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PHYSICOCHEMICAL AND POLLEN ANALYSIS OF WESTERN GHATS HONEY OF KARNATAKA, SOUTH INDIA

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ABSTRACT
The present investigation was undertaken to determine the physicochemical properties and pollen analysis of 30 honey samples collected from different locations of Western Ghats of Karnataka, South India, out of which 8 samples were found to be unifloral and 22 were multifloral. In unifloral honey samples, *Sapindus laurifolia*, *Areca catechu, Acacia* sp., *Mangifera indica, Terminalia bellerica, Syzygium* sp., *Pongamia pinnata*, and *Eucalyptus* sp., were predominant pollen types. These samples were also analyzed for several physicochemical parameters such as moisture, ash, pH, total acidity, total sugar, proteins, alkaloids, and phenols. This type of physicochemical and pollen analysis of honey samples favors the possibility of utilizing the rich flora of Western Ghats for good quality honey.

KEY WORDS: Multifloral honey, physicochemical characteristics, Pollen analysis, Unifloral honey, Western Ghats.

INTRODUCTION

Honey is a carbohydrate rich naturally complex product produced by honeybees from floral nectar. Honey has been used by all civilizations as nutrient food and in traditional medicine. Honey has different physical, chemical and pollen spectra depending on the floral sources from which it has been collected. The quality of honey depends on various physiological factors such as climate, soil, etc. (Asif 2002 & White *et al*., 1964). Honey contains sugar, proteins, moisture, vitamins, minerals, enzymes, polyphenols and flavonoids (Al-manary *et al.* 2002**).** Because of this unique and complex nature, honey is proved to be useful in the treatment of burns, wounds, skin ulcers, as an antioxidant, and in the treatment of external eye diseases (McCathy 1995 & Balasubramanyam 2011). Furthermore, honey is a highly valuable ingredient in condiments, beverages, sauces and sweets (Rasmussen *et.al.* 2008). Unifloral honeys of a particular origin may be greatly accepted by consumers, so the importance of understanding the composition of honey from human point of view is valuable (Seijo 1997**).** In fact numerous studies have been reported on physical, chemical, and melissopalynological parameters of honeys from all over the world (Azeredo *et al*., 2003, Downey *et. al*., 2005, Finola *et. al*., 2007, Al *et.al*., 2009, Xesus *et.al*., 2010). The scientific literature revealed that not much information is available with respect to physicochemical characteristic of honeys from Western Ghats of Karnataka, which is one of the major regions of honey production and mega biodiversity hotspot in India.The purpose of this study was to investigate some physicochemical parameters such as moisture, ash, pH, total acid, and total sugar, proteins, alkaloids, phenols and microscopical analysis of honey collected from different regions of Western Ghats in Karnataka, South India.

MATERIALS AND METHODS

Honey sampling

Honey samples were collected by beekeepers from 30 different locations in Western Ghats of Karnataka. The study covers the physicochemical characterization and melissopalynological studies of pollen present in honeys.

Pollen analysis

All the samples were subjected to pollen analysis as per the method of Louveaux *et al*. (1978) with the aim to confirm the floral origin of honey samples. Pollen analysis is based on the extraction of pollen grains from 5 g of crude honey. The sample was dissolved in distilled water and the sediment was mixed with acetolysis mixture and incubated in water bath, then centrifuged and decanted. After this, sediment of pollen grains was mixed with glycerin. The examination of pollen slides were carried out with a light microscope at 400x and 1000x for identification the pollen type. The pollen grains were counted to determine the relative frequency of the different pollen type in honey samples. Pollen identification was made based on the relevant literature and reference pollen slides.

