Quality evaluation of Indian bee (*Apis cerana indica* F.) honey in perception to enhance market potentiality

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**ABSTRACT**

The commonly cultivated honey from the Indian bees (*Apis cerana indica* F.) was selected in the raw unprocessed form to study its physicochemical characteristics and antioxidant property. Various physiochemical parameters like moisture, electrical conductivity, acidity, hydroxy methyl furfural content and sugar profile were analyzed and compared with the composition criteria of honey set by EU Directive. It was found that Indian bee honey has acceptable quality limits so that it can be upgraded to the European markets. The radical scavenging activity of honey tested using DPPH assay showed that its percentage radical scavenging activity was 95.55% at 500µg concentration (p< 0.05). Hence due to the high therapeutic potential it can also be utilized in the food industries as a functional food ingredient.

**Key words:** Antioxidant activity, DPPH assay, Physicochemical characteristics.

**INTRODUCTION**

Honey is a natural delicious viscous sweetener made by bees. The process of making honey begins when the bees feast on flowers, collecting the flower nectar in their mouths. This then mixes with special enzymes in the bee’s saliva and turns it into honey (Jones, 2001; FHIS, 2012).

Honey is a complete food with all major nutrients and a wide array of minor nutrients. Apart from the nutritional profile of honey has also been associated with improved antioxidant capacity, modulation of immune system, antimicrobial activity, and influence on lipid values and regulation of glycemic responses. Honey could be considered as a supplement which holds good for people belonging to all age groups (Al-Quassemi and Robinson, 2003; Gheldof and Engeseth, 2002).

The global production of honey increased by 10(%), in the years 2005-2010 it has been ranging from 1.4 million metric tons (MTs) to 1.54 million MTs. This is due to the increased consumption rate of honey in the world (Mishra, 2000).

The total honey production in India is estimated to be 65,000MT during 2010-2011 (National Honey Board- 2011). In spite of having an excellent rate of production honey in India the per capita honey consumption is estimated to be 5.25g which is very less when compared globally.

Out of total production over 80% of honey is directly consumed as household, about 10% used in traditional medicine or used in Ayurvedic preparations and hardly 10% of honey is consumed either as food or products like fruit processing, bakery, confectionery, allopathic medicine and other products.

As there is a growing demand for Indian honey in the global market and hence more and more people are attracted towards this agribusiness, as it is recognized as a lucrative income generating activity. Hence there is a clear cut necessity to undertake quality evaluation of Indian bee honey scientifically and to identify a scope for value addition of the same as a strategy to increase its market potentiality.

**MATERIALS AND METHODS**

**Honey sample:** Among the major five species of honeybees in India, *Apis cerana indica* F. bee honey or locally called as Indian bee honey was selected for the study because it is the most preferred bee species by the bee farmers in Kerala. The raw unprocessed honey was collected from the southern zone of Kerala.

The quality of collected honey sample was analyzed by studying the physicochemical characteristics. Apart from which their major nutrients, microbial contamination and antioxidant property were also analyzed to identify its potentiality to become a sole ingredient in food industry.

**Determination of physicochemical characteristics:** The major physicochemical characteristics assessed were pH, moisture, electrical conductivity, specific gravity, ash content, TSS, free acidity, HMF according to AOAC (1999).

**Determination of sugars:** The major analysis done in this category are total sugars and reducing sugars, sucrose content, fructose glucose ratio, true glucose per cent and true fructose per cent according to AOAC (1999).

**Determination of nutrients:** The macronutrient analyzed was carbohydrate using Anthrone method. Atomic absorption spectrometry using hydride vapour generator was used to...
Determine mineral content (Fe, Mn, Zn & Cu). The ash of honey sample was dissolved in 100ml of 2N HCL. Calcium was estimated by the method of Sadasivam and Manickam (1992).

**Determination of microbial contamination**: Sample was assessed for the presence of various microorganisms that include bacteria, yeast, mould, actinomycts and coliforms. Serial dilution of the samples followed by spread plating were employed to estimate the population of viable microorganisms in the honey (Johnson and Curl, 1972).

**Determination of antioxidant property**

**Total polyphenols**: The Folin–Ciocalteu method was used for the determination of the total phenolics (Slinkard and Singleton, 1977). Phenols react with phosphomolybdic acid in folin–ciocalteau reagent in alkaline medium and produce molybdenum blue complex. According to this principle various concentrations of the prepared extracts when react with the folin – ciocalteau reagent and 10% NaCo3 solution give shades of blue colour which was measured at 725nm. Gallic acid was used as standard. All the tests were performed in quadruples. The results were determined from the standard curve and were expressed as gallic acid equivalent (mg/g of extracted compound).

