

Analysis of Physico-Chemical Properties of Honey of Stingless Bee (Hymenoptera: Apidae: Meliponinae) of Southern Karnataka, India

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ABSTRACT

Stingless bee (Hymenoptera: Apidae: Meliponinae) honey is a dark black coloured nutritious and medicinally valued substance, produced by stingless bee species by using the nectar and pollen of blossoms of various plant species. Stingless bee honey exhibits variation in its physical properties, chemical composition and nutritional quality. Therefore, present investigation was conducted to assess the physical parameters and chemical constituents in the stingless bee honey collected from Mysore, Chamarajanagar and Kodagu districts of southern Karnataka. Results revealed significant differences with respect to the physical parameters and chemical constituents between the stingless bee honey samples. The pH ranged from 3.42 to 3.55, electrical conductivity ranged from 0.668 to 1.412 mS/cm, specific gravity was in between 1.35 and 1.38 g/cm³, absorbance at 660 nm was 0.39 to 1.696. The turbidity ranked between 0.09 to 0.38 NTU and the moisture with the honey was in between 30.85 to 40.8 g/100 g. Moreover, the chemical parameters such as glucose content in the honey was ranged between 11.0 to 17.0 g/100 g, fructose was 30.0 to 40.0 g/100 g with protein 237.14 to 595.48 mg/100 g. However, the total dissolved solids were ranged in between 285.2 and 613.6 in the honey samples. Further, minerals like, calcium, magnesium, sodium, potassium, iron, copper, chromium, zinc, manganese and phosphorous content in terms of parts per million did vary significantly between the honey samples collected from Mysore, Chamarajanagar and Kodagu districts of southern Karnataka. Furthermore, vitamins such 'C', 'B6', thiamine, riboflavin, niacin, biotin and lipoic acid were also indicated significant variations in the honey samples. Thus, physical parameters and chemical constituents are unique to the honey sample, collected from various districts which likes at different agro-climatic regions and demonstrated the influence of locally available flora and climate of that geographical region. During the present investigation, region specific uniqueness of stingless bee honey of southern Karnataka was recorded and fulfilled the requirement of honey analysis for quality and human consumption as like *Apis* species honey.

Keywords: Stingless bee honey, Physical, Chemical parameters, Southern Karnataka.

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INTRODUCTION

Honeybees produce honey which is a sweet substance used by man for various pharmacological purposes and human consumption as well. *Apis* and non-*Apis* species

like stingless bees produce honey by using the nectar and pollen of blossoms or plants,^[1] which are collected, transformed and combined with specific digestive enzymes of their own, then stored in the honey combs/pots to ripen and mature.^[2] More than 300 honey types are available in the international market across the world.^[3] The colour, flavour, minerals and vitamins content of honey depended on the flowers from which honeybees/stingless bees gather the nectar,^[4,5] geographical and climatic conditions.^[6,7] This results a great variation in physical and chemical composition of honey.^[3,7-10]

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China is the largest producer of honey in the world, producing 4,85,960 tonnes annually.^[11] India produced more than 1,33,200 metric tonnes of honey during 2021-2022.^[12] Of which major share comes mainly from the colonies of *Apis* species and only little amount of honey is produced from stingless bee species.^[13] Interestingly, stingless bee honey has high nutritional and therapeutic value compared to the honey produced by *Apis* species. However, published reports on the physico-chemical constituents of stingless bee honey are sparse^[14] have reported the analysis of physico-chemical properties of stingless bee honey^[15,16] and^[7] have reported the physico-chemical parameters of stingless bee honey from Brazil, Thailand and other parts of the world^[3] have provided a comprehensive review about physico-chemical properties of stingless bee honey from different parts of the world. Further,^[10] have reported the analysis of physico-chemical properties of stingless bee honey from Ethiopia. However, in India,^[17] has published the comparative account on physico-chemical characters of *Apis* and stingless bee honey. Later, published reports on the analysis of stingless bee honey for its physical and chemical parameters are poor. However,^[18-20] have recorded the physico-chemical properties of different floral honeys including stingless bee honey from Bangalore and other geographical locations of Kerala and Karnataka.

