A Comparative Analytical Study of Fresh Honey Collected from Honey Comb of Apis Mellifera and Market Samples of Honey

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i37A31993

Editor(s):
(1) Dr. Mohamed Fawzy Ramadan Hassanien, Zagazig University, Egypt.

Reviewer(s):
(1) Tajamul Rashid, Hamdard Institute of Medical Sciences & Research, India.
(2) Vijayashree Mathad, Narayana Health, India.

Complete Peer review History: https://www.sdiarticle4.com/review-history/71154

Received 10 May 2021
Accepted 14 July 2021
Published 15 July 2021

ABSTRACT

Honey is a natural product which was known to ancient seers of Ayurveda since Veda period. The nutritional and therapeutic benefits of honey were well known and also documented in authentic texts of Ayurveda. However the abundant availability of honey has gone decreasing since industrialization and deforestation. It has resulted in artificial culture and marketing of honey. The quality of such honey is matter of concern as it is easy to adulterate honey but is very hard to find out the difference between natural and adulterated honey. Considering this issue, present work has been planned to compare freshly collected natural honey and marketed samples of honey. For the comparative analytical study four different samples were collected. Three samples of three different pharmacies were procured from market (honey of branded Ayurvedic companies) and compared.
1. INTRODUCTION

Honey is known as Madhu or Kshaudra in Ayurveda compendia and contains a number of nutritionally important substances which support good health and recovery from few ailments. It is mixed with a variety of herbs and made into compound for curing various ailments. Honey is natural sweet substance produced by honey bees, especially by the species of *Apis mellifera* which is collected and transformed by combining specific substance of their own, deposit, dehydrate, store, & leave in honeycombs to ripen & mature. Honey is made from nectar contained in cultivated as well as wild flowers. According to Charaka Samhita which is an ancient Sanskrit text on Ayurveda medicine, there are four different types of honey such as Makshika, Bhamara, Kshaudra and Paittaka. Makshika is considered as the best type of honey which has color similar to sesame oil. It is produced by reddish variety of honey bees. Bhamara honey is produced by the Bhamara type of bees. It is heavy and white in color. Kshaudra honey is brown in color and produced by a small type of honey bee. Paittaka honey is produced by a large type of bees and the color is similar to ghee [1].

According to Ayurvedic system of medicine honey is Madhur (sweet) and Kashaya (astringent) in Rasa (Madhur is predominat Rasa and Kashaya is less predominant Rasa), Ruksa in Guna (property), Sheet in Veerya (potency). Immature honey leads to aggravations of Tridosha (three physical body humors) and mature honey restore these three Doshas in its equilibrium state. Newly formed honey increases the body weight and old honey decreases the body fat and thus body weight [2-3]. It is used as Anupana (is a fluid vehicle taken with or after medicine or eating and which assists in quick absorption and assimilation of the medicines. Anupana provides a medium of administration with acceptability and palatability. Sometimes, Anupanas are also used to produce an antidotal effect [4]. It is also used as probiotic [5].

Analysis of food, chemicals and medicines have key role in quality control, quality assurance as well as understanding and drawing suppositions regarding their pharmacokinetic and pharmacodynamic activities. Honey is dietary supplement, a chemical as it contains enzymes which get added from saliva of honey bees and it is medicine too. Therefore, along with physico-chemical analysis, the quality control parameters of honey essential include nutritional value analysis and special test which known as HMF value. Physico-chemical parameter provides overall quality information while nutritional and HMF value analysis provides vital details regarding quality of honey. Hence present work was planned on comparative analytical study by these three angles to find out differences in fresh honey collected from honey comb of *Apis Mellifera* and market samples of honey with objectives to assess the physical properties of fresh honey and marketed sample honey, to assess the chemical properties of fresh honey & marketed sample honey, to assess the fresh Honey & marketed honey by traditional techniques and to compare the physicochemical properties of fresh Honey & marketed sample honey.

2. MATERIALS AND METHODS

Materials: Collection of fresh Honey & marketed sample honey: For the comparative study of various analytical parameters of quality control for honey, four different samples were collected. Three samples of three different pharmacies were procured from market (honey of branded Ayurvedic companies) in original packing condition of nearly same period, while fresh honey sample was collected from the honeycomb of *Apis mellifera* from the herbal garden of Mahatma Gandhi Ayurveda College Hospital & Research Centre (MGACHRC), Salod Hospital & Research Centre (MGACHRC), Salod
The fresh collected sample was filtered three times to remove any contaminations. The honey was kept in air tight glass container to avoid moisture absorption.

