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Physicochemical Properties, Antioxidant Features of Honey Samples from Ecological Niches of Western Maharashtra

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Abstract: ***The physico-chemical and antimicrobial properties of 10 samples of honey obtained from Western Maharashtra, India were investigated. The physico-chemical properties evaluated were colour , pH, free acidity, electrical conductivity, moisture, ash, phenol , invertase activity, proline, Hydroxy Methyl Furfural (HMF), total protein and carbohydrate as well as reducing sugar, antioxidant activity and pollen analysis. Results showed a range of honey colors ranging from light to dark amber. The pH values ranged from 3.8 to 4.4. The low pH of honey is known to inhibit the presence and growth of microorganisms. The moisture content in the investigated samples ranged from 17 to 70.6 %. Further, free acidity of some samples were within permissible limits (50meq/kg). Ash content ranged from 0.50 g to 100 g-1 and the electrical conductivity was computed to be at 0.64 mScm-. HMF values ranged from 7.4 to 22.5mg/kg and phenol content was found to vary between the ranges of 0.037 to 0.051. Antioxidant activities were evaluated by determining the 1,1-diphenyl-2-picryl-hydrazyl(DPPH) quantity and they were found to be significant.***

Keywords: Honey, Physico-chemical, HMF (Hydroxy Methyl Furfural), Antioxidant

1. Introduction

Honey is a natural food produced in particular by *Apis mellifera* and *Apis dorsata* bees from nectar or secretion of flowers. Honey has a content of 80-85 % carbohydrates, 15-17 % water, 0.3 % proteins, 0.2 % ashes, and minor quantities of amino-acids and vitamins as well as few other components in low levels of concentration. [1]. interestingly, honey has been cited in the Quran, a Holy book for Muslims (Section 16 Verse 68-69), highlighting its intrinsic medicinal properties. Honey contains a variety of phytochemicals and other substances such as organic acids, vitamins and enzymes which may serve as a source of dietary antioxidants. The antioxidant property is probably the most important property of honey and is significantly affected by the presence of flavonoids, phenolic acids, ascorbic acid, catalase, peroxidase, carotenoids and products of Maillard reactions [2].High levels of flavonoids, phenolic acids, ascorbic acid, catalase, peroxidase and carotenoids ensure a high level of antioxidants in honey that is the hallmark of its effect as a medical natural product [3].

Honey consumption by human has been reported to increase total plasma antioxoidant as well as reducing capacity which can be immuno and/or carcino- protective in nature. The quality of honey is mainly determined by its sensorial, chemical, physical and microbiological characteristics. The criteria for ensuring quality honey have been specified by the EC Directive 2001/110 [4]. The major criteria are moisture content, electrical conductivity, ash content, reducing and non-reducing sugars, free acidity, diastase activity and HMF content [5].

The antioxidant properties of honey are derived from both enzymatic (*e.g.*, catalase, glucose oxidase and peroxidase) and non-enzymatic substances (*e.g.*, ascorbic acid, α-tocopherol, carotenoids, amino acids, proteins, Maillard reaction products, flavonoids and phenolic acids) [5–7]. The amount and type of these antioxidants are largely dependent on the floral source or honey variety, and there exist a correlation between antioxidant activity with total phenolic content which has been abundantly established in literature [5, 7]. In India, although honey is produced and consumed on large scale, there is lack of significant void in knowledge pertaining to its properties, characteristics and specialties. Therefore the objective of the current study was to investigate the physical, chemical and antioxidant properties of honey in order to better understand this natural product with reference to its medicinal properties.

2. Materials and Methods

Collection of Honey Sample: The honey sample was collected from different sites in Maharshtra as shown in Figure 1 and Table 1.

Table 1: Name of regions from where the samples were collected

Samples	Region
S1	Mahabaleshwar
S2	Gaganbavda
S3	Radhanagari
S4	Kolhapur
S5	Ambevadi
S6	Hadapsar(Pune)
S7	CBRI(Pune)
S8	Fab India(Pune)
S9	Mhasla(konkan)
S10	Konkan