The International Honey Commission (IHC) has proposed certain constituents as quality criteria,^[21] which include: moisture content, electrical conductivity, sugar content, minerals, vitamins, etc.,^[3] These constituents depended on the nectar collected from different flowering plants^[6,22] and locally prevailed factors.^[3,15,23] Recent past, several published reports at national and international level described^[3] and insisted the necessity for regular analysis of honey to maintain quality and to provide contamination-free honey to consumers. The Indian Centre for Science and Environment (CSE) has surveyed samples from several national honey brands in India that were intended for export in 2010 and found the honey samples contaminated with antibiotics and heavy metals.^[24] Hence, regular analysis of honey is essential to maintain quality standards in the honey as per Codex Alimentarius.^[21] The physicochemical parameters of stingless bee honey have not been explored well in Karnataka.^[20] Hence, the present investigation was conducted to analyze the physico-chemical parameters and commonly occurring vitamins in the stingless bee honey in southern Karnataka.

MATERIALS AND METHODS

Collection and preparation of honey sample

The stingless bee honey collected from Meliponiculturists at Mysore, Chamarajanagar and Kodagu districts during 2021-2022. Samples were stored separately in airtight plastic containers in dark place until their analysis by following standard methods during the present investigation. Physical parameters such as pH, electrical conductivity, specific gravity, absorbance, turbidity and moisture contents were analyzed.

pH

5 mg honey dissolved in 95 mL distilled water and used that solution for pH determination as per the description of.^[25]

Electrical Conductivity (EC)

20 g honey was dissolved in 100 mL double distilled water to measure electrical conductivity at 27°C.^[26]

Specific gravity

The specific gravity was measured using formula specific gravity of honey = $C - A / D - A$, where, C = Weight of specific gravity of bottle with honey, A = Weight of specific gravity of bottle and D = Weight of specific gravity bottle with water as described.^[27]

Absorbance and turbidity of honey

Honey samples obtained from Mysore, Chamarajanagar and Kodagu were measured as per.^[28] Moreover, the moisture content was analyzed using standard methods.

Moisture

It is determined by using a refractometer. The moisture content of honey is expressed in grams per 100 g honey sample. Further, the chemical parameters such as glucose, fructose, protein, total dissolved solids, minerals and vitamins were analyzed using standard methods.

Estimation of Glucose

Glucose was estimated in the honey by GOD-POD kinetic assay kit of Auto span.^[28,29]

Estimation of Fructose: Fructose was estimated as per^[30]

Estimation of Protein

10 micro litre honey sample was diluted in 10 mL of double distilled water and prepared sample as per the method described by^[31] and read in 660 nm using ELICO Spectrophotometer SL 177 as per.^[31] Further, Total ten minerals such as Calcium (Ca), Magnesium (Mg), Sodium

(Na), Potassium (K), Iron (Fe), Copper (Cu), Chromium (Cr), Zinc (Zn), Manganese (Mn) and Phosphorous (P) were analyzed by using five-millilitre honey samples dissolved in 45 mL of distilled water by maintaining five replicates from each district and finally prepared the sample as per the description of^[32] taken the readings using an Atomic Absorption Spectrophotometer. Furthermore, seven vitamins namely: Vitamin 'C', Thiamine, Riboflavin, Niacin, Biotin, Lipoic Acid and Vitamin 'B6' were analyzed using standard methods. Collected data was systematically compiled and analyzed using standard statistical methods as per.^[33]

RESULTS

Results of physico-chemical analyses of stingless bee honey are presented in Tables 1 to 4. Total 6 physical parameters namely: pH, electrical conductivity, specific gravity, absorbance, turbidity and moisture content in the stingless bee honey samples collected from Mysore, Chamarajanagar and Kodagu districts of southern Karnataka are presented in Table 1.

pH

The pH of stingless bee honey from different districts of southern Karnataka were ranged from 3.42 to 3.55 and the analysis of variance of stingless bee honey between these districts indicated significant variation ($F=116.8$; $p>0.001$) (Table 1). Comparatively, stingless bee honey collected from Kodagu district had little higher pH (3.55 ± 0.01) than that of the Chamarajanagar and Mysore districts (Table 1).

