INDIAN JOURNAL OF SCIENCE AND TECHNOLOGY

RESEARCH ARTICLE

Physicochemical properties of Honey from Traditional Beekeepers of Sohra

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OPEN ACCESS

Received: 09.06.2021

Accepted: 02.07.2021

Published: 17.07.2021

Citation: Kharkamni B (2021) Physicochemical properties of Honey from Traditional Beekeepers of Sohra . Indian Journal of Science and Technology 14(25): 2111-2118. https://doi.org/10.17485/IJST/v14i25.1055

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Funding: Principal and Governing Body, Lady Keane College, Shillong, for the funding received for the project

Competing Interests: None

Published By Indian Society for Education and Environment (iSee)

ISSN
Print: 0974-6846
Electronic: 0974-5645

Abstract

Objective: To determine the physicochemical properties of honey to help ascertain its quality. The properties investigated in the present work are specific gravity, refractive index, moisture content, total solids, pH, free acidity, electrical conductivity, and hydroxymethylfurfural (HMF). **Methods/Statistical analysis:** To determine properties of honey such as refractive index, moisture content, pH, free acidity, electrical conductivity, and hydroxymethylfurfural, the procedures prescribed by the International Honey Commission were followed. Specific gravity was determined using the method described by Rebiai et al. Total solids was calculated by the formula given by Amin et al. Statistical analyses were carried out in Microsoft Excel 2016, and all readings were presented as mean±standard deviation. Correlation studies were also used wherever applicable. **Findings:** The moisture content lies in the range (16.6±0.4) g/100g to (19.4±0.3) g/100g. The total solids of all honey samples were above 80%. The pH varies from (3.59±0.02) to (3.73±0.01). The free acidity recorded a minimum of (32.3±1.5) meq/kg and a maximum of (42.0±2.0) meq/kg. The honey samples recorded electrical conductivities between (0.51±0.15) mS/cmand (0.61±0.25) mS/cm. The HMF varies from (8.9±0.2)mg/kg to (34.2±0.2) mg/kg. All the properties that were measured were well within the range prescribed by international agencies responsible for the quality control of Honey. **Novelty:** Adulteration of honey is a major concern as it affects the health and well being of a person. The recent study by the Centre for Science and Environment, New Delhi which reported adulteration of honey by eight household brands in the Indian market is a cause of alarm. A noteworthy feature of the current study was that it highlighted the superior quality of honey from traditional bee-keepers of Sohra, which even have the potential for export. No adulteration was detected relative to the parameters being investigated.

Keywords: Sohra; Bee; Honey; Physicochemical properties; Quality; Adulteration

1 Introduction

Honey is a naturally sweet product produced by bees from the nectar of plants or honeydew. It is imbibed with antimicrobial [(1)] and anti-inflammatory properties [(2)].

The chemical composition of honey is predominantly carbohydrates. Additionally, honey also contains proteins, enzymes, organic acids, minerals, vitamins, amino acids, and water. Honey is used worldwide for its health benefits, but it is also one of the most adulterated food products. Adulteration of honey posed serious health issues to an individual. Honey adulteration generally raises the blood sugar of an individual and has a damaging effect on the vital organs of the body, especially the liver and kidneys [3]. Recent studies by the Centre for Science and Environment, New Delhi reported that eleven out of thirteen brands of honey in India failed the purity criteria for honey [4]. Out of these thirteen brands, eight were well-known brands in the Indian market. Studies in Mexico [5], also reported non-compliance of honey samples with international regulations. Several international agencies, such as the Codex Alimentarius Commission, the International Honey Commission, etc., provide a detailed insight into quality control for honey. According to such agencies, several physical and chemical parameters are used to define the quality of honey and check possible adulteration. Many countries have set up regulations or adopted guidelines of reputed international agencies mentioned above when it comes to quality control for honey. In India, the Food Safety and Standards Authority of India (FSSAI) is the government agency that monitors and set up regulations for quality control of food products, including honey. However, honey from traditional beekeepers of Meghalaya is not subjected to any form of quality control. Das et al. did a quality evaluation of honey from West Garo Hills of Meghalaya [6]. It was observed that the ash content of honey samples from this area was not at par by national as well as international standards. However, the authors pointed out the need for government intervention to create awareness amongst the people to adopt scientific techniques of bee keeping. Mahnot et al. also did a quality assessment of honey from Shillong, Mawsynram and Sohra [7]. Their work focused more on the bioactive properties of honey and reported that Sohra and Shillong honey have high quality standards. However, the villages from Sohra, from which the honey samples were procured, was not mentioned in their work. Geographical location is important as the flora of that region will play a crucial role in the botanical origin of honey. Physical and chemical properties of honey are affected by several factors such as temperature, shelf life, presence of adulterants, method of storage, amount of moisture etc. The objective of the current study is thus to determine the physicochemical parameters to help ascertain the quality of honey and check for possible adulteration. The properties investigated in the present work are specific gravity, refractive index, moisture content, total solids, pH, free acidity, electrical conductivity, and HMF.

