sterilized dark polyethylene flasks under refrigeration (8°C).

Table 1: Honey samples with respective collection data

Samples	Town	Color	Samples	Town	Color
AP1	Anantapur	Dark amber	**KL21**	Kottayam	White
AP2	Eluru	Light amber	**KL22**	Palakkad	Dark amber
AP3	Warangal	Light amber	**KL23**	Thiruvananthapuram	Light amber
AP4	Hyderabad	Light amber	**KL24**	Kollam	White
AP5	Tirupati	Dark amber	**KL25**	Ernakulam	Light amber
AP6	Guntakul	Light amber	**KL26**	Alappuzha	White
AP7	Nellore	Light amber	**KL27**	Chittarikkal	Light amber
AP8	Rajahmundry	Dark amber	**KL28**	Calicut	White
AP9	Vijayawada	Light amber	**KL29**	Adoor	White
AP10	Visakhapatnam	Light amber	**KL30**	Kochi	Dark amber
KA11	Indi	Light amber	**TN31**	Coimbatore	Light amber
KA12	Hassan	Light amber	**TN32**	Theni	Light amber
KA13	Mysore	Light amber	**TN33**	Salem	Light amber
KA14	Kannur	Amber	**TN34**	Sathyamangalam	Dark amber
KA15	Tumkur	Dark amber	**TN35**	Namakkal	Light amber
KA16	Sagara	Light amber	**TN36**	Kanyakumari	Light amber
KA17	Hassan	Light amber	**TN37**	Kovilpatti	Dark amber
KA18	Sulya	Light amber	**TN38**	Thiruvarur	Dark amber
KA19	Shrirangapattana	Dark amber	**TN39**	Hosur	Dark amber
KA20	Bangalore	Light amber	**TN40**	Vellore	Light amber

2.2. Chemicals and reagents

All the chemicals were of the highest analytic degree. Prior to all measurements, the samples were previously homogenized and were sonicated for 10 min (45°C) until the complete dissolution of the sugar crystals.

2.3. Physical properties

2.3.1. Color and Moisture content

The collected samples were diluted in water (1:1; w/v) and measured at 635 nm [28]. Moisture content was determined from the refractive index of the honey. A digital refractometer (NR 101 Spain), that can be thermostated at 20°C, regularly calibrated with distilled water or with another certified reference material [12].

2.3.2. Total solids

The Percentage of total solids of each sample was calculated by using the following formula: Total solids (%) = 100 - Moisture content [13].

2.3.3. Water Insoluble Solids Content

Twenty grams of honey was weighed and dissolved in a suitable quantity of distilled water at 80°C and mixed well. The test sample was filtered through a previously dried and weighed fine sintered glass crucible and washed thoroughly with hot water (80°C) until free from sugar. The crucible was dried for one hour at 130°C, cooled and weighed to the nearest 0.1 mg. Finally, the result was expressed as percent water-insoluble solids.

$$\text{Insoluble solids \% by mass} = \frac{(M1-M2)}{W} \times 100$$

Where,

M1 = Mass of the residue and the crucible; M2 = Mass of the crucible; W = Mass of the test portion.

2.3.4. pH

A pH meter (HI 98127, Hanna instruments, Mauritius) was used to measure the pH of a 10% (w/v) solution of honey prepared in double distilled water [12].

2.3.5. Free acidity

The acidity of honey was determined by volumetric method. Ten grams of honey were dissolved in 75 ml of distilled water and solution was titrated with 0.1 M NaOH to pH 8.30. Acidity is expressed in millie quivalents/kg honey (mEq/kg)

2.3.6. Electrical conductivity (EC)

EC were estimated each honey samples using a conductivity meter for a 20 % (w/v) solution of honey suspended in double distilled water [12].