Electrical conductivity (EC)

It was in between 0.668 and 1.41mS/m in stingless bee honey of southern Karnataka. The EC was very high (1.41 ± 1.65 mS/m) in the honey collected from Chamarajanagar district than that of other districts, but it was very less (0.668 ± 1.06 mS/m) in the honey collected from Kodagu district (Table 1).

Specific gravity

It was high (1.38 ± 0.001 g/cm³) in the stingless bee honey samples collected from Mysore and Chamarajanagar districts and it was less in 1.35 ± 0.001 g/cm³ in the honey sample collected from Kodagu district. The analysis of variance of stingless bee honey between these districts indicated significant variation ($F=4986.0$; $p>0.001$) (Table 1).

Absorbance

The Optical Density (OD) at 660nm was read for stingless bee honey and the OD ranged between 0.39 and 1.696 for the honey samples collected from different districts of southern Karnataka. The OD was high (1.696 ± 0.008) in the honey sample collected from Mysore and it was very less (0.39 ± 0.009) in the honey sample collected from Kodagu district. Analysis of variance of stingless bee honey between these districts indicated significant variation ($F=55734.0$; $p>0.001$) (Table 1).

Turbidity

Nephelometric turbidity units for turbidly for the stingless bee honey samples ranged in between 0.09 and 0.38. It was high (0.38 ± 0.002) in the honey collected from Mysore district and very less (0.09 ± 0.001) in the honey of Kodagu district (Table 1). Analysis of variance of stingless bee honey between these districts indicated significant variation ($F=50306.0$; $p>0.001$) (Table 1).

Moisture

Interestingly, the moisture content was high (4.08 ± 0.005) in the honey of Kodagu district and it was followed by 3.85 ± 0.01 each in the honey of Mysore and Chamarajanagar districts (Table 1). Analysis of variance of moisture content in the honey between these districts indicated significant variation ($F=669.4$; $p>0.001$) (Table 1). Thus, moisture content varied significantly between the stingless bee honey samples collected from different districts of southern Karnataka.

Table 1: Physical parameters of stingless bee honey of Southern Karnataka.

Sl. No.	District	Physical parameters of stingless bee honey					
		pH	Electrical Conductivity (mS/m)	Specific Gravity (g/cm ³)	Absorbance (at 660 nm)	Turbidity (NTU)	Moisture (g/100 g)
1.	Mysore	3.42±0.014	1.290±2.68	1.38±0.001	1.696±0.008	0.38±0.002	30.85±0.001
2.	Chamarajanagar	3.5±0.01	1.41±1.65	1.38±0.001	1.259±0.004	0.28±0.001	30.85±0.016
3.	Kodagu	3.55±0.01	0.668±1.06	1.35±0.001	0.39±0.009	0.09±0.001	40.80±0.005
	'F' value	116.8	2153.0	4986.0	55734.0	50306.0	669.4

Note: Each value is mean of five observations. All the 'F' values are significant at 0.001% level. mS/m: milliSiemens per meter; NTU: Nephelometric Turbidity Units.

Chemical properties

During the present investigation, total four chemical constituents such as glucose, fructose, protein and Total Dissolved Solids (TDS) were analyzed in the stingless bee honey samples. The glucose content was ranged in between 0.011 and 0.17 mg/g and indicated significant difference ($F=5807$; $p>0.001$) among the different districts. Moreover, the glucose content was high (0.017 ± 77.18 mg/g) in the honey of Mysore district and it was followed by 0.013 ± 0.001 mg/g in the honey of Chamarajanagar district and 0.011 ± 101.16 mg/g in the honey of Kodagu district (Table 2). However, the fructose content was little higher compared to glucose and it was ranged in between 0.03 to 0.04mg/g in the honey and there existed a significant difference ($F=15496.0$; $p>0.001$) between different districts of southern Karnataka (Table 2).