2 Materials and Methods

2.1 Study Area

Sohra, located about 54 km southwest of Shillong, the state capital, has been selected as the study area. It is one of the most popular tourist destinations in Meghalaya and is located at 25.3^0N latitude and 91.7^0E longitude. Sohra is known for its beautiful and picturesque locations, which have captivated tourists worldwide. Honey from this region is also considered one of the best in the state. Frequently the honey is sold in local markets, but it is also sold under different brands such as Zizira, Meghalaya Natural Honey, Lemon Honey to name a few. The weather in the region is rainy and humid in the summer and dry and misty in winter. A significant part of the region's geographical area consists of hills and valleys with various flora from orchids to ferns.

The villages from Sohra which were selected for this study were presented in Table 1.

Table 1. Latitudes and Longitudes of the villages inSohra

Sample No	Village Name	Longitude	Latitude
1	Nongsteng	25° 28′ 88.2″ N	91° 65′ 73.9″ E
2	Mawphu	25° 31′ 77.3″ N	91° 65′ 32.8″ E
3	Umblai	25° 31′ 22.1″ N	91° 66′ 14.5″ E
4	Khatarshnong	25° 38′ 60.0″ N	91° 77′ 81.5″ E
5	Mawlong	25° 21′ 35.2″ N	91° 69′ 44.5″ E
6	Nongriat	25° 23′ 81.0″ N	91° 67′ 93.6″ E
7	Nongtraw	25° 37′ 94.5″ N	91° 76′ 37.4″ E
8	Sohbar	25° 21′ 08.8″ N	91° 74′ 85.5″ E

2.2 Experimental Methods

Honeys were collected from at least three beekeepers of each of the villages mentioned above and brought to the Department of Physics, Lady Keane College, Shillong, for analysis. Any visible contaminants were removed, and the honey samples were stirred at room temperature and then passed through a 0.5 mm sieve [8]. The honey samples were then gently pressed with a spatula

through the sieve and collected in glass bottles. All the chemicals used for the analysis of honey samples were of analytical grade quality.

2.2.1 Specific gravity

The specific gravity of honey was determined with the help of a specific gravity bottle[9] and is defined by equation (1)

$$Specific\ Gravity\ =\ \frac{Weight\ of\ specific\ gravity\ bottle\ (50\ ml)\ filled\ with\ honey}{Weight\ of\ the\ same\ bottle\ filled\ with\ water} \tag{1}$$

2.2.2 Refractive index and Moisture

The Refractive index of honey was determined by an Abbe's Refractometer[8]. Moisture content (in gm) per 100 gm of honey was obtained by the formula developed by Wedmore[10] from the data of Chataway[11] and given by equation (2)

$$W = \frac{\bar{1}.73190 - log(RI - 1)}{0.002243} \tag{2}$$

Where W is the moisture content, and RI is the refractive index.

2.2.3 Total Solids

The total solid in honey expressed in percentage[12] was determined from equation (3)

$$\text{Total solids } = 100 \text{ - Moisture content} \tag{3}$$

2.2.4 PH

The pH of honey was determined by a Digital pH Meter 335 (SYSTRONICS). 10 g of the honey sample was taken and dissolved in 75 ml of distilled water. The pH electrodes were then immersed in the solution, and the pH value was noted[8].