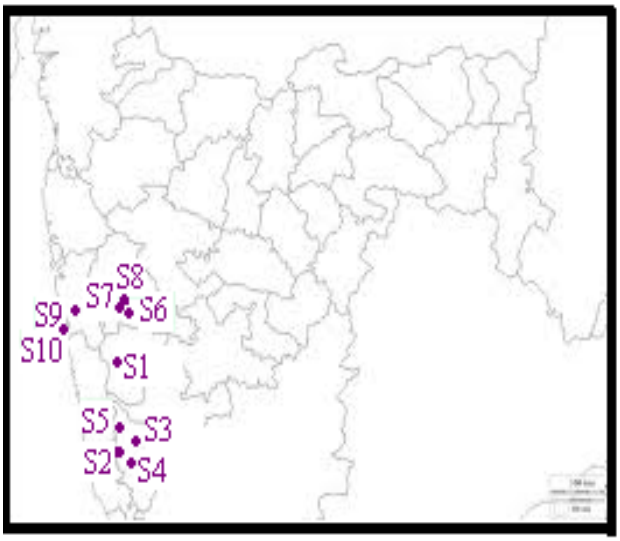

Figure 1: The places from where honey sample was collected

Physico-chemical analysis:

2.2.1 Colour Determination

The color intensity of the honey samples was measured according to the Pfund classifier.

2.2.2 Electrical conductivity

An EC meter was calibrated as per manufacturer's instructions and the electrode was dipped in honey solutions. [9].

2.2.3 Determination of moisture (refractometric method):

2.24 pH, Free acidity : pH was measure by pH meter in a solution containing 1g of honey sample in 20 ml of distilled water according to Association of Official Analytical Chemists (1990) [10].

2.2.5 Phenol Content: Phenol content was done by Folin ciocalteau method [11].

2.2.6 Estimation of Protein: Proteins in honey samples were estimated by Folin - Ciocalteau method at 720 nm.

2.2.7 Estimation of Total Carbohydrate content: Total Carbohydrate content of honey samples were estimated by Phenol Sulfuric acid Method at 480 nm.

2.2.8 Estimation of Reducing Sugars by DNSA method: Reducing Sugars of honey samples were estimated by DNSA method at 530nm.

2.2.9 Determination of Invertase activity: lnvertase activity was determined using the Siegenthaier method

2.3.0 Determination of hydroxyl methylfurfural after White: Determination of hydroxymethylfurfural (HMF) was done using the Winkler method.

2.3.1 Determination of antioxidant activity with the DPPH radical scavenging method: The antioxidant properties of each honey sample were also studied by evaluating the free radical-scavenging activity of the DPPH radical. The determination was based on the method proposed by Ferreira et al. [12].

3. Results

3.1 Physicochemical analysis

The analysis of physical characteristics of different honey samples is given in Table 2. The maximum values for different parameters is highlighted in red and the minimum value is highlighted in green. The parameters are also shown separately in figures 2.6

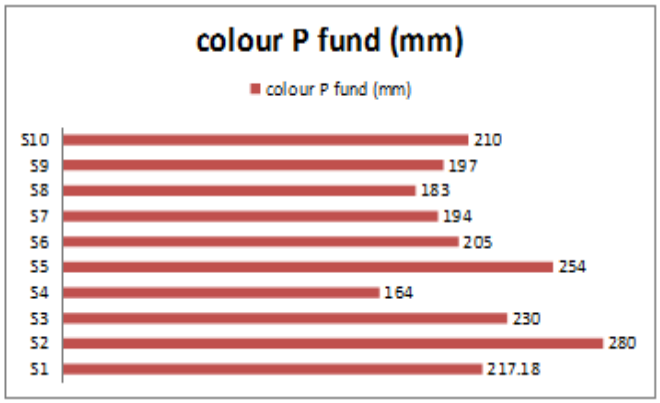

Figure 2: COLOUR Pfund(mm) of different honey samples

Table 2: The various parameters tested for **Physico-chemical analysis of Honey samples**

SAMPLES	COLOUR Pfund(mm)	pH	FREE ACIDITY	CONDUCTIVITY (mho) x10	REFRACTIVE INDEX (u)	Moisture R CONTENT(%)	ASH CONTENT (g/100g)
S1	217.18	4.0	5.9	0.543	1.494	27.7	0.04
S2	280	4.3	6.4	0.693	1.477	23.8	0.03
S3	230	4.4	3.8	0.601	1.469	27	0.04
S4	164	4.4	5.5	0.674	1.482	21.8	0.03
S5	254	4.3	6.4	0.596	1.482	21.8	0.03
S6	205	3.9	19.2	1.690	1.473	25.4	0.08
S7	194	4.0	4.5	0.379	1.470	26.6	0.06
S8	183	3.97	5.1	0.058	1.486	20.2	0.08
S9	197	3.88	10.2	0.088	1.482	21.8	0.08
S10	210	3.97	11.9	0.198	1.450	17	0.07
Mean	213.4	4.11	7.89	0.552	1.476	27.6	0.05