Protein

The concentration of protein was high (595.48 ± 15.8 mg/g) in the honey collected from Chamarajanagar district and it was 431.32 ± 15.8 mg/g less in the honey sample of Kodagu. However, protein content was less (237.14 ± 2.51 mg/g) in the honey sample collected from Kodagu and showed significant variation ($F=1190.0$; $p>0.001$) (Table 2). Further, the Total Dissolved Solids (TDS) in the honey samples were ranged in between 285.2 and 613.6. The honey sample obtained from Chamarajanagar district indicated highest (613.6 ± 0.54

TDS and lowest (285.2 ± 0.44 TDS) was in the honey sample obtained from Kodagu district and indicated significant variation ($F=52505$; $p>0.001$) (Table 2).

Minerals

Table 3 shows the minerals composition in stingless bee honey collected from Mysore, Chamarajanagar and Kodagu districts of southern Karnataka. Honey samples were screened for 10 minerals namely: calcium, magnesium, sodium, potassium, iron, copper, chromium, zinc manganese and phosphorous. Excepting chromium all the minerals were recorded in mg per 100 g of honey, whereas, chromium was found Below Detection Limit (BDL). The potassium (677.76 ± 19.33 to 2298.3 ± 3.55 mg/100 g), calcium ranged from 113.73 ± 12.86 to 361.87 ± 33.93 mg/100 g. It was followed by magnesium, ranging from 52.28 ± 1.89 to 150.69 ± 0.55 mg/100 g. The sodium was ranging from 28.13 ± 4.34 to 68.45 ± 2.06 mg/100 g. Similarly, other minerals such as iron (2.74 ± 0.63 to 185.3 ± 2.00 mg/100 g), copper (0.23 ± 0.10 to 0.54 ± 0.57 mg/100 g), chromium (BDL and 0.69 ± 0.55 mg/100 g), zinc (1.27 ± 0.12 to 2.76 ± 1.35 mg/100 g), manganese (1.82 ± 0.67 to 13.36 ± 1.51 mg/100 g) and phosphorous (32.9 ± 1.99 to 275.59 ± 4.97 mg/100 g) presence in the stingless bee honey indicated considerable variations (Table 3). Analysis of the variance of these minerals in the stingless bee honey indicated significant differences between the different districts of southern Karnataka

Table 2: Chemical parameters of stingless bee honey of southern Karnataka.

Sl. No.	District	Chemical parameters of stingless bee honey			
		Glucose (g/100 g)	Fructose (g/100 g)	Protein (mg/100 g)	Total Dissolved Solids (TDS) (mg/100 g)
1.	Mysore	17.0±7.18	30.0±7.82	431.32±15.8	574.8±2.94
2.	Chamarajanagar	13.00±7.71	30.0±7.72	595.48±12.11	613.6±0.54
3.	Kodagu	11.0± 10.12	40.0±17.49	237.14±2.51	285.2±0.44
	'F' value	5807.0	15496.0	1190.0	52505.0

Note: Each value is mean of five observations. All the 'F' values are significant at 0.001% level. g=gram.

Table 3: Minerals composition of stingless bee honey of southern Karnataka.

Sl. No.	District	Minerals composition of stingless bee honey (mg/100g)									
		Ca	Mg	Na	K	Fe	Cu	Cr	Zn	Mn	P
1.	Mysore	113.73±12.86	52.28±1.89	68.45±2.06	677.76±19.33	41.02±1.00	0.39±0.59	0.21±0.52	1.68±0.57	2.37±0.62	91.25±0.89
2.	C. Nagar	361.87±33.93	150.69±0.55	54.16±3.62	2298.3±3.55	185.3±2.00	0.54±0.57	0.69±0.55	2.76±1.35	13.36±1.51	275.59±4.97
3.	Kodagu	115.61±1.37	77.4±1.47	28.13±4.34	692.38±2.45	2.74±0.63	0.23±0.10	BDL±0.00	1.27±0.12	1.82±0.67	32.9±1.99
	'F' value	139.0	3869.0	103.8	19893.0	15417.0	0.303NS	BDS	3.482*	120.1	4885.0