**DPPH assay**: DPPH radical scavenging activity of honey extract was determined according to the method given by Sreejayan and Rao (1996). To 1ml of the extract at various concentrations (100-500mg/ml) in methanol, 1ml of DPPH was added. It was then incubated at 37°C for 30 minutes. The absorbance of the test mixture was read at 517nm using, Cyberlab, a double beam spectrophotometer. The percentage inhibition of DPPH radical was calculated by comparing the result of the test with that of control (methanol and 1 ml DPPH) using the formula:

\[
\text{Percentage inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100
\]

**RESULT AND DISCUSSION**

**Physicochemical characteristics**: Physicochemical characteristics of honey are its major criteria to enter in to the export market. pH of the honey was found to be 3.55, acidic which is expected to have a flavour enhancement property due to the presence of organic acids like gluconic acids in it. But certain studies also opined that the low pH of honey tends to impact a sour taste which makes the honey unpalatable (Gulfraz et al., 2010; Gomes et al., 2010).

According to the national standards Agmark, ISI, PFA Act the ash content of honey should be 0.5 per cent (Shamala and Jyothi, 1999). In the present investigation it was found that the ash content of *Apis cerana indica* was 0.22%. The electric conductivity was 0.41 ms/cm. The higher electrical conductivity content can be due to their relatively higher ash content. The higher ash content relates to the presence of higher inorganic residues after honey carbonization (Malika et al., 2005). Honey of the same floral source can vary due to seasonal climatic variations and to different geographical origins (Anklam, 1998).

Even though the honey was collected from the southern zone of Kerala which has a wet and maritime tropical climate the moisture content was found to be with in the limits of 16.6%. Moisture is one of the important criteria in setting honey quality standards. Moisture content of any honey may differ according to the region from which it is collected.

Specific gravity is another physico chemical characteristic of honey which helps to correlate with water activity of the same. It was found that the taken honey sample had a specific gravity of 1.36. The specific gravity of honey depends on its water activity. If the water activity is 15% then specific gravity of honey is 1.435 and for 18% of water activity it is 1.4171. In the current study the specific gravity of honey was 1.36 and therefore its water activity will be below 15%. Hence honey has been utilized in the preparation of IMF products, which has therapeutic importance, despite being somewhat odd in flavour.

The free acidity of honey was determined to be 0.16gm of formic acid per 100gm of sample. Acidity is a property of honey which explains two aspects of honey viz., the presence of organic acids in honey and on the other side it also explains the quality of honey. Generally honey tends to have acidity due to the presence of a number of organic acids in it namely, formic, acetic, butyric, citric, gluconic,

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>EU standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>16.6</td>
<td>&lt; 20%</td>
</tr>
<tr>
<td>Electrical conductivity (ms/m)</td>
<td>0.41</td>
<td>Not more than 0.8ms/cm</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>0.22</td>
<td>-----</td>
</tr>
<tr>
<td>pH</td>
<td>3.55</td>
<td>-----</td>
</tr>
<tr>
<td>Total soluble solids (* Brix)</td>
<td>75.5</td>
<td>-----</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>1.36</td>
<td>-----</td>
</tr>
<tr>
<td>Hydroxy methyl furfural (mg/Kg)</td>
<td>30.01</td>
<td>&lt; 80mg/Kg</td>
</tr>
<tr>
<td>Free acidity (g/100g)</td>
<td>0.16</td>
<td>&lt;50meq acid/1000 gram</td>
</tr>
</tbody>
</table>
lactic, malic and succinic acid. High acidity can be an indicator of fermentation of sugars into organic acids. But the sample taken for the current study had a low level of acidity which shows its freshness.

The total soluble solids of honey sample was estimated to be 75.5° Brix using refractometer. Hydroxy methylfurfural content was analysed and found to be 30.01mg/Kg of sample which is within the limits of quality standards set by EU council directive. Hydroxymethyl furfural (HMF) is not yet considered as a harmful substance, but there are evidences for carcinogenic potential of other members of this class, hence EC Directive 2001/110 imposed permissible limits up to 80mg/Kg of honey. In the present study the amount of HMF is very low i.e., 30.01mg/Kg than the allowed limits of 80mg/Kg, which indicate that the honey is fresh and did not undergo much processing like overheating during preservation and storage.

**Sugar profile of Apis cerana indica:** The sugar profile of Indian bee honey were total sugars up to 65.21%, reducing sugars were up to 60.03%. The sucrose per cent was 4.92% and the F/G ratio was found to be 0.77%. The true glucose and fructose per cent were also estimated by calculation method and was found to be 35.01% and 27.04% respectively.

Hence the sugar profile of honey in present study revealed that the amount of total sugars and sucrose content were according to EU Directive composition criteria for honey. Sucrose content was found to be slightly higher but still it is within the prescribed range of EU Directive.

**Nutrient analysis:** The major nutrient present in honey are supposed to be carbohydrates which provide it about 300Kcal per 100gm of honey. In present study the carbohydrate content was found to be 80g/100g of honey. 95% of carbohydrates found in honey are fermentable, which is an acceptable characteristic of honey for its use in many bakery applications. The carbohydrate composition of honey also aids in other wide functional applications like its use for flavour enhancement, colour development and extended shelf life alone and when added to foods.