3.2 Determination of Invertase activity and hydroxyl methylfurfural after White in Table 3.

Table 3: Determination of Invertase activity and hydroxyl methylfurfural after White

SAMPLE	PROLINE (mg/kg)	INVERTASE (Units/kg)	INVERTASE NUMBER	PHENOL CONTENT (mg / kg)	HMF CONTENT (mg/kg)
S1	256	3.65	0.49	0.051	15.71
S2	2880	5.34	0.73	0.043	22.45
S3	3200	7.15	0.97	0.044	10.47
S4	960	32.7	4.45	0.045	9.73
S5	3368	15.4	2.09	0.054	17
S6	6400	9.5	1.29	0.045	11.22
S7	3555	72.4	9.86	0.037	14.96
S8	1600	15.8	2.16	0.042	7.4
S9	5760	11.5	1.55	0.047	17.96
S10	6400	4.4	0.6	0.043	19.4
Mean	3437.9	17.77	2.419	0.0451	14.63

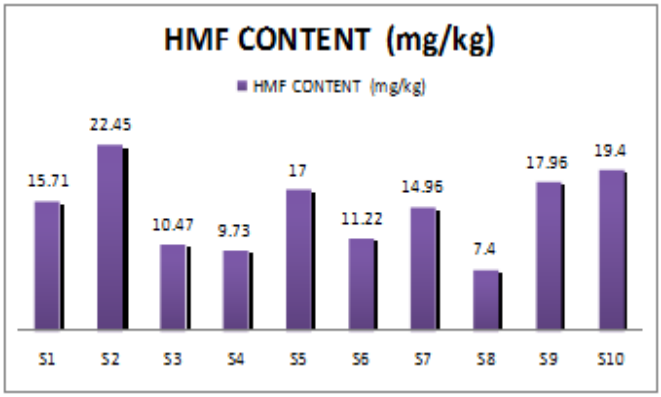

Figure 3: HMF content of different Honey samples

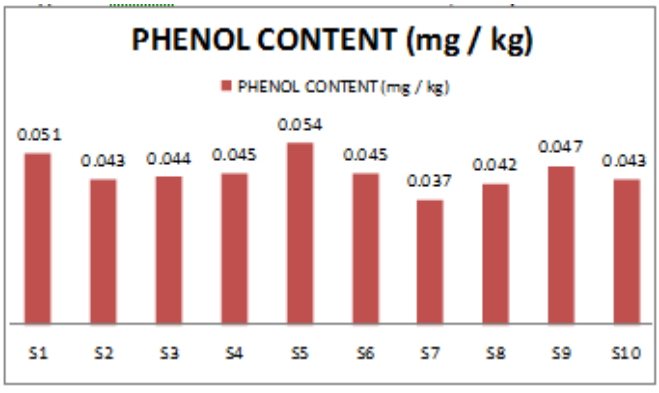

Figure 4: Phenol content of different Honey samples

3.3 Total reducing and non-reducing sugars: The total sugar and non-reducing sugar content of different honey sample are summarized in Table 4.

Table 4: Total sugar content, reducing sugar and Sucrose content of different honey samples

sample	*Total sugar content (%) (g/ml)*	*Reducing sugar (%) g/g*	*Sucrose (%)*
S1	62.45	63.34	2.54
S2	63.23	63.24	2.34
S3	57	59.34	2.45
S4	58.90	67	2
S5	65	65	1.89
S6	54	62.1	2.29
S7	68	62	1.76
S8	65	61	2.90
S9	59	56	2.45
S10	56.50	53	2.22
Mean	60.02	52.1	2.23

The Total Sugar content (%) g/ml, mean of all samples was found to be of 60.02 g/ml and mean reducing content was found to 52.1g/g with the mean Sucrose content of all honey samples was 2.23%.

3.4 Result of antioxidant activity with the DPPH radical scavenging: The result of antioxidant activity of different Honey samples done by the DPPH radical scavenging assay are given in Table 5.the standard used was Ascorbic acid.

4. Discussion

4.1Physical Analysis:

4.11pH

Honey is characteristically acidic, with a pH ranging between 3.2 and 4.5. All of our honey samples were acidic, with pH values ranging between 3.6 and 4.1. Because it has been reported that low pH inhibits the presence and growth of microorganisms, honey may have the potential to be used as good antibacterial agents.

4.12 Moisture content

The percentage of moisture content in the investigated samples ranged from 17.19 to 27, which are above 20, the maximum limit for the moisture content as per the Codex standard for honey.

