A Comparative Study on the Chemical Composition, Chemical Characterization and Floral Studies of Raw and Processed Honey Samples of *Apis* Species

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**Abstract:** Honey is a substance made from nectar and sweet deposits from plants are gathered, modified and stored in the honey comb by the honey bees. In this case *Apis* species are of importance, due to the changes in viscosity, the consistency of the honey can be fluid, viscous or partly or entirely crystallized. Similarly, depending on the plant origin, the flavor, aroma, chemical composition and the count of pollen varies. No pollen or constituent particular to honey may be removed except where there are unavoidable circumstances in the removal of foreign inorganic or organic matter. The objective of my project emphasizes the study of chemical composition of raw and processed honey, to study the chemical characterization of raw and processed honey, to identify the floral and geographical origin of honey samples collected from *Apis* species and to draw a conclusion on the importance of raw and processed honey samples collected from *Apis* species. The tabulated results meet the Codex Alimentarius Standards. The comparison is made between the raw honey and the processed honey samples and conclusions are drawn to identify the better out of it. The raw honey samples were found to be effective than the processed honey samples and the methods used for labelling of the raw honey samples were cost effective, accurate and time efficient and according to the Codex Alimentarius standards.

**Key words:** Codex alimentarius • Chemical composition • Melissopalynology • HMF • Raw and processed honey

**INTRODUCTION**

Honey bees are natural social insects that live in hives. They feed on nectar (a sweet liquid made from flowers) which is converted through chemical process into honey [1]. Indian honey bee names include *Apis cerena*, *Apis dorsata*, *Apis mellifera* and *Apis florea*. Compounds like enzymes are added to the nectar, complex carbohydrate molecules are broken into simple sugars and then to acids. The most commonly found acid in the honey is gluconic acid and is the reason why the honey is acidic. Due to the variation in concentration of the acid the acidity of the honey varies and thus leads us to the variation in pH values [1]. Due to the different acidic content in different honey the flavors range from mild to pungent, the color range from colorless to dark brown and hence there is a change in taste in different types of honey.

Honey consists of 80-85% carbohydrates, 15-17% water, 0.3% proteins, 0.2% ashes and other minor quantities of components at low levels of concentration [3]. The principle difference in concentration of the honey comes from the floral origin, change in sugar concentration and minor compounds such as acids, heavy metals and minerals [6]. Blossom honey comes from nectar of flowers from plants and the Honeydew honey comes mainly from excretion of plant sucking insects on the living parts of plants or secretions of living parts of plants.
Melissopalynology is the study of pollen contained in honey and, in particular, the pollen's source. By studying the pollen in a sample of honey, it is possible to gain evidence of the geographical location and genus of the plants that the honey bees visited, although honey may also contain airborne pollens from anemophilous plants, spores and dust due to attraction by the electrostatic charge of bees [10].

The scope of Microscopical analysis of honey provides information about the geographical and botanical origin of honey and also additionally tells about the contamination of honey with brood, dust, soot etc. The pollen analysis can be done in 2 methods namely Qualitative and Quantitative method of pollen analysis [7].

The present study was carried out to investigate the chemical, composition, characterization and floral comparison between raw and processed honey samples.

**MATERIALS AND METHODS**

The samples were packaged in jars of 500g with bottling date between December 12 and February 13. All samples were filtered in advance for the removal of body parts since they were directly extracted from the honey comb. The samples were stored in the packages in dark at a temperature of the laboratory (25±2°C) for analysis. All processes were carried out within two weeks from obtaining the sample.

The pH of the honey samples collected was determined using pH meter [1]. For the invertase activity the substrate used is p-NPG (p-Nitro phenyl-alfa-D-glucopyranosid) which is disintegrated by the invertase enzyme from honey to glucose and p-nitrophenol. To stop the reaction the pH is modified to 9.5, by this the nitrophenol is transformed to nitrophenol anion. This is determined spectrophotometrically at 400 nm. The invertase activity from the measured absorbency is multiplied by 158.94 and calculated to a kilo of honey (U/kg). The value is indicated as IN (Invertase Number). The IN indicates the amount of sucrose per gram hydrolysed in one hour by the enzymes contained in 100 g of honey under lab conditions, [5][6]. The diastase activity was calculated as DN (Diastase Number). DN expresses units of diastase activity. One unit is defined as the amount of the enzyme that will convert 0.01 g of starch to the prescribed end point in 1 hour at 40°C under the test conditions. It was evaluated calorimetrically at 600 nm [4].