Micronutrients like calcium, iron, zinc, manganese and copper were analyzed in this study. The amount of vitamins and minerals are small and the contribution of honey to the recommended daily intake of the different trace elements is marginal. The micronutrient profiles of Apis cerana indica were as shown in the Table 2.

**Table 2.** Minerals of *Apis cerana indica* honey.

<table>
<thead>
<tr>
<th>Element</th>
<th>Mean value ± SD (µg/100g of honey sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>3600 ± 0.48</td>
</tr>
<tr>
<td>Iron</td>
<td>284.76±0.03</td>
</tr>
<tr>
<td>Manganese</td>
<td>154.33±0.02</td>
</tr>
<tr>
<td>Zinc</td>
<td>96.86 ± 0.02</td>
</tr>
<tr>
<td>Copper</td>
<td>16.66 ± 0.008</td>
</tr>
</tbody>
</table>

In spite of the presence of these micronutrients in marginal levels there is possibility for its application for contracting micronutrient deficiency. Several in vitro studies have been conducted in this regard (Bogdanov et al., 2007).

**Microbial contamination:** Microbial contamination of the honey sample was tested for the presence of bacteria, yeast, actinomycetes, fungus and coliforms. The result was an insignificant growth of these microbes in the honey sample analyzed in serial dilution. Even though Indian bee honey contains higher amounts of moisture the higher stability and keeping quality are due to its high acidity and low pH which in inhibiting the presence and growth of micro-organisms (Tumin et al., 2005). The enzyme systems like glucose oxidase and high content of reducing sugars were also found to discourage the growth of microbes like molds and aerobic bacteria in honey (White et al., 1962).

**Antioxidant property:** Honey was found to be a rich source of antioxidants may be due to the presence of phenolic compounds in it. Hence in the present investigation the total polyphenolic compounds present in honey was estimated using Folin–Ciocalteu method and was found to be 1053.75mg/Kg of sample.

As the honey analyzed was rich in phenolic compounds its antioxidant activity was tested using DPPH assay. The radical scavenging activity of honey on hydroxy radicals was compared with the antioxidant activity of a standard antioxidant *quercetin*. It was clear from the below table that the honey from *Apis cerana indica* bees have a similar antioxidant activity of 78.69% to 95.55% when compared with commercial antioxidant *quercetin* from 87.81% to 97.48%.

**Table 3.** DPPH radical scavenging activity of Indian honey and quercetin

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Indian honey</th>
<th>Quercetin</th>
</tr>
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<tbody>
<tr>
<td>100</td>
<td>78.69±0.015</td>
<td>87.81±0.12</td>
</tr>
<tr>
<td>200</td>
<td>82.43±0.021</td>
<td>90.19±0.026</td>
</tr>
<tr>
<td>300</td>
<td>85.76±0.024</td>
<td>93.51±0.16</td>
</tr>
<tr>
<td>400</td>
<td>90.82±0.028</td>
<td>95.59±0.11</td>
</tr>
<tr>
<td>500</td>
<td>95.55±0.18</td>
<td>97.48±0.08</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of four replicates

<table>
<thead>
<tr>
<th>Samples</th>
<th>F calculated</th>
<th>F tabulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>234.638</td>
<td>4.170</td>
<td></td>
</tr>
<tr>
<td>2353.812</td>
<td>2.689</td>
<td></td>
</tr>
<tr>
<td>71.883</td>
<td>2.689</td>
<td></td>
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</tbody>
</table>

DPPH is nitrogen centered radical which has been extensively used to test the free radical scavenging ability of various substances. DPPH assay is most frequently used antioxidant assay because the antioxidant potential of honey has been shown to be directly associated with its phenolic acid and flavonoid content. The percentage of scavenging
shown by Indian bee honey (95.55%) at 500 µg/ml in this study is higher than what was previously reported for Indian honey sample (57.5%) (Khalil et al., 2012).

In the present study honey sample showed an increasing DPPH scavenging with the concentration of honey extract. The antioxidant activity of honey might be due to its proton radical scavenging action by the antioxidant compounds present in the sample. The equivalent DPPH scavenging activity of this honey when compared with standard quercetin is due to the presence of high content of phenolic compounds like chrysin, apigenin and catechin (Schramm et al., 2003).

**CONCLUSION**

On the basis of the present study it can be concluded that raw Apis cerana indica bee honey are rich nutritionally as well as medicinally. Kerala being a major tropical ecosystem is characterized by rich biodiversity with abundant scope for natural products. Though honey is a natural product it should be pointed out that the records of honey as functional health food and uses of other honey-bee products are still incipient. Hence it is a necessity to study the health potentiality of honey with respect to Indian bee species to increase its per capita consumption rate and also to maximize its value addition in food industries.

**REFERENCES**


