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**RESEARCH ARTICLE**

# QUALITY ANALYSIS OF COMMERCIAL HONEYS IN INDIAN MARKET

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**ABSTRACT**

Investigation of 10 commercial honeys from India was analyzed for quality according to the Bureau of Indian Standards (BIS) and Agricultural Marketing Adviser to the Government of India (AGMARK). Moisture content, total reducing sugars (TRS), sucrose, fructose-glucose ratio (FGR), acidity, Fiehe's test, hydroxymethyl furfural (HMF) content, total pollen counts (TPC) and absorbance of honey samples were examined. The aim of the present work was to analyze the physical and chemical quality of some Indian honeys and find out whether they meet national standards of honey specifications. It was observed that two honeys were suspicious with respect to their quality as these honeys failed to meet the standards for TRS, sucrose, FGR, HMF and absorbance. Two honeys were genuine and were in accordance with the Indian standards, whereas six honeys showed increased level of HMF due to ageing or increased moisture content or possibly due to adulteration with external additives. The study recommends Indian honey manufacturers to preserve honey quality not only by following the Standards, but by taking care and precautions for mishandling, improper storage conditions and temperature factors which are responsible for the loss of genuineness of honey and poor quality. Also the study suggests consuming commercial honey within 18 months following its packing.



## INTRODUCTION

Honey is a natural food mainly composed of water, sugars and other minor substances such as organic acids, amino acids, proteins, minerals, vitamins, lipids, lactone and nitrogenous compounds (Echingo and Takenaka, 1974), (Dustmann, 1993). Honey has been used in vital alternative medicines of Ayurveda and daily consumption of honey is encouraged to promote good health (Joshi and Godbole, 1970). As a natural product, honey experiences relatively high price. Taking into account the traditional and contemporary uses of honey, we can interpret that there is an increasing demand and interest by consumers for high quality honey and honey products. Honey consumption has increased during the past decade due to consumers' preference for natural and pure products. Increasing demand for honey has led to a shortage, which in turn led to serious problems to honey industry. Honey has been for a long time a target for adulteration. Dishonest traders started making money by adding cheap components to honey and sold them at high prices in the market. Different types of commercial honeys vary in its composition, shelf life, cost and packing. The aim of the present work was to analyze the physical and chemical quality of some Indian honeys and find out whether they meet national standards of honey specifications.

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Thus, 10 commercial honeys randomly selected from market were analyzed for moisture content, TRS, sucrose, FGR, acidity, Fiehe's test, HMF content, total counts of pollen and absorbance.

## MATERIAL AND METHODS

### Collection of honey samples

Commercial honey samples were randomly selected from the market from various states of India viz. Maharashtra, Uttar Pradesh, Himachal Pradesh and Madhya Pradesh. According to the standards of AGMARK and BIS (1994) the honey samples were analyzed for various physical and chemical parameters such as moisture content, TRS, sucrose, FGR, acidity, Fiehe's test, HMF test, TPC and absorbance.

### Determination of moisture content

Abbe's refractometer was used to determine the moisture content of honey (Bogdanov et al., 1999). The Refractive Index (RI) is sensitive to temperature, so the readings were generally done at a standard temperature of 20ºC. If the determination was made at temperature other than 20ºC, the results were adjusted by using a correction factor provided in special tables that have been developed for honey, which express the percentage of water in relation to the RI. As the relationship determined with the help of a refractometer is not

exactly same for every sugar, special tables have been developed for honey, which expresses the percentage of water in relation to the Refractive Index (Wedmore, 1955).

**Determination of Total Reducing Sugars (TRS)** (BIS, 1994)

TRS of honeys was determined using Fehling's Test. TRS (percent by mass) was calculated using the formula:

$$\text{Total reducing sugars, percent by mass} = \frac{250 \times 100 \times S}{H \times M}$$

where S = strength of $CuSO_4$ solution

H = volume (ml) of honey solution required for titration, and

M = mass (g) of honey.