The moisture content of honey is determined using refractometer. The sample was subjected to the refractometer 3 times and the average was calculated [2]. The ash content was determined by using 5 grams of each honey sample collected was placed in a china dish and subjected to a muffle furnace at a temperature of 660°C and incinerated until it turned into ash and the values were tabulated.

The sugar and amino acid was determined by using High Performance Liquid Chromatography (HPLC). The honey samples collected were subjected to the HPLC equipment for analysis of the amount of reducing sugars to find out the different types of amino acid present in the sample [7].

Hydroxymethylfurfural (HMF) was done by using 5 grams of honey sample dissolving in 25 ml of deionised water. 0.5 ml of Carrez Solution I (150 mg/ml potassium ferrocyanide) was added to the sample and mixed well. 0.5 ml of Carrez Solution II (300 mg/ml zinc acetate) was then added to the sample and mixed well. The sample was brought to a final volume of 50 ml with deionised water using a drop of alcohol to suppress surface foam. The sample was filtered and the first 10 ml of filtrate was discarded. 5 ml of the remaining filtrate was transferred into each of two test tubes. A reference sample was prepared by adding 5 ml of 0.20% sodium bisulfite to one test tube of filtrate. A test sample was prepared by adding 5 ml of deionised water to the other test tube of filtrate. Both samples were mixed well with a vortex mixer. The absorbance of the test sample was measured against the reference sample at 284 nm and 336 nm. The absorbance of a clarified aqueous honey solution was measured against a reference solution of the same honey in which the 284 nm chromophore of HMF was destroyed by bisulphite [9].

The pollen grains present in the honey samples is the only basis of identification of the plant source. The microscopic analysis of the honey samples reveals their botanical origin. The method proposed by International Commission for Bee Botany [10] was used with a little alteration in the present study to determine the quantitative and qualitative Microscopical analysis then slides were numbered and preserved for microscopic examination. The microscopic examination of the slides prepared from the honey sediments helps to helps to determine the botanical origin of honey. An attempt was made to identify the pollen up to species level. “Predominant pollen” constituting pollen count of more than 45%, “Secondary Pollen” (16-45%), “Important
Minor Pollen” (3-15%), “Minor Pollen” (< 3%). The honey samples were classified as Unifloral honey if the honey contains pollen, mainly from a certain plant species in other words if the pollen of that species is predominant ie, above 45%. The pollen grains counted by scanning the whole slide or slides prepared (depending on the quality of the sediment) are expressed in percentage frequencies represented in Table 2.

RESULTS

The results of chemical characteristics of different honey samples are summarized in the Table 1 with statistical significance.

The pH of the honey samples were acidic compared to the processed honey samples which justifies that it has better gluconic acid which helps in the digestion of glucose content easily Figure 1. Highest was 5.6 and lowest was 4.6. The moisture content of the raw honey sample is not more than 20% which satisfies the Codex Alimentarius standards [11] which conclude that the samples are not adulterated, highest was 25.0% and lowest moisture content was 13.0% Figure 2. The high diastase activity in the raw honey samples suggests us that the conversion of the starch in the body after consumption will be higher than the processed honey sample Figure 3. The Schade units ranged from 5 to 7 well within the range of 8 as per Codex Alimentarius Standards [11]. The low values of invertase number in the processed honey samples conclude that the conversion of sucrose into simpler sugars is low in the processed honey after consumption Figure 4. The ash content of the raw honey samples are higher than the processed honey samples since they are directly extracted from the honey comb and also it satisfies the Codex Alimentarius standards [11] less than 0.01g/5g of the honey sample Figure 5.

During HPLC studies the samples were compared to analyse the presence of sucrose. For control sample sucrose standard 1mg/ml was taken and run through HPLC and graphs were obtained. They were obtained around the 4.2 to 4.5th min concluding the presence of the sucrose as per the standard references [7] Figure 6-10. The HMF of the processed honey samples was higher than the raw honey samples, the highest value was 74mg/kg and the lowest HMF content was 13.824mg/kg [9].