PHYSICOCHEMICAL ANALYSIS

Moisture content: For the estimation of moisture content, 5g of each honey samples were put in a flat dish and dried in the oven at 105 ^{0}C for 3 hrs. Covered and cooled in desiccator and weighed. The samples were re-dried for one hr in the oven, cooled and reweighed. The process was repeated at one hr drying intervals until a constant weight was obtained (William,et. al. , 2009). Determination of ash content, 5gms of each sample was taken in a crucible and ashed in a muffle furnace at 600^{o}C, cooled and weighed (AOAC 1990). Ash percentage was calculated. The pH value of honey samples were measured using a digital pH meter with solution prepared with 10g of honey in 75ml of distilled water (AOAC, 1990). Free acidity

was determined by Titrimetric method (AOAC 1990). 10g of honey samples were homogenized with 75 ml of distilled water and filtered. The solution was titrated by adding 0.05M NaoH and stopped at pH 8.5. Acidity (milliequivalent of acid per kg of honey) was determined as 10 times the volume of NaoH used in titration. Total sugar content was assessed calorimetrically by anthrone methods described in laboratory manual (Jayaraman 1981). Protein content of honey samples was determined following the Method of Lowry *et al*., 1951. Total phenol was calorimetrically determined by following the method based on Folin-Ciocalteu's reagent (Slinnkard & Singleton, 1977). Alkaloid content was determined according to Harbon, 1973

RESULT AND DISCUSSION

Pollen analysis

Table 1 shows the occurrence of honey types and pollen count. Honeys are always classified as unifloral and multifloral depending on pollen frequency. Unifloral honeys were considered those with dominant pollen type over 45%. In this study, 8 out of 30 honey samples were unifloral and 22 were multifloral.

TABLE 1: Honey samples collected from Western Ghats for pollen analysis

Sl.No.	Location of honey samples	Honey type	Colour	Pollen count	Group
1.	Kushalnagara	Multifloral	Amber	122000	V
2.	Nagarahole	Multifloral	Yellow	58400	IV
3.	Talakaveri	Multifloral	Dark yellow	132000	V
4.	Madkeri	Multifloral	Yellow	125000	V
5.	Sringeri	Multifloral	Yellow	124000	V
6.	Horanadu	Multifloral	Amber	123000	V
7.	Sagara	Multifloral	Dark yellow	66000	IV
8.	Tirthalli	Multifloral	Dark yellow	55700	IV
9.	Hosanagara	Multifloral	Light amber	141000	V
10.	Puttur	Unifloral (*Syzygium* sp.)	Light amber	100000	V
11.	Sulya	Multifloral	Yellow	115000	V
12.	Kundapura	Unifloral (*Acacia* sp.)	Dark yellow	60800	IV
13.	Dharmasthala	Multifloral	Dark yellow	130000	V
14.	Sakaleshpura	Unifloral *(Terminalia bellarica)*	Dark amber	108500	V
15.	B R Hills	Multifloral	Dark amber	123000	V
16	Chamarajanagara	Unifloral *(Pongamia pinnata)*	Light yellow	62500	IV
17.	Bandipur	Multifloral	Amber	120000	V
18.	Mysore	Unifloral (*Eucalyptus* sp.)	Amber	62500	IV
19.	Siddapura	Unifloral (*Areca catechu)*	Light amber	60800	IV
20.	Yellapura	Multifloral	Amber	139000	V
21.	Gokarna	Multifloral	Dark amber	133000	V
22.	Honnavara	Unifloral (*Sapindus* sp.)	Light yellow	102000	V
23.	Talaguppa	Unifloral *(Mangifera indica)*	Dark amber	105000	V
24.	Jamboti	Multifloral	Dark yellow	64800	IV
25.	Belgaum	Multifloral	Amber	118400	V
26.	Dandeli	Multifloral	Yellow	120000	V
27.	Castle Rock	Multifloral	Light yellow	98000	IV
28.	Vittla	Multifloral	Light yellow	65800	IV
29.	Karwara	Multifloral	Light yellow	51600	IV
30.	Gundlupet	Multifloral	Yellow	125000	V