2.2.5 Free acidity

The free acidity of honey was determined by the method of titration. 10 g of the honey sample was taken and dissolved in 75 ml of distilled water. The resultant solution was titrated with a 0.1 M NaOH solution till pH 8.3 was achieved[8]. The free acidity of honey was expressed in milli equivalents/kg.

2.2.6 Electrical conductivity

The electrical conductivity of honey was measured by a Digital Conductivity Meter 304 (SYSTRONICS).

20 g dry matter of honey was taken in 100 ml distilled water and electrodes of the conductivity meter were dipped in the solution and readings were noted[8]. The electrical conductivity was expressed in $mScm^{-1}$.

2.2.7 HMF

HMF was determined by the method suggested by White[13] and approved by the International Honey Commission[8]. The instrument for the purpose was a compact double-beam spectrophotometer Shimadzu UV-1800 UV/Visible Scanning Spectrophotometer.

2.3 Statistical analysis

All readings were performed in triplicates and presented as mean±standard deviations. Correlation studies were also used where ever applicable. The statistical analyses were carried out in Microsoft Excel 2016.

Table 2. Physical Properties of honey

Sample No	Village Name	Specific Gravity	Refractive Index at 20^0C	Moisture(g/100g)	Total solids in %
1	Nongsteng	1.258±0.002	1.492±0.001	17.8±0.4	82.2±0.4
2	Mawphu	1.265±0.001	1.495±0.001	16.6±0.4	83.4±0.4
3	Umblai	1.264±0.003	1.493±0.002	17.5±0.6	82.5±0.6
4	Khatarshnong	1.267±0.003	1.492±0.001	17.7±0.2	82.3±0.2
5	Mawlong	1.265±0.002	1.493±0.001	17.1±0.2	82.9±0.2
6	Nongriat	1.266±0.001	1.492±0.001	17.5±0.2	82.5±0.2
7	Nongtraw	1.323±0.001	1.488±0.001	19.4±0.3	80.6±0.4
8	Sohbar	1.267±0.004	1.493±0.001	17.5±0.2	82.5±0.2

Table 3. Chemical Properties of honey

Sample No	Village Name	pH	Free Acidity(meq/kg)	Electrical Conductivity (mS/cm)	HMF (mg/kg)
1	Nongsteng	3.63±0.02	39.3±1.2	0.61± 0.25	8.9±0.2
2	Mawphu	3.59±0.02	37.0±1.0	0.51±0.2	18.6±0.3
3	Umblai	3.65±0.01	39.7±1.5	0.55±0.2	10.5±0.3
4	Khatarshnong	3.67±0.01	42.0±2.0	0.51±0.15	31.2±0.2
5	Mawlong	3.64±0.02	38.7±1.5	0.52±0.25	15.8±0.3
6	Nongriat	3.73±0.01	34.7±1.5	0.55±0.4	21.6±0.3
7	Nongtraw	3.69±0.02	37.0±2.0	0.57±0.25	32.6±0.2
8	Sohbar	3.72±0.01	32.3±1.5	0.55±0.38	34.2±0.2

3 Results and Discussion

3.1 Specific Gravity

The specific gravity of honey samples collected from areas around Sohra varies from (1.258±0.002) to (1.323±0.001) as shown in Table 2. The values obtained in this study were similar to the works of Sunkesula et al. for honey samples from Southern India (1.04-1.53)[14]. Honey samples from all villages except Nongtraw have specific gravity similar to Apis florae honey from the southern zone of Kerala[15]. On the contrary, specific gravity from Nongtraw was similar to the raw Trigona honey from Kerala[15].

3.2 Refractive Index and Moisture Content

The refractive index lies in the range (1.488±0.001) to (1.495±0.001) as shown in Table 2. The result was similar to the studies of honey samples from Eastern Romania by Albu et al. (1.485-1.499)[16]. Similar values were also recorded by Mahnot et al.[7] for Shillong honey (1.4885) and Sohra honey (1.4982).