2.4. Biochemical properties

2.4.1. Total sugar content

Total sugar content of honey was determined calorimetrically by the anthrone method [14]. The

amount of total sugar was calculated from the standard curve of glucose.

$$\text{Total Sugar (\%)} = \frac{\text{Calculated Sugar (g)}}{\text{Weight of Honey (g)}} \times 100$$

2.4.2. Reducing sugar content

Reducing sugar content of the honey was determined by dinitrosalicylic acid method [15].

$$\text{Total Reducing Sugar (\%)} = \frac{\text{Calculated Reducing Sugar (g)}}{\text{Weight of Honey (g)}} \times 100$$

2.4.3. Sucrose content

Sucrose content was determined by inversion, adding 10 mL of dilute HCl, 50 mL of diluted honey solution and water in a 100 mL volumetric flask. The solution was then heated in a water bath, cooled and diluted to the mark. Five milliliters (5 mL) of standardized Fehling's solutions A and B were transferred to a 250 mL Erlenmeyer flask containing 7.0 mL of water and 15.0 mL of diluted honey solution. The Erlenmeyer flask was heated and 1.0 mL of methylene blue (0.2%) was added. Titration was carried out by adding the diluted honey solution until the indicator decolorizes.

2.4.4. Fructose content

Percentage of fructose can be calculated by applying simple formula written as under:

$$\text{Fructose (\%)} = \frac{\text{Total Reducing Sugar (g)} - \text{Total Sugar (g)}}{0.925} \times 100$$

2.4.5. Protein content

Protein content of collected honey samples were measured by Lowry's method [16].

2.5. Antioxidant activity

2.5.1. Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of the extracts was determined according to the method reported by Klein *et al,* [29]. The reaction mixture contained 1.0 mL of different concentration of extracts (20-100 µg/mL), 1.0 mL of iron-EDTA solution (0.13% ferrous ammonium sulphate 0.26% EDTA), 0.5 mL of 0.018% EDTA, 1.0 mL of DMSO (0.85% in 0.1 mol/L phosphate buffer pH 7.4) and 0.5 mL of 0.22% ascorbic acid. The tubes were capped tightly and heated in a water bath at 80-90°C for 15 min, the reaction was terminated by adding 1.0 mL of ice-cold TCA (17.5%). To the above reaction mixture 3.0 mL of Nash reagent (75.0 g of ammonium acetate, 3.0 mL of glacial acetic acid and 2.0 mL of acetyl acetone were mixed and distilled water was added to a total volume of 1 L) was added and incubated at room temperature for 15 min for color development. The intensity of the yellow color formed was measured at 412 nm against a reagent blank. Ascorbic acid and gallic acid were used as standards. The percentage of inhibition was determined by comparing test with standard.

$$\text{Hydroxyl radical (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{\text{Absorbance of control}} \times 100$$

3. RESULTS AND DISCUSSION

The density values for the honey samples investigated ranged from 1.04-1.53 g/cm^3. Tamilnadu honey samples showed maximum density compared to other collected samples. These values were higher than the values 1.425, 1.366, 1.398kg/m^2 obtained from Kachia, Zonkwa, Gidan-Waya respectively [17]. The density and moisture content are correlated and are used as a measure of adulteration in honey. As water content in honey increases, relative density decreases. High moisture could increase honey fermentation by certain osmotolerant yeasts [1] thus reduces honey's storage shelf life time.

In the present study, moisture content of all samples analyzed was within the range of acceptable international standard *i.e.* 20%. The total solids of the honey ranged from 81.0-87.4%. Honey from the Kerala had the maximum, whereas that from the Andhra Pradesh had the lowest percentage of total solids. The water insoluble solids content ranged from 0.12-0.17 %. All the honey samples showed acidic pH values, ranged between 4.25-4.62. These values are equal the range (4.3-6.0) that was reported for Nigerian honeys [18] but comparable to the range of 3.2 and 4.5 as reported by White and Landis, and to the range of 3.5 and 3.7 [19]. In general, the acidic pH value of honey inhibits the microorganism's activities [4] as most grow around pH 7. Free acidity in all tested samples was below 35mEq/kg. This information indicated the absence of unwanted fermentation [20]. Electrical conductivity, closely related to the concentration of mineral and organic acids, Values of electrical conductivity in the investigated honey samples were between 0.68 and 0.87 ms/cm (table 2).

The sugar is the main constituent that governs honey property and its content is firmly related to the degree

of maturity and botanical origin of honey. The maximum concentration of sugar was found in Kerala honey samples (86.4±2.41 %) and minimum in Karnataka (80.2±2.70 %) similar finding were recorded by other researchers on honeys [21, 22]. The range of reducing sugar content was 68.4-75.9 %, highest level was noted for Karnataka honey samples compared to samples of other region, which is similar to the international standard *i.e.* 60-100 g for honey [23]. The fructose content was found to be higher than that of the sucrose. Leon Ruiz*et al.* stated [24] that the sugar composition had discriminant capacity as markers of honey. Contrary to this, the findings of Munstedt *et al.*, [25] described that in honeys; the composition of fructose was higher than that of sucrose (Table 3).