**Determination of sucrose (BIS, 1994)**

The difference between the results of reducing-sugar determinations before and after hydrolysis is a measure of the content of non-reducing sugars. Determination of sucrose from honeys was done by taking 100 ml of the stock honey solution. To it 1ml of concentrated HCl was added and the solution was heated to boil. The solution was then kept aside overnight. This inverted honey solution was neutralized with sodium carbonate and the total reducing sugars were determined using the formula:

Sucrose, percent by mass = [(reducing sugars after inversion, percent by mass) – (reducing sugars before inversion, percent by mass)] × 0.95.

**Determination of Fructose-Glucose Ratio (FGR) (BIS, 1994)**

Iodine solution (0.05 N), NaOH Solution (0.1 N), concentrated $H_2SO_4$ and Standard Sodium Thiosulphate Solution ($Na_2S_2O_3$ - 0.05 N) were the reagents used. Honey solution (50 ml) was pipetted in a 250-ml stoppered flask. To it 40 ml of iodine solution and 25 ml of NaOH solution was added and kept in dark for 20 min. Then 5 ml of concentrated $H_2SO_4$ was added. The excess of iodine was titrated immediately against standard $Na_2S_2O_3$ solution. A blank was conducted using 50 ml of honey solution. Glucose percent by mass was calculated using the formula,

$$\text{Approximate glucose Percent by mass (w)} = \frac{(B - S) \times 0.004502 \times 100}{a}$$

where B = volume of $Na_2S_2O_3$ solution required for the blank,

S = volume of $Na_2S_2O_3$ solution required for the sample, and

a = mass of honey taken for test.

$$\text{Approximate fructose, percent by mass (x)} = \frac{\text{Approximate total reducing sugars, percent - w}}{0.925}$$

True glucose, percent by mass (y) = w – 0.012 x

$$\text{True fructose, percent by mass (z)} = \frac{\text{Approximate reducing sugars, percent} - y}{0.925}$$

True reducing sugars, percent by mass = y + z

$$\text{Fructose-glucose ratio} = \frac{\text{True fructose, percent by mass (z)}}{\text{True glucose, percent by mass (y)}}$$

**Determination of acidity (BIS, 1994)**

Standard NaOH Solution (0.05 N) and phenolphthalein indicator solution were the reagents used to determine acidity of honeys. In a flask 10g of the honey sample was thoroughly dissolved in 75 ml of distilled water and titrated against 0.05N NaOH using 4-5 drops of neutralized phenolphthalein solution where pink colour of indicator should persist for atleast 10 sec. Blank was determined using distilled water and indicator and this value was used to correct the volume of standard NaOH solution used. Calculation was carried out as follows:

$$\text{Acidity (as formic acid), Percent by mass} = \frac{0.23 \times V}{M}$$

where V = corrected volume of 0.05 N NaOH solution required for titration; M = mass (g) of the test sample.

**Determination of Fiehe's Test (BIS, 1994)**

The HMF test is carried out only when Fiehe's test is positive. Resorcinol solution was prepared by dissolving 1g resublimed resorcinol in 100 ml HCl. in a mortar 5g of honey sample was mixed with 10 ml of ether. The ether extract was decanted in a porcelain dish for evaporation to dryness at room temperature. A drop of freshly prepared resorcinol solution was added to the residue. Positive reaction for HMF is indicated by immediate appearance of cherry red colour, whereas a negative reaction is indicated by a faint pink colour disappearing after a short time.