Note: Each value is mean of five observations. All the 'F' values are significant at 0.001% level. PPM: Parts Per Million; BDL: Below Detection Limit. * Value is not significant. Ca: Calcium; Mg: Magnesium; Na: Sodium; K: Potassium; Fe: Iron; Cu: Copper; Cr: Chromium; Zn: Zinc; Mn: Manganese and P: Phosphorous.

Table 4: Vitamins composition of stingless bee honey of Southern Karnataka.

Sl. No.	District	Vitamins of stingless bee honey of southern Karnataka (in IUs/ μ g)						
		'C'	Thiamine	Riboflavin	Niacin	Biotin	Lipoic Acid	'B6'
1.	Mysore	15.46 \pm 0.00	43060 \pm 1425.48	627.69 \pm 33.70	476.88 \pm 0.72	889.56 \pm 6.04	102480 \pm 178.88	5365.62 \pm 8.55
2.	Chamarajanagar	10.66 \pm 0.00	54260 \pm 944.45	267.69 \pm 25.27	529.01 \pm 1.63	851.71 \pm 0.00	77920 \pm 334.66	4399.99 \pm 25.67
3.	Kodagu	5.86 \pm 0.00	49300 \pm 1634.62	104.61 \pm 4.21	463.11 \pm 1.36	824.67 \pm 0.00	107360 \pm 536.65	4243.74 \pm 25.68
	'F' value	84.43	0.00	599.3	3550.0	436.0	8644.0	3978.0

(Table 4). During the present investigation, Potassium, Sodium, Calcium and Magnesium were detected as major minerals in stingless bee honey collected from different districts of southern Karnataka. However, during the present investigation all the minerals found in detection limits (Parts per Million) except chromium. Since, mineral content is used as an important index, associated with the environment, where nectar and pollen are collected by honeybees.

Vitamins

Table 4 shows the vitamins composition in stingless bee honey collected from Mysore, Chamarajanagar and Kodagu districts of southern Karnataka. Total seven vitamins were screened and most of them were differed in their IU content. Vitamin 'C' and 'B6', thiamine, riboflavin, niacin, biotin and lipoic acid were found in international units and their content was ranged in between 5.86 \pm 0.00 and 15.46 \pm 0.00, 4243.74 \pm 25.68 and 5365.62 \pm 8.55, 43060 \pm 1425.48 and 54260 \pm 944.45, 104.61 \pm 4.21 and 677.76 \pm 33.70, 463.11 \pm 1.36 and 529.01 \pm 1.63, 824.67 \pm 0.00 and 889.56 \pm 6.04, 77920 \pm 334.66 and 107360 \pm 536.65 international units respectively (Table 4). Analysis of variance of different vitamins contents in the stingless bee honey collected from Mysore, Chamarajanagar and Kodagu districts indicated significant differences excepting thiamine (Table 4).

DISCUSSION

Physical properties

Usually, honey is acidic in nature^[18] and shows a pH ranging from 3.2 to 4.5.^[3] However, the average pH should be ranged from 3.9 to 6.5^[34] or 3.4 to 6.10 in a honey.^[21,35] Since, honey pH is considered as one of the useful parameters to determine the geographical origin of honey.^[3] The pH measure is the total concentration of hydrogen ions that provides information on the strength of acidity^[18] that indirectly indicates the rich ash content^[36] and minerals like calcium, sodium, potassium and other ash constituents.^[18] Interestingly, low pH in honey help avoid microbial contamination. But, the