**Sample codes**: Codes were given to the collected honey samples (Table 1).

**Analytical parameters**: Proximate analysis carried out on the honey samples to determine their organoleptic characters, physico-chemical parameters and microbial bioburden. Descriptive Statistics was used for data analysis.

**Traditional methods**:

I. Flame test- cotton wick is prepared and soaked in honey and burned in open air. Wick with original honey burns without sound like oil or ghee wick.

II. Thumb test: Few drops of honey taken on thumb and rub with index finger and feel the touch. Original honey feels slimy.

III. Water test: Few drops of honey put in bowl containing water. Original honey sinks at the bottom. Adulterated honey is soluble in water.

IV. Heat test: one tea spoon honey heated on gas flame. Original honey liquefies on heating.

**Observation and results**: The collected four samples of honey have almost similar organoleptic characters i.e. colour, odour, taste and touch. (Table 2) Similarly, observations of the traditional methods of testing honey by flame test (burning of honey), thumb test (putting thumb on surface of honey), water test (putting a drop of honey in water) and heat test (heating little quantity of honey in a vessel) are also found almost similar among natural honey and marketed samples of honey. (Table 3) In physico-chemical analysis, parameters such as Specific gravity, Loss on drying at 105°C, Brix test, Total ash, Fructose and Glucose proportion have comparatively less value in NH compared to BH, DH and PH. On the other hand moisture content in NH is more as compared to other samples. (Table 4)

In analysis of nutritional value, protein is not detected in NH and PH. Fat and crude fibers are not detected in all samples. The detected amount of protein in BH and DH is also negligible compared to daily requirement of protein. As honey is flower nectar collected by bees and gets broken down into simple sugar which is stored inside the honeycomb. Hence protein, fat and fibers are either nil or negligible. The Hydroxymethyl Furfural (HMF) value is found minimum in PH and higher in BH sample. (Table 5) Vitamin content such as Riboflavin, Niacin, Pantothemic acid, Pyridoxine and Folate are found within normal range in all four samples. (Table 6) Major difference is detected in mineral proportion in all four samples. The decreasing order of mineral proportion among the samples is NH, DH, BH and PH. (Table 7) As most of these minerals comes under category of essential micronutrients hence the observation indicate NH as best quality sample.

**Table 1. Codes of honey samples**

| S.N. | Honey sample collected from source | Code |
|------|----------------------------------|------|
| 1.   | Naturally collected honey from honey comb | NH |
| 2.   | Sample B* | BH |
| 3.   | Sample D* | DH |
| 4.   | Sample P* | PH |

*Brand name and pharmacy name are kept confidential*

**Table 2. Organoleptic characters of natural honey and marketed samples of honey**

| S.N. | Honey Code | Colour | Odour | Taste | Touch |
|------|------------|--------|-------|-------|-------|
| 01   | NH         | Light yellowish | Characteristic | Sweet | Smooth |
| 02   | BH         | Light yellowish* | Characteristic | Sweet* | Smooth |
| 03   | DH         | Light yellowish* | Characteristic | Sweet* | Smooth |
| 04   | PH         | Light yellowish | Characteristic | Sweet | Smooth |

*Tests for these samples were different organoleptically but not enough different to express them in available terms*
Table 3. Test reports by traditional methods of natural honey and marketed samples of honey

| S.N. | Name of Method | NH | BH | DH | PH |
|------|----------------|----|----|----|----|
| 01   | Flame test     | Wick burns without sound | Wick burns with faint sound | Wick burns with faint sound | Wick burns without sound |
| 02   | Thumb test     | Feel slimy | Less slimy compared to NH | Less slimy compared to NH | Less slimy compared to NH |
| 03   | Water test     | Sinks to bottom | Sinks to bottom | Sinks to bottom | Sinks to bottom |
| 04   | Heat test      | Liquefied | Liquefied | Liquefied | Liquefied |

