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Research Article

Physiochemical Properties of Indian Honey from Different Floral Origins

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ABSTRACT

Honey is a natural sweet substance in taste produced by honey bees, from the honey dew and nectars of plant flowers. The present study investigates certain important physicochemical characteristics of honey samples of different floral origins which are very useful for characterization of honey. The values obtained for electrical conductivity (0.38–1.12 mS/cm), moisture content (19.61-23.99%), pH (3.80-4.07), ash content (0.14-0.56%) and specific gravity (1.35-1.43g/cm^2), were within the criteria set by National and International regulations of quality. The quality of honey depends upon the botanical origins, handling, transportation and storage conditions.

Key words: Bee honey, Electrical conductivity, Physical properties, Chemical properties

Introduction

Honey is defined as the natural substance sweet in taste produced by honey bees from the nectars of plant flowers and honey dew (Codex Alimentations, 2001). The honey is a valuable nutrient product containing large amounts of various sugars and small amounts of amino acids, lipids, vitamins and minerals (Cereser and Laux, 2010). The natural honey is a supersaturated sugar solution with 99% of its contents are sugar and water only (Siddiqui, 1970; Doner, 1977). The properties and compositions of bee honey depend on geographical floral origin, season, environmental factors and treatment of beekeepers (Da Costa Leite *et al*., 2000; Kaskoniene *et al*., 2010). To maintain purity and quality of honey in order to safeguard the consumer health, various national and international regulations have been formulated on the basis of physicochemical properties of honey (Codex Alimentations, 2001; EC, 2001; FSSAI, 2011). Bogdanov *et al.* (2004) found more than 22 different types of sugars in honey; however, fructose and glucose are the major sugar content. Primary sugars existed in honey are fructose and glucose, and in nectar honey the fructose content should exceed that of glucose (Zafar *et al.,* 2008). Fructose/glucose ratio indicates the ability of honey to crystallize (White and Doner, 1980; Buba *et al.,* 2013).

The quality of honey is a prime factor for consumers as the adulteration of honey has increased since last many years for quick economical gain. The honey can be adulterated easily by adding certain amount of sucrose, commercial glucose and artificial color etc. These artificial honeys often have similar taste and physical appearance of natural honeys, but they lack the medicinal and nutritional properties as that of natural honey. Hence, it becomes important to study the physicochemical properties of honey to assess its quality. The physicochemical properties of honey that can be easily determined have been found to be helpful for comparison of natural honey samples and also serves as important indicators that can help to distinguish natural honey from artificial honey

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(James *et al.*, 2009). The International Honey Commission (IHC) has therefore proposed certain quality factors like moisture content, sugar, acidity and viscosity for honey. Moisture content and acidity of honey play an important role in determining the overall characteristics of honey and final assessment of its quality (Nadezda *et al.*, 2014). Higher the moisture contents in honey, greater the possibility of yeast fermentation and thus the change of flavour and color of honey.

The low moisture content protects honey from microbiological activity and thus it can be preserved for longer periods (AL-Naji and Hujazy, 1982; Akhtar *et al*., 2014). Electrical conductivity of honey shows great variability according to the floral origin and can be used for differentiating between honey samples (Nigussie *et al.*, 2012). Total Soluble Solids (TSS) is a measure of the combined content of all inorganic and organic substances in honey in molecular, ionized or colloidal solution form and can be used to determine the honey purity (Khalil *et al*., 2012).

India has a long history of apicultural practices owing to its rich biodiversity. With more than 2.5 lakh beekeepers and an annual production of around 94,500 metric tons; India is now emerging as a major producer and exporter of honey with total market worth is around INR 20.0 billion (NBB, 2020). Although honey due to its multi-faceted beneficial properties and myriad uses is being consumed by millions of people in India, but comprehensive information regarding physicochemical properties of honey originating from different floral sources in the Punjab state of India is scanty. The purpose of this study was to evaluate the physicochemical parameters of honey originating from different floral sources in the Punjab state of India.

Materials and Methods

Honey samples

Honey samples of local origin were collected from reputed vendors in Ludhiana and Jalandhar districts of Punjab state of India representing sunflower honey, mustard honey and multifloral honey and all were harvested in 2022. All samples were stored at (-16 ±2°C) till further analysis to avoid the effect of laboratory conditions on the chemical composition and physical properties of honey samples. The samples were equilibrated to room temperature before the analyses were performed.

Electrical conductivity

Electrical conductivity of honey samples under analysis was measured using the DiST (Dissolved Solids Tester) by Hanna instruments (HI 98311) and a 20% (w/v) solution of honey was suspended in milli-Q water. Electrical conductivity was measured at constant temperature of 22^{o}C maintained using water bath.

Specific gravity

The specific gravity (density) of honey was determined as per the method given by BIS (1994). The empty (50 ml) specific gravity bottle was initially weighed and then filled upto the mark with distilled water and again weighed. Now, the water was replaced with honey sample and again weighed.