optimum pH for most organisms is in between 7.2 and 7.4. Surprisingly, during the present investigation, honey samples indicated a pH in between 3.42 and 3.55 which was little less compared to the standard pH prescribed by the^[18,35] have recorded very less pH i.e., 3.54 to 3.76 in multifloral honey collected from the colonies of *Apis* species at few areas of southern Karnataka. But, during the present study, the stingless bee honey shows little less pH compared to the honey of *Apis* species^[35] and others.^[3] The electrical conductivity in the stingless bee honey samples was not similar compared to the earlier published reports but below the maximum limit as indicated by the international standard.^[3,37] The Yemeni and Egyptian honey samples had the EC 4.18 \pm 1.98 mS/cm and Saudi and Indian (Kashmiri) honey samples have indicated the lower EC 0.53 and 0.67 mS/cm respectively.^[37] The multifloral honey of *A. dorsata* in Mysore district showed EC 0.67mS/cm and it was less 0.65 and 0.58mS/cm respectively in the *A. dorsata* honey collected from the Chamarajanagar and Kodagu districts^[38,39] have reported the EC in the range of 0.33 to 0.94ms/cm in Indian honeys. EC is closely related to the concentration of minerals and organic acids and show great variability according to the floral origin.^[40,41] Since, Mysore, Chamarajanagar and Kodagu districts have varied edaphic factors, floral source and local climate,^[42] perhaps all these prevailed different conditions might have influenced the variation of electrical conductivity in the honey of stingless bee. Thus, stingless bee honey revealed variation in its electrical conductivity. Similar type of observations was reported by^[18,37-43] and our observations are on par with the published reports of^[3] The specific gravity is another vital physical parameter, used to determine the honey quality.^[44] During the present investigation, the specific gravity ranged in between 1.35 to 1.38 g/cm² in the honey of stingless bees. In the honey of *Apis* species, the specific gravity would be ranged from 1.33 to 1.36 g/cm² in unifloral honey,^[43] 1.32 to 1.45 g/cm² for various unifloral and multifloral honeys^[38,42,44] have recorded lowest 1.52 specific gravity with an average of 1.40 \pm 0.03 g/cm² in *Apis* species from Karnataka.^[18] However, stingless bee honey of southern Karnataka

showed little higher value of specific gravity compared to *Apis* species as reported by.^[18] In the honey of stingless bee collected from southern Karnataka, specific gravity was almost nearer to the earlier reports published by ^[3, 38, 42 & 44]. Perhaps, time of storage, harvesting methods, extraction from the comb and ripening process might have influenced the specific gravity and that could result in the variation of specific gravity in different type of honeys ^[3, 43]. Further, the absorbance and turbidity of honey indeed specific parameters, used to identify the colour and status of honey.^[45] The stingless bee (e.g. *Tetragonula irridipennis*) honey is dark amber colour^[18] due to the presence of tyrosine and tryptophan.^[17] Moreover, honey colour usually ranges from light yellow to amber, dark amber and black in extreme cases and sometimes even green or red blue in *Apis* species.^[37] However, the colour of stingless bee honey was dark black with most of the samples collected from southern Karnataka. The colour variation is mainly based on the flora, geographical origin and climate.^[46] During the present study, stingless bee honey samples revealed dark black colour with an absorbance 0.39 to 1.696 at 660 nm and the turbidity 0.09 to 0.38 NTU. Hence, the available stingless bee honey in this region nearer to the standards of IHC^[3,21,34] have recorded highest moisture content (19.80 to 20.83%) in the honey of stingless bee (e.g. *Tetragonula irridipennis*) compared to *Apis* species.^[18] Furthermore, moisture content is affected by climate, season and moisture content of original plant nectar.^[21] Moreover, the moisture content is depended on the harvesting season, degree of honey maturity, ripeness, processing techniques and storage conditions.^[18] Surprisingly, during the present study, 30.85 to 40.85 g/100 g moisture content recorded and indicated the highest moisture content compared to the earlier published reports^[20] have recorded 20.07 to 23.34% of moisture in *A. cerana* honey^[15] have recorded 25.99 to 36.89% moisture in the honey of stingless bee species in Brazil and it was higher than the threshold for Brazilian and international standards. As stingless bee honey shows high hygroscopic behaviour ^[15] preserved even if the environment where stingless bees live have low humidity ^[47]. Perhaps, prevailed varied geographical conditions at southern Karnataka might have interfered with moisture content in the flower and the hygroscopic behaviour of honey of stingless bees and resulted to have high moisture content compared to the earlier published reports. Thus, our observations are on par with the observations of.^[15,18,20,47]