The moisture content of the honey samples from Mawphu recorded a minimum of (16.6±0.4) g/100g, and those from Nongtraw recorded a maximum of (19.4±0.3) g/100g as shown in Table 2. The moisture content of honey samples in the current investigation were similar to those of Moroccan honey in a study by Bouhlali et al. (14.55-20.99%)[17]. The honey samples from Punjab and Khyber Pakhtunkhwa provinces of Pakistan also reported similar values of moisture content (16.32-19.91%)[18]. Albu et al. also reported moisture content in the same range for honey samples from Eastern Romania (15.20-20.77%)[16]. Nedji et al. also reported moisture content in the same range for Algerian honeys (17.88-18.38%)[19]. Saudi (15.64%), Yemeni (16.28%) and Egyptian (18.32%) honeys also have similar moisture content as reported by Sohaimy et al.[20]. Malaysian honeys also have moisture content in the same range (11.59 to 19.06%)[21]. Honey samples from around India also reported similar values of moisture; like the southern zones of Kerala by Krishnasree et al. (10.03-19.79%)[15], Kannad Taluka from Aurangabad by Bhalchandra (15.96 to 18.23%)[22], those from Varanasi district by Sahney et al. (13.38-21.44%)[23], Sohra and Shillong by Mahnot et al. (15.40-22.8%)[7]. Das et al. reported moisture content in the range of 18.8% to 19.3% for honey samples from West Garo Hills, Meghalaya[6]. Moisture content in honey is very crucial in quality control. The more moisture in honey, the more likely it will be spoiled due to yeast fermentation[24]. The Codex Alimentarius Commission set an upper limit of 20% for

Table 4. Comparison of physicochemical properties of Sohra's Honey with honeys from different regions of India and some other Countries

	Honey origin	Specific gravity	Refractive index at 20^0C	Moisture (g/100g)	Total Solid in %	pH	Free Acidity (meq/kg)	Electrical Conductivity (mS/cm)	HMF (mg/kg)
1	Present Study	1.258-1.323	1.488-1.495	16.6-19.4	80.6-83.4	3.59-3.73	32.3-42.00	0.51-0.61	8.9-34.2
2	Das et al [West Garo Hills of Meghalaya, India]	NA	NA	18.8-19.3	76.85-76.99	4.43-4.49	NA	0.321-0.322	0.05-17.7
3	Mahnot et al [North East India]	NA	1.4795-1.4982	15.4-22.8	NA	3.37-4.00	10.52-54.41	NA	49.87-297.93
4	Sunkesula et al [Southern India]	1.04-1.53	NA	11.5-16.4	81.0-87.4	4.25-4.62	22.4-38.4	0.68-0.87	NA
5	Krishnasree et al [Southern zone of Kerala, India]	1.28-1.40	NA	10.03-19.79	75.5-79	3.49-4.45	0.15-0.68	0.26-0.56	3.4-30.01
6	Albu et al [Eastern Romania]	1.414-1.450	1.485-1.499	15.20-20.77	79.23-84.80	3.673-5.503	2.4-50	0.13-0.679	NA
7	Bouhlali et al [Morocco]	NA	NA	14.55-20.99	NA	3.54-4.9	10.93-36.67	0.137-0.824	8.3-68.92
8	Khan et al [Pakistan]	NA	NA	16.32-19.91	NA	3.22-5.18	3.97-24.77	0.286-0.591	4.71-37.25
9	Nedji et al [Algeria]	NA	NA	17.88-18.38	NA	3.77-4.6	NA	0.29-0.41	NA
10	Sohaimy et al [Egypt, Yemen, Saudi Arabia and India]	NA	NA	14.73 -18.32	NA	4.114 – 4.637	NA	0.53 -4.18	NA
11	Moniruzzaman et al [Malaysia]	NA	NA	11.59-19.06	NA	3.83-4.1	NA	0.41-0.79	6.07-67.94
12	Bhalchandra et al [Aurangabad district Maharashtra, India]	NA	NA	15.96-18.23	NA	3.8-4.2	NA	0.07-0.8	9.28-20.9
13	Sahney et al [Varanasi district U.P, India]	NA	NA	13.38-21.44	NA	3.7-4.7	NA	0.24-1.15	NA
14	Brown et al [Trinidad and Tobago]	1.34-1.45	NA	16.9-32.4	NA	2.88-3.91	NA	NA	NA
15	Kavapurayil et al [Kerala, India]	1.302-1.420	NA	22.6-26.2	NA	3.47-3.76	NA	NA	11.52-203.52
16	Nayik et al [Kashmir Valley, India]	NA	NA	18.2-19.11	80.89-81.8	3.52-3.78	NA	0.25-0.79	5.49-22.64
17	Kumar et al [Northern India]	1.39-1.42	1.4814-1.4906	18.37-22.00	NA	3.81-4.85	14.83-40.17	0.28-1.00	3.65-23.16
18	Islam et al [Bangladesh]	NA	NA	17.19-19.19	NA	3.6-4.1	NA	0.2-0.8	3.06-703.1
19	Rane et al [Western Maharashtra, India]	NA	1.450-1.494	17-27.7	NA	3.88-4.4	3.8-19.2	0.058-1.69	7.4-22.45
20	Almasi et al [Southern Karnataka, India]	1.40-1.41	NA	18.10-18.50	NA	4.16-4.66	NA	0.53-0.8	NA
21	Abdulkhaliq et al [West Bank,Palestine]	1.422-1.43	NA	14.5-19.0	NA	3.03-5.98	NA	NA	2.1-34.2