Table 4. Physico chemical parameters of natural and marketed samples of honey (per 100 gm)

| S.N. | Name of Parameter | NH | BH | DH | PH |
|------|------------------|----|----|----|----|
| 01   | Specific gravity | 0.934 kg/m³ | 0.955 kg/m³ | 0.988 kg/m³ | 0.992 kg/m³ |
| 02   | Loss on drying at 105 °C | 6.21 w/w | 5.82 w/w | 6.13 w/w | 5.79 w/w |
| 03   | Total ash | 0.50 % | 0.65 % | 0.67 % | 0.61 % |
| 04   | Brix test | 81 °Bx | 85 °Bx | 85 °Bx | 86 °Bx |
| 05   | pH | 3.34 | 3.41 | 3.37 | 3.38 |
| 06   | Moist content | 20% | 17% | 19% | 18% |
| 07   | Fructose | 41% | 48% | 48% | 45% |
| 08   | Glucose | 36% | 37% | 39% | 38% |

Table 5. Nutritional value and HMF value of natural and marketed samples of honey (per 100 gm)

| S.N. | Nutritional value | NH | BH | DH | PH |
|------|-------------------|----|----|----|----|
| 01   | Protein | 0.0 % | 0.015 % | 0.02 % | 0.0 % |
| 02   | Fat | 0.0 % | 0.0 % | 0.0 % | 0.0 % |
| 03   | Carbohydrates | 81.64 % | 83.22 % | 84.74 % | 85.88 % |
| 04   | Total minerals | 0.44 % | 0.19 % | 0.23 % | 0.17 % |
| 05   | Crude fibers | 0.0 % | 0.0 % | 0.0 % | 0.0 % |
| 06   | Total energy | 326.55 Kcal | 355.81 Kcal | 369.35 Kcal | 343.51 Kcal |
| 07   | Hydroxymethyl Furfural (HMF) | 22.34 mg/kg | 39.25 mg/kg | 37.48 mg/kg | 19.85 mg/kg |

Table 6. Vitamin contents in of natural honey and marketed samples of honey (mg per 100 gm)

| S.N. | Vitamins | Normal value | NH | BH | DH | PH |
|------|----------|--------------|----|----|----|----|
| 01   | Riboflavin (Vit B<sub>2</sub>) | 0.01 – 0.02 | 0.012 | 0.013 | 0.011 | 0.013 |
| 02   | Niacin (Vit B<sub>3</sub>) | 0.10 – 0.20 | 0.17 | 0.09 | 0.11 | 0.14 |
| 03   | Pantothemic acid (Vit B<sub>5</sub>) | 0.02 – 0.11 | 0.08 | 0.07 | 0.09 | 0.08 |
| 04   | Pyridoxine (Vit B<sub>6</sub>) | 0.01 – 0.32 | 0.18 | 0.21 | 0.19 | 0.22 |
| 05   | Folate (Vit B<sub>9</sub>) | 0.002 – 0.01 | 0.001 | - | 0.001 | - |
|      | Total    | 0.443 | 0.383 | 0.402 | 0.453 |

*Obtained from Bogdanov S et al [4]

3. DISCUSSION

Honey is a product obtained from honeycomb as a thick, golden liquid produced by industrious bees from nectar of flowering plants and is saved inside the beehive. Nectar is a sweet juice presents in flowers and while collection of nectar by honey bees, it get mixed with honey bee extra stomach enzymes that transform its chemical composition and pH [6]. Therefore, chemical nature of honey varies based on types of flowers from which nectar is collected, season, geographical location and species of honey bees. However, honey prepared by specific type
Glucose and fructose also contribute in characteristics to the honey. Present that imparts the physical and nutritional were the principal sugars found in the honey (~38%) and glucose (~31%) and sucrose (~1%) as well as presence of solid content.

Specific gravity is measured to indicate thickness, however these have limitations when the differences in samples are minor or chemically stable.

Table 7. Mineral contents of natural honey and marketed samples of honey (mg per 100 gm)

| S.N. | Minerals | Normal value* | NH   | BH   | DH   | PH   |
|------|----------|---------------|------|------|------|------|
| 01   | Calcium  | 3 – 31        | 14   | 18   | 21   | 16   |
| 02   | Iron     | 0.03 – 4      | 1.5  | 0.56 | 1.2  | 0.7  |
| 03   | Magnesium| 0.7 – 13      | 07   | 03   | 05   | 07   |
| 04   | Phosphorus| 2 – 15      | 09   | 11   | 08   | 10   |
| 05   | Potassium| 40 – 3500     | 399  | 148  | 183  | 127  |
| 06   | Sodium   | 1.6 – 17      | 08   | 09   | 11   | 08   |
| 07   | Zinc     | 0.05 – 2      | 1.5  | 0.44 | 0.8  | 1.3  |
| Total|          | 440 mg        | 190 mg| 230 mg| 170 mg|      |