Table 1: Statistical analysis of the collected honey sample

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>pH</th>
<th>Moisture Content (%)</th>
<th>Ash content (g/5g)</th>
<th>Diastase Activity (no unit)</th>
<th>Invertase Activity (no unit)</th>
<th>HMF (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP1</td>
<td>5.6</td>
<td>21.6</td>
<td>0.0079</td>
<td>21.667±0.52</td>
<td>3.3 ±0.1632</td>
<td>64.448±0.1567</td>
</tr>
<tr>
<td>AP2</td>
<td>5.4</td>
<td>25.0</td>
<td>0.0056</td>
<td>23.633±0.73</td>
<td>4.11±0.0852</td>
<td>74.112±0.1436</td>
</tr>
<tr>
<td>ADR0</td>
<td>5.2</td>
<td>16.2</td>
<td>0.0067</td>
<td>26.066±0.09</td>
<td>15.667±0.16</td>
<td>49.344±0.7183</td>
</tr>
<tr>
<td>ACR1</td>
<td>4.8</td>
<td>14.0</td>
<td>0.0053</td>
<td>27.5±0.35</td>
<td>16.266±0.1885</td>
<td>31.104±1.567</td>
</tr>
<tr>
<td>ACR2</td>
<td>4.6</td>
<td>13.0</td>
<td>0.0082</td>
<td>28.2±0.74</td>
<td>18.533±0.4714</td>
<td>13.824±0.2715</td>
</tr>
</tbody>
</table>

Table 2: Spectrum of Pollen types analysed from different Honey samples

<table>
<thead>
<tr>
<th>SLNo</th>
<th>Sample no</th>
<th>Place of collection</th>
<th>Floral types</th>
<th>Predominant</th>
<th>Secondary pollen</th>
<th>Important minor pollen</th>
<th>Minor pollen</th>
<th>Absolute pollen count (APC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>ADR0</td>
<td>Thanjavur</td>
<td>Uni floral</td>
<td>Cocos nucifera</td>
<td>Cucumis sativas</td>
<td>Dendrotheca falcata, Hopla glabra</td>
<td>Iroa nigricans, Leucas hirta</td>
<td>142</td>
</tr>
<tr>
<td>2.</td>
<td>ACR2</td>
<td>Bangalore</td>
<td>Multi floral</td>
<td>Gossypium sp., Hysterus rosa sinensis</td>
<td>Leucas hirta, Pupalia lapaceae</td>
<td>Papaliu lapaceae</td>
<td>Grass pollen</td>
<td>230</td>
</tr>
<tr>
<td>3.</td>
<td>ACR1</td>
<td>Bangalore</td>
<td>Uni floral</td>
<td>Eucalyptus globulus</td>
<td>Greedella robusta, Ludwigia species, Mandiera indica</td>
<td>Pongaiu glabra, Psidium guajava</td>
<td>Syzygium jambos</td>
<td>550</td>
</tr>
</tbody>
</table>

Fig. 1: Graphical Representation of pH values obtained
Fig. 2: Graphical Representation of Moisture % (by mass) values obtained

Diastase activity

Fig. 3: Graphical Representation of DN for the collected honey Samples

Invertase Activity

Fig. 4: Graphical Representation of IN for the collected honey Samples
Fig. 5: Graphical Representation of ash content for the collected honey samples

Fig. 6: Sucrose analysis of sample ADR0 using HPLC

Fig. 7: Sucrose analysis of sample ACR1 using HPLC
Fig. 8: Sucrose analysis of sample ACR2 using HPLC

Fig. 9: Sucrose analysis of sample AP1 using HPLC

Fig. 10: Sucrose analysis of sample AP2 using HPLC
For the floral studies honey samples were subjected to the Erdtman’s analysis and was examined under microscope to quantify and qualify the pollen isolated and the details are summarized in Table 2 which explains the differentiation between the Unifloral and Multifloral honey samples [10].

**DISCUSSION**

The present study reports on chemical characteristics which were in accordance with the published results. The pH values ranged from 4.6 to 5.6 which were in accordance with the results of [1]. The moisture content varied from 13 to 25 percent [2]. The minimum range of moisture content is due to low rate of fermentation. The HMF content, sucrose levels and Reducing sugars were within the range of Current Codex Standard [11] and confirmed the freshness of samples [7, 9]. The ash content were also within the range in accordance with [1] and [3]. In conclusion, chemical characteristics of all the raw honey samples showed much closer values of Codex Standard [11] indicating good quality. However, the low standards were noticed for the honey samples which were processed.

The honey samples ADR0, ACR1 and ACR2 were processed and the Absolute Pollen Count (APC) was calculated and thus was tabulated. The samples ACR1 and ACR2 have comparatively more pollen count. The processed honey samples AP1 and AP2 were also subjected to the APC study, since the pollen isolate was very minimum the pollen in the slide were not found intact and conclusion led to the processed honey samples were void of pollen content [10].

**REFERENCES**