moisture content [25], and it was observed that all honey samples from Sohra have moisture content less than 20%. A correlation coefficient of 0.7 between specific gravity and moisture content indicates a strong association between the two variables.

3.3 Total Solids

The total solids in honey samples from Sohra were above 80%, samples from Nongtraw recorded a minimum of (80.6±0.4)%, and those from Mawphu recorded a maximum of (83.4±0.4)% as shown in Table 2. Honey samples from Eastern Romania by Albu et al. [16] also reported similar range of total solids (79.23–84.80%) as well as studies in southern India by Sunkesula et al. (81.0-87.4%) [14]. However, studies of honey samples from West Garo Hills of Meghalaya reported total solids in the range 76.85% - 76.99%.

3.4 PH and Free Acidity

The pH of honey samples from Sohra varies from (3.59±0.02) to (3.73±0.01) as shown in Table 3. The pH values also fall within the recommended range of 3.40 – 6.10 [25]. Similar results were obtained by Brown et al. for honey from Trinidad and Tobago [26]. The values reported in the present study were also identical to honey samples from the West Bank of Palestine (3.03-5.98) [27]. Several workers from India also reported pH value in the same range as the present study such as the different parts of Kerala by Krishnasree et al. (3.49-4.45) [15] and Kavapurayil et al. (3.47 and 3.76) [28] and Jammu and Kashmir by Nayik et al. (3.52-3.78) [29]. However, Mahnot et al. reported pH of 3.9 for Sohra honey and pH 4 for Shillong honey [7]. Das et al. reported pH values in the range of 4.43-4.49 for honey samples from West Garo Hills, Meghalaya [6]. Honey samples from Mawphu were the most acidic with a pH value of (3.59±0.02), and those from Nongriat were the least acidic with a pH value of (3.73±0.01).

The free acidity lies in the range of (32.3±1.5) meq/kg – (42.0±2.0) meq/kg as shown in Table 3. The values were identical to the linden (12.70–49.80 meq/kg) and multifloral honeys (15.60–50.00 meq/kg) from Eastern Romania by Albu et al. [16]. Moroccan honeys also have free acidity almost in the same range (10.93-36.67 meq/kg) [17]. Similar results were also obtained by Kumar et al. for Indian honeys (14.83-40.17 meq/kg) [30] and by Sunkesula et al. for honey samples from southern India (22.4-38.4 meq/kg) [14]. Mahnot et al. [7] reported free acidity of 10.52 meq/kg for Sohra honey and 16.06 meq/kg for Shillong honey. Fermentation of honey increases its acidity, so the latter plays a crucial role in the quality control of honey. The Codex Alimentarius Commission recommended that the free acidity must not exceed 50 meq/kg [25], and all honey samples from Sohra areas have a free acidity less than the recommended value. The low pH of honey also indicates its effective antimicrobial action [31].