Chemical properties

Amount of glucose in stingless bee honey was less than that of fructose. Similar types of observations were

reported by.^[25,48,49] Fructose is one of the major sugars, present more in the honey compared to glucose, because the nectar is floral in origin, collected by honeybees. So, glucose content is less compared to fructose whether it is *Apis* honey or stingless bee honey, as both the species are depended on flower nectar for their carbohydrate source. Further, protein content in a honey is depended on the flora of the region^[45] and the pollen and nectar gathering ability of the stingless bees.^[50] Perhaps, locally available flora mightn't similar between different districts of southern Karnataka and stingless bees couldn't get similar type of nectar and pollen during their forage. Hence, variations in the protein content of stingless bee honey resulted. Protein content of a honey is geographical region specific that helps reveal the quality in terms of protein composition.^[50] *Apis* species honey shows 5 mg/g and 1.91 to 4.69 mg/mL of protein content in India.^[6,51] However, during the present investigations, the protein content in the stingless bee honey was more than that of the honey obtained from the colonies of *Apis* species. Further,^[3] have reported 14 minerals in a honey of stingless bees collected from different parts of the world. Surprisingly, no specific standards have been set in terms of the mineral composition of stingless bee honey.^[3] The most abundant minerals in stingless bee honey were found to be Potassium (K), ranging from 45.9 mg/100 g to 1357.76 mg/100 g. This is followed by Sodium (Na), ranging from 0.78 mg/100 g to 589.7 mg/100 g. Next is Calcium (Ca), ranging from 3.85 mg/100 g to 199.6 mg/100 g and finally Magnesium (Mg), ranging from 1.15 mg/100 g to 73.76 mg/100 g.^[3] During the present investigation, Potassium, Sodium, Calcium and Magnesium were detected as major minerals in stingless bee honey collected from different districts of southern Karnataka. Thus, our observations are similar to the observations of.^[3] Have reported few minerals (e.g. chromium, copper, iron, manganese, magnesium and) in unifloral honey of *Apis* species (e.g. Lychee, Clover and Jamun honey) collected from different parts of India and found below detection limits. ^[1,38,52] However, during the present investigation, all the minerals found in detection limits (Parts Per Million) except chromium. Since, mineral content is used as an important index, associated with the environment, where the nectar and pollen are collected by honeybees. Surprisingly, minerals such as calcium, iron, magnesium, sodium and potassium would elevate the nutritional value of honey and considered their presence as potential indicator of geographical origin of honey.^[38] Therefore, minerals are important to the living system, which play a crucial role in biological systems. Few minerals help support the