Table 2: Physical properties of analyzed honey samples

Parameter	Average value of honey samples (M±SD)			
	Andhra pradesh	Karnataka	Kerala	Tamilnadu
Density (g/cm^2)	1.07±0.04	1.20±0.07	1.04±0.05	1.53±0.03
Moisture (%)	11.5±1.20	13.9±0.98	16.4±1.09	14.47±1.12
Total solids (%)	81.0±2.45	86.7±1.51	87.4±2.67	85.6±1.94
Water insoluble solids (%)	0.12±0.002	0.15±0.001	0.17±0.005	0.14±0.004
pH	4.37±0.98	4.55±0.47	4.62±0.63	4.25±0.87
Free acidity (mEq/kg)	38.4±1.28	32.7±1.67	26.6±1.41	22.4±1.72
Electrical conductivity (mS/cm)	0.84±0.06	0.68±0.04	0.74±0.07	0.87±0.08

Values are expressed Mean ± Standard Deviation; n=10

Table 3: Biochemical properties of analyzed honey samples

Parameter	Average value of honey samples (M±SD)			
	Andhra pradesh	Karnataka	Kerala	Tamilnadu
Total sugar content (%)	84.2±1.21	80.2±2.70	86.4±2.41	85.1±2.35
Reducing sugar content (%)	71.4±2.88	75.9±1.73	72.7±2.10	68.4±1.54
Sucrose content (%)	6.17±0.94	5.80±0.47	7.01±0.76	6.64±0.91
Fructose content (%)	22.7±1.34	25.9±1.25	26.5±0.87	23.1±1.18
Protein content (%)	0.70±0.004	0.45±0.002	0.52±0.003	0.38±0.005

Values are expressed Mean ± Standard Deviation; n=10

The hydroxyl radical scavenging effect can provide the overall hydrogen/electron donating activity of honey as well, like other dietary foods. The value of the HRS in honey samples was determined and given in Fig. 1.

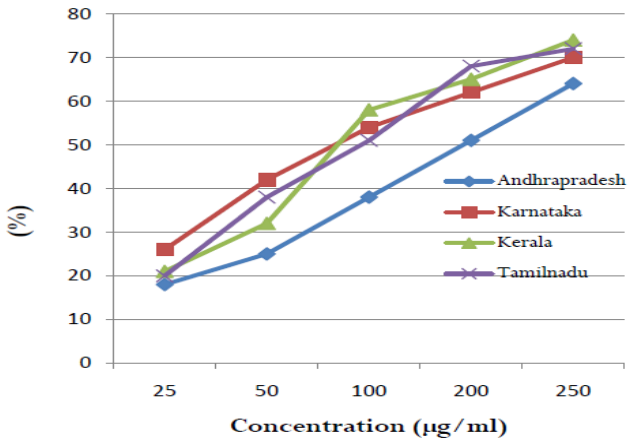

Fig. 1: Hydroxyl radical scavenging assay

The honey solutions exhibited varying degrees of scavenging capacity ranging from 64-74%, maximum scavenging activity (74%) was noted in Kerala honey samples. The decreasing absorbance also accompanied by a discoloration of HRS purple color [26]. Klein and Cohen [27] demonstrated that dark honeys had HRS inhibition values above 70% and light honeys demonstrated inhibition values below than 40%. Interestingly, although the Kerala honey sample was not dark, it generally measured high antioxidant activity.

4. CONCLUSION

In the present study, 40 different honey samples were investigated for their physico-biochemical and bioactive properties.Honey has powerful immune system booster. Its antioxidant and antibacterial properties help toimprove digestive system. Our results clearly noted all the collected honey sample physico-biochemical

properties were within standard. The antioxidant activity of honey can be attributed to the presence of antioxidant compounds, and to possible synergies between additional food constituents. There is potential for natural antioxidants to replace synthetic compounds in food systems to improve consumer perception.

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