The multifloral honeys analyzed were characterized by high pollen diversity with many pollen types, whereas unifloral honeys analysed were characterized by low pollen diversity. The unifloral honeys were from Puttur (*Syzygium* sp.), Kundapura (*Acacia* sp.), Sakaleshpura (*Terminalia bellerica),* Chamarajanagara *(Pongamia pinnata),* Mysore *(Eucalyptus* sp.), Siddapura (*Areca catechu*), Honnavara (*Sapindus laurifolia) and* Talaguppa *(Mangifera indica*). The absolute pollen per gram of honey was in the range of 51,600 to 1, 41,000. The minimum pollen count was found in Karwar honey sample and maximum pollen count was observed in Hosanagara honey sample. 36.6% of honeys belonging to group IV were considered to be very rich in pollen content and

63.4% belonging to group V were extremely rich in pollen count. The color of honey samples in present work range from pale yellow to dark amber depending on floral sources and composition (Table 1). In general, lighter colors are associated with delicate flavors and darker colors with strong flavors and less attractive appearance. Color is thus a factor in grading and marketing honey.

Physicochemical parameters

Table 2 summarizes the result obtained from physicochemical analysis of honey samples. The amount of moisture content in the honey is an important factor in determining the quality of honey in the world honey trade. Honey having high water content is more likely to ferment. A maximum of 21% of moisture was suggested as a standard (Bogdanov 1999). It is also an important factor in determining its storage quality. In the present study, moisture content varied from 7.4% to 32.5%. Honeys from Sringeri (32.50%), Yellapura (31.67%), Gundlupet (31.02%) and the unifloral honey *Syzygium* sp., (30.0%) from Puttur exceeded the permitted limit of Codex Alimentarius (2001). This may be because of premature extraction of honey. Low moisture content helps to protect honey from microbial activity and can be preserved for long period.

The ash content varied from 0.16% to 0.85%, the highest ash value were observed in the honey sample collected from Gokarna (0.85%) and lowest was observed in the sample from Gundlupet (0.16%). The present study supports the report of Al *et al.,* (2009), light colored honey usually have low ash content than the dark colored honey generally which have higher ash content. According to Bogdanov (1999), the pH of honey should be varying from 3.2 to 4.5. In the present study, the honeys pH value varied from 3.2 to 4.77. Honeys of *Mangifera indica* (4.77) from Talaguppa, *Areca catechu* (4.65) from Siddapura and Gokarna (4.60) samples were outside the range, which have been shown to have high pH value. Low pH of honey inhibits the presence and growth of microorganisms and increases the shelf life of honey (Terrab *et al*., 2002)**.**

TABLE 2: Results of physicochemical analysis

Sl.No	Location of honey samples	pH	Ash %	Moisture %	Acidity Meq/kg	Total sugar %	Protein mg/g	Phenol mg/g	Alkaloid %
1.	Kushalnagara	4.0	0.35	22.56	8.8	80.3	0.70	0.60	6.6
2.	Nagarahole	3.63	0.52	15.40	9.0	70.0	0.34	0.32	10.0
3.	Talakaveri	4.08	0.45	12.64	8.0	73.6	0.85	0.40	13.8
4.	Madkeri	3.86	0.45	12.64	18.0	72.1	0.65	0.27	12.0
5.	Sringeri	3.78	0.65	32.50	9.0	77.5	0.75	0.92	7.5
6.	Horanadu	3.45	0.75	11.51	9.0	76.8	0.65	0.53	15.4
7.	Sagara	3.50	0.58	18.21	8.0	73.2	0.55	0.96	8.2
8.	Tirthalli	4.30	0.42	7.40	7.0	78.6	0.30	0.66	11.0
9.	Hosanagara	4.09	0.67	19.46	22.0	78.2	0.95	0.47	7.4
10.	Puttur	4.12	0.69	30.00	20.0	80.0	0.67	0.41	9.4
11.	Sulya	4.33	0.17	18.46	9.0	73.1	0.70	0.49	17.8
12.	Kundapura	4.42	0.53	18.60	9.0	79.8	0.40	0.64	15.2
13.	Dharmasthala	3.48	0.60	15.54	20.0	75.0	0.80	0.67	10.6
14.	Sakaleshpura	3.96	0.49	12.40	39.0	70.5	0.70	0.65	8.2
15.	B R Hills	4.23	0.79	15.47	22.0	79.8	0.72	0.52	16.8
16	Chamarajnagar	3.57	0.55	18.60	12.0	70.5	0.38	0.53	4.2
17.	Bandipur	3.20	0.42	18.98	22.0	76.6	0.60	0.62	12.0
18.	Mysore	4.42	0.50	11.80	12.0	70.5	0.47	0.40	12.0
19.	Siddapura	4.65	0.38	10.80	8.0	71.1	0.33	0.40	19.0
20.	Yellapura	3.58	0.80	31.67	18.0	81.6	0.92	0.73	2.8
21.	Gokarna	4.60	0.85	16.38	18.0	64.2	0.85	0.74	2.6
22.	Honnavara	4.44	0.43	8.00	13.0	72.0	0.85	0.20	8.1
23.	Talaguppa	4.77	0.48	9.80	31.0	79.8	0.78	0.84	9.2
24.	Jamboti	4.31	0.23	21.42	20.0	81.6	0.50	0.36	10.0
25.	Belgaum	4.37	0.37	25.02	18.1	81.1	0.70	0.31	9.6
26.	Dandeli	4.59	0.70	15.69	19.0	69.4	0.70	0.51	9.4
27.	Castle Rock	4.16	0.54	16.12	18.0	72.1	0.62	0.73	12.2
28.	Vittla	3.95	0.48	19.02	21.0	73.5	0.50	0.66	2.0
29.	Karwara	3.92	0.49	10.71	10.0	78.1	0.22	0.87	3.8
30.	Gundlupet	3.68	0.16	31.02	22.0	74.2	0.82	0.79	13.4