4.13 Color analysis: The color characteristics of the honey samples are presented in graph. According to the USDA, honey samples with Pfund values less than 8 are classified as"water white", between 9 and 17 mm are classified as "extra white", between 18 and 34 mm as "white", between 35 and 50 mm as "extra light amber", between 51 and 85 mm as "light amber", between 86 and 114 as"amber" and greater than 114 as dark amber. Based on this classification, all honey samples were classified as dark amber.

4.14 Electrical conductivity: EC is an indicator of the botanical origin of honey. It has been reported that blossom honeys and mixtures of blossom and honeydew honeys ideally should have EC values of less than 0.8 mS/cm according to the European Union. EC values of honey samples varied between 0.2 and 0.8 which is within the range recommended by the European Union.

4.15 HMF Concentration: HMF concentration is widely recognized as a parameter of honey freshness. This is because it is absent in fresh honeys, and its levels tend to increase during processing and/or due to aging. HMF values of honey sample is within the range of 7 22 mg/kg that do not exceeded the limits (80 mg/kg) established by European Community regulations.

4.2 Biochemical Analysis

4.2.1 Sugar content

The total sugar content of the samples ranged from 42.8% to 60.6%.

4.2.2 Total phenolics: The phenolic acid content determined using Gallic acid as standard was between 152.4 and 688.5 mg Gallic acid/kg (r2 = 0.995). :

4.2.3 Proline: Proline is reported to mainly originate from bee salivary secretions during the conversion of nectar into honey. Protein levels are dependent on the type offlora that the bees visit and thus may be variable. Proline concentrations are an indicator of honey quality and of adulteration (suspected if proline levels are below 183 mg/kg).All of our samples generally had high proline levels (above 183 mg/kg), indicating that the quality of the honey is good.

4.2.4 DPPH

DPPH is a stable nitrogen centered radical and has been widely used to test the free radical scavenging ability of various samples. The higher the DPPH scavenging activity, the higher is the antioxidant activity of the sample. The percentage of DPPH scavenging activity of honey indicating that they have good antioxidant activities.

Table 5: Result of antioxidant activity done with the DPPH radical scavenging Assay

Samples	*IC 50 Values (µg/ml)*
Sample 1	29
Sample 2	29
Sample 3	18
Sample 4	18.5
Sample 5	16
Sample 6	16
Sample 7	15
Sample 8	15
Sample 9	19
Sample 10	18
Ascorbic acid	5.14

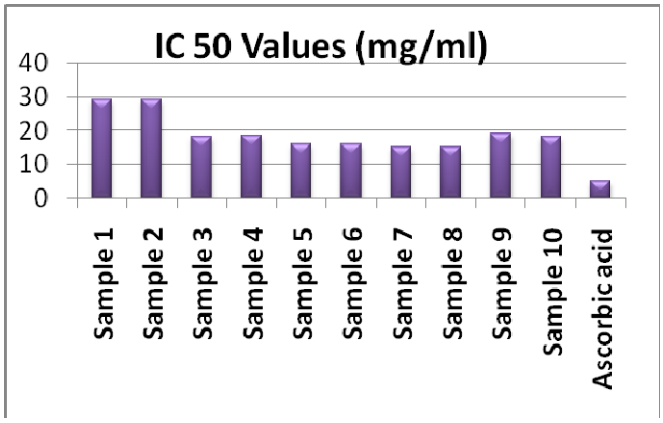

Figure 5: Graphical representation of antioxidant activity done with the DPPH radical scavenging Assay

5. Conclusion

This is the first study to investigate the physicochemical and antioxidant properties of Westren Maharastra honeys more elaborately. This study showed that Westren Maharastra honey samples have high antioxidant potential, as indicated by their high phenolic, flavonoid, and ascorbic acid and proline contents. 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.

Table 6: All the Parameter normal range and sample range

Parameters	*Normal Range*	*Sample Range*
COLOUR(mm Pfund)	>114(Amber Honey)	164-280
pH	3.2 - 4.5	3.8-4.4
FREE ACIDITY(meq /kg)	20-200	38-192
ELECTRICAL CONDUCTIVITY(mS cm-1)	0.09-0.80	0.058-1.690
MOISTURE CONTENT (%)	18-18.5	17-70.6
ASH CONTENT(% (w/w))	0.005-0.05	0.03-0.08
PROLINE CONTENT(mg/kg)	180 Min	256-6400
INVERTASE NUMBER	4-10	0.49-9.86
PHENOL CONTENT(mg gallic acid/ kg)	0.1-1.44	0.037-0.051
HMF CONTENT(mg/ kg)	5-40	7.4-22.45

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Author Profile

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