**Determination of HMF content (BIS, 1994)**

Barbituric Acid solution and þ-Toluidine solution were used to determine HMF content in honeys. Test sample was prepared by weighing 10g of honey sample and dissolved thoroughly in 50 ml distilled water. The sample should be tested after preparation without delay. Photometric determination was done by pipetting 2ml honey solution followed by 5ml þ-toluidine solution in 2 test tubes each. In one test tube 1ml distilled water was added that served as blank and in the other 1ml barbituric acid solution was added and were mixed thoroughly. Addition of reagents was done without interval and finished in 1-2 min. The extinction of sample was read against the blank at 550 nm using a 1cm cell, immediately after the maximum value was reached. Calculation was done using the formula:

$$\text{mg/100 g HMF} = \frac{\text{Absorbance}}{\text{Thickness of Layer}} \times 19.2$$

Results were expressed as mg HMF/kg honey.

**Determination of Total Pollen Count (Lakshmi and Suryanarayana, 1997)**

Ten gm of honey was weighed and dissolved in 40ml distilled water. The solution was centrifuged in four centrifuge tubes for 10 min at 3000 rpm. Without disturbing the sediment supernatant liquid was carefully removed from all the four tubes with a pipette and transferred to one graduated centrifuge tube. To this tube a drop of 0.5% aqueous basic fuschin was added to stain the grains and was centrifuged again. After centrifugation the supernatant liquid

**Table 1: Analysis of the physical and chemical parameters of some Indian honeys**

| No. | Name of honeys (State) | Moisture percent by mass | TRS percent by mass | Sucrose percent by mass | FGR | Acidity percent by mass | Fiehe's Test | HMF mg/kg | TPC/g of honey | A 660 nm % |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Max. 22 | Min.65 | Max.5.0 | Min.1.0 | Max.0.2 | | Max.80 | Max. 50000 | Max.0.3 |
| 1. | Amrut Agmark honey (MH) | 19.63 ± 0.03 (0.058) | 76.33 ± 0.21 (0.36) | 1.53 ± 0.009 (0.015) | 1.053 ± 0.002 (0.006) | 0.126 ± 0.0003 (0.0006) | + + + | 81.98 ± 0.04 (0.07) | 36000 ± 1528 (2646) | 0.146 ± 0.0000 (0.0000) |