Acidity is an important quality criterion. The total acidity of all the honey samples analyzed in this study was within the prescribed limits of 40 meq kg as proposed by Codex Alimentarius Commission (2001). Unifloral honey *Terminalia bellerica* from Sakleshpura has a higher level of total acidity (39meq kg) and *Syzygium* sp. from Putter has lower acidity (7.0 meq kg). Multifloral honey samples of Bandipur and Gundlupet showed highest acidity (22.0

meq kg), whereas honey samples from Kushalnagara, Talakaveri and Siddapura showed lowest acidity (8.0 meq kg). The variation of this factor is due to source of nectar and climatic conditions of the area (Asif *et al*., 2002**).** High acidity of honey also plays an important role in preventing bacterial growth. Sugars are the main constituent of honey comprising about 90% of honey dry weight. The sugar spectrum of honey depends on the sugar present in nectar and enzyme present in bees. The highest content of total sugar was found to be 81.6% in Yellapura and Jamboti honeys and 81.1% in Belgaum honey sample.

In the present study, the protein content of analysed honey samples ranged between 0.22 mg/100 g to 0.95 mg/100 g. The result obtained depends on the pollen count of these collected honeys which was successfully used for the determination of botanical origin (Baroni *et al.,* 2002). Hosanagara honey showed higher content of protein. The total alkaloid detected in honey samples in the present study was 2.0% to 19.0%. Phenols are an important group of compounds with respect to the appearance and functional properties of honey (Al- mammary *et al***.,** 2002). These compounds in honey are the main constituents which have protective effect against reactive oxygen species production. In the present study, the total phenolic content varied with the highest value obtained being 96 mg/100 g honey. Dark colored honeys are reported to contain more phenols (Amiot *et al.,* 1989). Honey is produced in every country of the world and it is an important energy food. Honey quality is assessed largely on the basis of its color, flavor, physicochemical composition and pollen spectrum. Based on the results obtained, it is concluded that the 75% of chemical composition of honey samples collected from Western Ghats confirmed the requirement of Codex Alimentarius 2001. Generally multifloral honey has mixed flavor and aroma compared to unifloral honey which has unique flavor and aroma. The Western Ghats of Karnataka has been considered as a potential area for commercial honey production because of its rich diversity of flowering plants of apicultural importance. From the economic point of view, the assessment of floral origin and physiochemical properties add to quality and commercial value of the honey. In conclusion, honeys of Western Ghats are of good quality and can be used as supplementary food and also in traditional medicine.

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