physiological and biochemical reactions, which induce general metabolism, blood circulation and influence as catalysts in reproductive process. Thus, mineral content of the honey enhance the nutritional status in turn help benefit the consumers. Hence, stingless bee honey possesses all the minerals which are required by man and found to be useful for human consumption. Similarly, the vitamins composition in stingless bee honey collected from Mysore, Chamarajanagar and Kodagu districts of southern Karnataka indicated significant differences excepting thiamine. Honey contains more than 200 distinct chemical compounds. Honey collected from different geographical regions exhibit specific composition and properties, which are unique and dependent on the geographical origin of nectar that the bee collected, prevailed local climate^[6] and harvesting and storage methods.^[15,53] Therefore, regular analysis of stingless bee honey for physico-chemical parameters is important to update the quality standard of stingless bee honey^[3&15]. To produce quality stingless bee honey, stingless bees rearing in the name of 'Meliponiculture' undertaken by many farmers who have made this activity more popular in Neotropical regions of Central and South America, Thailand, Malaysia, Nigeria and Australia.^[3] However, in India, Meliponiculture is being practised since many years, despite undocumented traditional Meliponiculture at different geographical regions in a sporadic way; it is difficult to popularize this activity unlike 'Apiculture'. Therefore, Meliponiculture need more encouragement, should be undertaken on large scale basis and the honey produced by stingless bees to be regularly analyzed for its physico-chemical parameters as per HIC to validate the quality standards for human consumption. Thus, our observations are in conformity with the observations of earlier workers.^[1-3,6-8,10,20,34,37,38,42,43,46,50,51-53] Stingless bee honey collected from different districts of Karnataka is meeting the standards of IHC and from the food safety point of view it is safe for human consumption. Our observations are in conformity with the previous studies made by.^[1,47-49] Thus, honey contains various chemical compounds, which present differently in different honey samples collected from different districts of southern Karnataka. Hence, each honey sample has specific chemical composition which is unique physical properties with little variations and depended on various prevailed floral, edaphic and climatic factors^[6,15,42] which are specific at different districts of southern Karnataka.

CONCLUSION

Stingless bee honey contains several distinct chemical compounds and exhibited different physical properties

which are unique to southern Karnataka. The chemical constituents of stingless bee honey varied considerably between different districts and indicated the influence of locally prevailed varied ecological conditions, available flora and uneven harvesting and preservation methods followed by Meliponiculturists at Mysore, Kodagu and Chamarajanagar districts. During the present investigation, it was witnessed with the results obtained in relation to physical properties and chemical constituents of honey of stingless bees. Therefore, regular analysis of honey for its physico-chemical parameters is necessitated to maintain the quality standards of honey as prescribed by the International Honey Commission (IHC) to meet the consumer's demand.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Total six physical parameters (e.g. pH, electrical conductivity, specific gravity, absorbance, turbidity and moisture content), four chemical constituents (e.g. glucose, fructose, protein and total dissolved solids), ten minerals (e.g. calcium, magnesium, sodium, potassium, iron, copper, chromium, zinc manganese and phosphorous) and seven vitamins (e.g. vitamin 'C', 'B6', thiamine, riboflavin, niacin, biotin and lipoic acid) were analyzed in the stingless bee honey samples collected from Mysore, Chamarajanagar and Kodagu districts of southern Karnataka. Interestingly, majority of the physical and chemical properties were indicated significant variation between different districts of southern Karnataka. The pH, EC, specific gravity, optical density, turbidity and moisture content was respectively 3.42 to 3.55, 0.668 to 1.41 mS/m, 1.35 ± 0.001 to 1.38 ± 0.001 g/cm², 0.39 to 1.696 (at 660 nm), 0.09 to 0.38 Nephelometric turbidity units and 3.85 ± 0.01 to 4.08 ± 0.005 in stingless bee honey. Moreover, the glucose, fructose, protein and Total Dissolved Solids (TDS) were ranged in between 0.011 and 0.17 mg/g, 0.03 to 0.04 mg/g, 237.14 ± 2.51 mg/g to

595.48±15.8 mg/g and 285.2 and 613.6. Further, except chromium, all the minerals were recorded in mg per 100 g of honey, whereas, chromium was found Below Detection Limit (BDL). Furthermore, the vitamin 'C' and 'B6', thiamine, riboflavin, niacin, biotin and lipoic acid content was ranged in between 5.86±0.00 and 15.46±0.00, 4243.74±25.68 and 5365.62±8.55, 43060±1425.48 and 54260±944.45, 104.61±4.21 and 677.76±33.70, 463.11±1.36 and 529.01±1.63, 824.67±0.00 and 889.56±6.04, 77920±334.66 and 107360±536.65 international units respectively. Thus, stingless bee honey is with various chemical constituents, exhibit specific physical properties and meets the quality standards with little variations.

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