*Obtained from Bogdanov S et al [4]

Ayurveda is an Indian system of medicine which has numerous references regarding quality control and quality assurance of natural products. These Ayurveda parameters are based on five sense organs so they have limitations but they are easy to access without any cost. Hence the collected samples viz NH, BH, DH and PH were first analyzed by Ayurveda testing methods. All these samples have almost similar colour, odour, taste and touch. There were some differences in these parameters but as matter of feeling, these differences can’t be expressed. Similarly due to lack of parameter measuring methods, the minor variations observed in Flame test, Thumb test, Water test and Heat test have overall given same results. Hence it can be interpreted that the Ayurveda testing methods are utilizable when the collected sample of honey have more differences, however these have limitations when the differences in samples are minor or chemically stable.

Specific gravity is measured to indicate thickness as well as presence of solid content [7]. Fructose (~38%) and glucose (~31%) and sucrose (~1%) were the principal sugars found in the honey present that imparts the physical and nutritional characteristics to the honey. Proportion of glucose and fructose also contribute in increasing specific gravity of honey [8]. However the analytical data represent that NH has lowest specific gravity, fructose and glucose. Brix test indicates proportion of sugar in a sample [9]. The Brix value for NH is lowest (81/100 gm) and highest in PH (86/100 gm) indicated more proportion of sugar in PH. This raises concern of sugar adulteration in natural honey to increase its palatability or shelf life or to increase product quantity. However it is difficult to make any confirm claim regarding natural and marketed samples of honey as the difference may also be due to different plant species from which honey bees has collected the honey.

Loss on drying at 105 °C indicates presence of moisture content which is found highest in NH and hence the weight change after drying in sample NH (6.21 w/w) is comparatively higher than other samples [10]. Free hydrogen and hydroxyl ions in solute is represented by pH. It can be affected by chemicals in present in a solution and thus it is an indicator of chemical differences. pH of solutions also helps in understanding their solubility and biological availability of nutrients such as phosphorus, nitrogen, and carbon [11], pH of all samples is slightly different and varies between 3 to 3.5 representing chemical and nutrient differences which is also confirmed by nutritional value analysis.

The nutritional values of honey could be altered. The quality and biochemical properties of honey are related to honey maturity, production methods, processing and storage conditions, climatic conditions as well as the nectar source of the honey. Crude fibers and fats are not detected in nutritional value analysis of all four samples. Protein and carbohydrates are maximum in DH, while total minerals are highest in NH. In view of nutrition, energy and trace mineral requirement for living beings, it can be said that NH and DH are better source of these
nutrients compare to BH and PH. Similarly, the comparative total proportion of vitamins is highest in NH (0.443) followed by PH (0.453), DH (0.402) and BH (0.383). The nutritional value and profile differ accordingly and can thus influence the value of a particular honey for healthiness of human being [12]. However, in present work based on all studied parameters, natural honey is found best compared to three market samples (DH, BH and PH).

5-hydroxymethylfurfural (HMF) is an organic compound found in honey and food products due to result of reduction mechanism in sugars as an effect of increased temperature or heating. Additionally, storage conditions also change HMF value and thus HMF is considered as quality testing parameter for honey [13]. HMF value is directly proportional to fructose: glucose ratio as well as moisture content [14]. The fructose: glucose ratio in NH, BH, DH and PH is 1.13:1, 1.29:1, 1.23:1 and 1.18:1 respectively. According to this ratio the decreasing order of HMF should be NH<PH<DH<BH. However, obtained decreasing order is PH<NH<DH<BH. Lower HMF value in sample PH compared to NH is due to less difference in fructose: glucose ratio and less moisture content. According previous research on Indian honey, the range for different samples was found between 13.80 to 36.86 [15]. In view of this reference, all studied samples in present work are of acceptable quality. However the difference in samples compared to fresh natural honey is directing that other samples might have been gone through some processing [16-19].

4. CONCLUSION

In the present work, fresh natural honey and collected market samples of honey showed differences in both nutritional value as well as HMF value which represent possibility of some processing. As the values of analytical parameters of honey changes based on conditions such as source of honey, collection season, temperature and storage condition, hence it feels the need of hour to create standard quality range of honey different aforementioned conditions and such conditions should be part on labeling of honey. This will help in accessing quality of marketed samples of honey.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

Author would like to thank DMIMSU for motivating and providing all necessary help for writing this article.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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