| 2. | Baidyanath Agmark honey (MH) | 19.13 ± 0.03 (0.058) | 70.45 ± 0.02 (0.03) | 1.47 ± 0.012 (0.021) | 1.077 ± 0.002 (0.006) | 0.127 ± 0.0003 (0.0006) | + + + | 116.16 ± 0.03 (0.05) | 29000 ± 2309 (4000) | 0.231 ± 0.0003 (0.0006) |
| 3. | UttraKhand Agmark honey (UP) | 21.23 ± 0.03 (0.058) | 77.30 ± 0.37 (0.64) | 0.76 ± 0.009 (0.015) | 1.383 ± 0.005 (0.006) | 0.185 ± 0.0000 (0.0000) | + + + | 92.54 ± 0.07 (0.12) | 39000 ± 2000 (3464) | 0.250 ± 0.0003 (0.0006) |
| 4. | Golden Agmark honey (UP) | 18.20 ± 0.00 (0.000) | 51.31 ± 0.09 (0.15) | 27.39 ± 0.071 (0.123) | 0.783 ± 0.002 (0.006) | 0.136 ± 0.0000 (0.0000) | + + + | 136.70 ± 0.25 (0.43) | 31000 ± 3464 (6000) | 0.037 ± 0.0003 (0.0006) |
| 5. | Indian Agmark honey (UP) | 19.03 ± 0.03 (0.058) | 56.42 ± 0.08 (0.14) | 27.89 ± 0.043 (0.075) | 0.933 ± 0.005 (0.006) | 0.073 ± 0.0033 (0.0058) | - | 122.08 ± 0.05 (0.09) | 15667 ± 1453 (2517) | 0.338 ± 0.0003 (0.0006) |
| 6. | Ambika honey (MH) | 19.77 ± 0.03 (0.058) | 82.72 ± 0.13 (0.22) | 1.34 ± 0.003 (0.006) | 1.177 ± 0.005 (0.006) | 0.128 ± 0.0000 (0.0000) | - | 65.28 ± 0.03 (0.05) | 43667 ± 882 (1528) | 0.280 ± 0.0003 (0.0006) |
| 7. | Pure Honey (MH) | 21.90 ± 0.00 (0.000) | 72.33 ± 0.29 (0.51) | 4.18 ± 0.018 (0.031) | 1.083 ± 0.007 (0.015) | 0.162 ± 0.0003 (0.0006) | + + + | 82.56 ± 0.02 (0.04) | 29667 ± 3180 (5508) | 0.278 ± 0.0000 (0.0000) |
| 8. | Vindhya Vaili honey (MP) | 21.30 ± 0.00 (0.000) | 75.62 ± 0.34 (0.59) | 0.36 ± 0.012 (0.020) | 1.497 ± 0.010 (0.012) | 0.304 ± 0.0003 (0.0006) | - | 46.27 ± 0.01 (0.02) | 37667 ± 2603 (4509) | 0.338 ± 0.0003 (0.0006) |
| 9. | Dabur honey (HP) | 17.83 ± 0.03 (0.058) | 79.03 ± 0.22 (0.38) | 4.44 ± 0.044 (0.076) | 1.403 ± 0.005 (0.006) | 0.213 ± 0.0003 (0.0006) | - | 4.99 ± 0.03 (0.06) | 25333 ± 1202 (2082) | 0.231 ± 0.0003 (0.0006) |
| 10. | Mehsons honey (UP) | 19.87 ± 0.03 (0.058) | 74.74 ± 0.27 (0.47) | 1.55 ± 0.047 (0.081) | 1.093 ± 0.002 (0.006) | 0.144 ± 0.0003 (0.0006) | - | 10.18 ± 0.00 (0.01) | 42000 ± 2309 (4000) | 0.237 ± 0.0003 (0.0006) |