3.5 Electrical Conductivity (EC)

The honey samples from Sohra recorded electrical conductivities between (0.51±0.15) mS/cmand (0.61±0.25) mS/cm as shown in Table 3. The EC values in the present investigation were well within the recommended range (less than 0.8 mS/cm) [25]. The EC values in the current study were similar to the linden (0.279–0.646 mS/cm) and multifloral honeys (0.210–0.679 mS/cm) from Eastern Romania by Albu et al. [16]. Malaysian honeys also recorded values of EC in the same range (0.41-0.79 mS/cm) [21]. A study from Bangladesh by Islam et al. (0.2-0.8 mS/cm) [32] also reported similar values. The values of EC obtained in this study were also similar to Saudi (0.53 mS/cm) honey by Sohaimy et al. [20]. Similar values from India were also reported such as honey from Maharashtra by Rane et al. (0.2 and 0.8 mS/cm) [33], Jammu and Kashmir by Nayik et al. (0.25-0.79 mS/cm) [29], Indian honeys by Kumar et al. (0.28-1.0 mS/cm) [30], southern Karnataka by Almasi et al. (0.53-0.8 mS/cm) [34] and those from Varanasi district by Sahney et al. (0.24-1.15 mS/cm) [23]. A linear relationship between ash content and electrical conductivity given by C = 0.14 + 1.74 A, where C is the electrical conductivity, and A is the ash content [35] shows the dependence of electrical conductivity on ash content. However, the Codex Alimentarius Commission [25] recommended replacing ash content with electrical conductivity to ascertain honey quality. The electrical conductivity is nowadays also used to differentiate between blossom honey and honeydew honey [25].

3.6 HMF

HMF is generally absent in fresh honeys but increases with storage due to two main factors, the storage temperature and the pH of the honey [36]. HMF varies from (8.9±0.2)mg/kg to (34.2±0.2)mg/kg. The honey samples from Punjab and Khyber Pakhtunkhwa provinces of Pakistan also reported HMF values in the same range (4.71-37.25 mg/kg) [18]. The values obtained in the present study were also identical to honey samples from the West Bank of Palestine (2.1–34.2 mg/kg) [27]. HMF values in the same range were reported in the southern zones of Kerala by Krishnasree et al. (3.4-30.01mg/Kg) [15]. Mahnot et al. reported HMF values of 94.41 mg/kg for Shillong honey and 49.87 mg/kg for Sohra honey [7]. The honey samples collected from

Nongsteng with the lowest HMF value of (8.9±0.2) mg/kg indicated the honey's freshness. Moreover, a correlation coefficient of 0.6 between HMF and pH values indicates a moderate association between the two variables. All honey samples have HMF values less than 40 mg/kg as recommended by the Codex Alimentarius Commission [25].

4 Conclusion

The present work aims to assess the quality of honey from Sohra by determining its physicochemical properties. The mean specific gravity of honey from different villages around Sohra was found to be (1.27±0.002). The moisture content of all honey samples was less than 20% which was well within the recommended limits. The low moisture content indicated the ability of honey to resist spoilage due to fermentation. The total solids in all honey samples were above 80%, which showed high stability upon storage. All honey samples from Sohra were highly acidic. However, both the pH and free acidity were well within the recommended range. The electrical conductivity of all honey samples was also below 0.8 mS/cm and within the recommended range. Moreover, all honey samples from Sohra are nectar or blossom honey as they have electrical conductivity less than 0.8 mS/cm [25]. HMF of the honey samples collected was also within the recommended limits (less than 40 mg/kg). The above analysis showed that honey from Sohra has superior quality and was found to comply with regulations of international agencies such as the Codex Alimentarius Commission or International Honey Commission.

The study has some drawbacks which need to be addressed in future investigations. Some of the future studies may include mapping the sugar profile of honey, identifying key factors responsible for antimicrobial and anti-inflammatory properties, and estimation of the mineral content in honey.

Acknowledgements

The author is thankful to the Principal and Governing Body, Lady Keane College, Shillong, for the funding received for the project. The author is also grateful to Dr. G. Syngai, Department of Biochemistry, Lady Keane College, Shillong, Dr. T. Mukhim and Prof. E. R. Dkhar, Department of Chemistry, Lady Keane College, Shillong, for their support during the work.

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