The values represent the mean ±SE (SD) of three determinations.
**MH**: Maharashtra, **UP**: Uttar Pradesh, **MP**: Madhya Pradesh, **HP**: Himachal Pradesh; **-** : Fiehe's test negative + + : Fiehe's test positive + + + : Fiehe's test strongly positivE

was taken out leaving 1ml. of the mixture for microscopical examination. Drop of this solution was placed on the Neubauer's Counting Chamber or haemocytometer. After placing the coverslip the slide was studied under the microscope.

**Determination of Absorbance of honey (BIS, 1994)**

Two gm of honey sample was dissolved in distilled water and the final solution was made to 10ml. The spectrophotometer was adjusted with distilled water in a cuvet at '0' absorbance or 100% transmittance at 660nm. Then honey solution was taken in the cuvet and the absorbance was directly read at the same wavelength.

**Statistical analysis**

In the present study statistical tests were applied to find out the mean, standard error and standard deviation values of all the tests performed. Results were evaluated using the Microsoft EXCEL 2000 software. In all cases the experiment was carried out in triplicate.

## RESULT AND DISCUSSION

In the present study 10 Indian honeys were investigated with an objective to analyze the physical and chemical attributes of selected honey samples. Evaluation of their quality was done based on concurrence with specifications set by AGMARK and BIS of which moisture content, TRS, sucrose, FGR, acidity, Fiehe's test, HMF test and TPC of the honeys were analyzed. Moisture content is one of the major factors which determine the quality of honey. Honey absorbs water when exposed to high relative humidity (RH) and gives off water when exposed to low RH (Sanford, 1982). Moisture content of honey helps to assess ripeness and shelf life, as honey with high moisture content may be spoiled by fermentation (Ruoff et al., 2005). Moisture content for A-grade honey is max. 22% by mass, and moisture content of all the assessed honeys were well maintained by the manufacturers and in accordance with the standards (Table 1), which was responsible for the shelf life of the honey and ultimately the quality. According to the Indian Standard Specification the TRS percent is minimum 65. Number of inexpensive sweeteners and syrups are now available and are used to replace the natural carbohydrates of honey (White, 1980). These sweeteners are illegally used by unscrupulous manufacturers or traders who seek large benefits by substituting expensive food products with low cost materials. Also there are certain adulterants which go undetected for adulteration (Recio *et al.,* 2006). In order to discontinue malpractice in the production of honey, a maximum limit of sucrose 5% by mass is permitted. FGR is used for the assessment of crystallization tendency of honey where higher ratio indicates liquid form and lower ratio indicates granulated honey (Austin**,** 1958), (Assil, 1991). Average FGR is 1.2 **:** 1 (White, 1978, 1980) and according to the Indian standards FGR is minimum 1. In our study we observed that 8 honeys met the standards for TRS, sucrose and FGR. But Golden Agmark honey and Indian Agmark honey from UP showed TRS and FGR exceptionally below the specified standard of min. 65% and min.1 respectively and sucrose exceptionally above the specified standard of max. 5% (Table 1). Honey regulations also depend on an important parameter 'total acidity' which can indicate the history of honey. Acidity signifies possible alcohol fermentation and production of acetic acid by bacterial action (Molan, 1996), (Kilchenmann and Amado, 2004). It has been documented that acidity of honey characterizes the presence of organic acids, lactones or

esters and inorganic ions such as phosphates and chlorides. Variation in acidity between different types of honeys is because of these constituents (Echingo and Takenaka, 1974), (Rodgers, 1979). Eight honeys retained acidity within the limits of BIS, 1994 (Table 1) whereas Vindhya Vaili honey (MP) and Dabur honey (HP) showed acidity exceeding the maxima of 0.2% by mass. This could be one of the important criterion for deterioration of honey quality. HMF is a break-down product of fructose which is formed slowly during storage and very quickly when honey is heated. Its presence is considered the main indicator of honey deterioration (Etzold and Lichtenberg, 2008). HMF indicates the freshness of honey. A trace of HMF (10mg/kg) is naturally present in honey. According to LaGrange and Sanders (1988) honey produced in subtropical climates has a high HMF value. So the standard for HMF content for Indian honeys is maximum 80mg/kg, higher than Codex Alimentarius Commission Standards (1995). Increase in HMF can be due to various reasons such as improper storage or handling, exposure to high temperature or ageing. But a large increase of HMF in honey could be due to overheating or due to the adulteration of honey with commercial invert sugar (Crane, 1980). Six honeys out of ten were positive for a qualitative preliminary test called Fiehe's test which was performed to detect presence or absence of HMF content in honey. Development of cherry red colour to the extract indicated a positive Fiehe's test. Increased level of HMF in Amrut Agmark honey, Baidyanath Agmark honey and Pure honey may be due to prolonged storage i.e. ageing because the test was performed on honeys whose date of expiration was finished leading to loss of freshness of honey. But in case of UttraKhand Agmark honey increased level of HMF may be due to high moisture content. In case of Golden Agmark honey and Indian Agmark honey discrepancy was observed in TRS, sucrose and FGR which clearly indicate that the honeys are certainly suspicious, and could perhaps be adulterated with external additives. Microscopic analysis of honeys revealed that all the honeys showed TPC within the maximum limits of value i.e. 50000. This is maintained by the manufacturers by using advanced ultrafiltration techniques. The drawback of this technique is it then becomes impossible to make out the geographical and botanical source of honeys. Absorbance of 8 honeys was within the BIS standards. Absorbance of Vindhya Vaili honey exceeded the maxima of 0.3 which may be due to increase of acidity level. In case of Indian Agmark honey, the absorbance was 0.338 which, according to Pfund scale of colour grading of honey illustrated that the honey is dark. On the whole, Indian Agmark honey was found to be a suspicious sample as TRS, FGR, sucrose content, total counts of pollens and absorbance do not meet the set standards and unfit for consumption.

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