Moisture content and Brix value

Moisture is the key criterion that determines the ability of honey to remain fresh and free of fermentation (Bogdanov *et al*., 2004). The moisture content was determined from the refractive index measurement of honey. The measurement of the refractive index for selected samples was performed using an Abbe refractometer (LABORATE, model RF001) connected to a thermostatic water bath that kept the temperature constant at 20°C. The sample was left in the refractometer to equilibrate thermally for two minutes and then the reading was performed. The reading of refractive index was noted from scale by adjusting the cross wire of telescope of Abbe refractometer on region distinguishing the dark and light region. The refractometer was then cleaned and dried before the measurement of the next sample. The repeatability of the refractive index determination was evaluated by performing three subsequent measurements under repeatability conditions on the same sample. Then, the moisture content was determined by using its relation with refractive index (n) given by (Jdayil *et al.*, 2002) as:

$$\text{Moisture content (\%)} = 608.277 - 397.473 \times n \quad \ldots(1)$$

Total soluble solids as °Brix were also measured using the hand refractometer with ranges of 50°Brix - 85°Brix. The refractometer was first standardized, the prism was then washed with distilled water and dried off with a soft tissue. A drop of honey was placed in the refractometer prism and reading was noted. The Brix of the sample was then calculated.

pH

A pH meter was (Hanna Instrument HI 73127, UK) used to measure the pH of a 10% (w/v) solution of honey prepared in milli-Q water. The calibration of pH meter was done before each reading with the standard buffer solutions.

Ash content

The ash content was determined indirectly from the measured electrical conductivity by using the following equation (Piazza *et al.*, 1991):

$$X1 = \frac{X2 - 0.143}{1.743} \quad \ldots(2)$$

where *X1* = ash value; *X2* = electrical conductivity in mS/cm at 22°C. Ash content is a quality criterion for botanical and geographical origin of honey.

Statistical analysis

All analyses were carried out in triplicates and the data were presented as means ± standard deviations. Analysis of variance (ANOVA) was used to compare the quantified variables in the samples of honey. The significance was calculated for $p < 0.05$. The statistical analyses were performed with the SAS 9.3.

Results and Discussion

Ash content and electrical conductivity (EC)

Ash content is a quality criterion for botanical and geographical origin of honey and it also differentiates nectar honey from honeydew honey (Khan *et al.*, 2016; White, 1978). In the present study, mustard honey showed the lowest ash content (0.14 ± 0.01 g/100gm) and while the multifloral honey has the highest values of ash content (0.56 ± 0.05 g/100 g) (see Table 2). The results found in this study were within the maximum limit established for honeys (< 0.6%) (FSSAI, 2011). Since, nectar honeys have a lower ash content (≤0.6%) than honeydew honeys (≤1.2%) (Feas *et al.*, 2010), therefore, the findings further substantiate that the tested samples in this study were nectar honeys.

The obtained value of electrical conductivity in the examined samples showed the highest value of 1.12 ± 0.08 mS/cm for multifloral honey and lowest value of *0.38 ± 0.03* mS/cm for mustard honey (see Table 1). EC is too a good criterion of the botanical origin of honey and it is used in routine honey control instead of ash content (Adenekan *et al.*, 2010). In general, the value of electrical conductivity depends on the concentration and mobility of ions present in the honey which in turn depends on the ash and acid content of honey; the higher ash and acid content, the higher the resulting conductivity. The mustard and sunflower honey were within the standard limit (not more than 0.8 mS/cm) but the multifloral honey is out of the standard limit (Codex Alimentations, 2001). Obtained results indicated that, the quality of mustard honey was better than sunflower and multifloral honey.

pH

The pH values of honey samples were measured and the obtained results confirmed that, all tested samples were acidic within the standard limit (pH 3.80–6.10) (Codex Alimentations, 2001) that insures honey samples' freshness. Among all honey types, sunflower honey is the most acidic (3.80 ± 0.05) followed by mustard (3.86 ± 0.03) and multifloral (4.07 ± 0.08) (see Table 1). The lowest acidity was

Table 1. pH, electrical conductivity and specific gravity of selected honey samples

Honey type	No. of Samples	pH	EC (mS/cm)	Specific gravity (gm/cm^2)
Sunflower	6	$3.80^{b} \pm 0.05$	0.48c ± 0.04	$1.39^{c} \pm 0.03$
Mustard	6	$3.86^{b} \pm 0.03$	0.38c ± 0.03	$1.43^{b} \pm 0.05$
Multifloral	6	$4.07^{a} \pm 0.08$	1.12a ± 0.08	$1.35^{a} \pm 0.03$

Table 2. Refractive index, ash content (%), moisture content (%) and Brix of selected honey samples

Honey type	No. of Samples	Refractive index	Ash (%)	Moisture content (%)	Brix (%)
Sunflower	6	$1.481^{a} \pm 0.05$	$0.19^{c} \pm 0.02$	$19.61^{b} \pm 0.86$	$78.37^{a} \pm 1.86$
Mustard	6	$1.478^{a} \pm 0.03$	$0.14^{b} \pm 0.01$	$20.81^{b} \pm 0.94$	$77.87^{a} \pm 1.39$
Multifloral	6	$1.470^{a} \pm 0.02$	$0.56^{a} \pm 0.05$	$23.99^{a} \pm 1.79$	$76.21^{a} \pm 0.98$

detected in multifloral honey. The acidity of both sunflower and mustard honey is higher than multifloral honey and which is statistically significant ($p < 0.05$). The high acidity of honey correlates with the fermentation of sugars present in the honey into organic acid, which is responsible for two important characteristics of honey: flavour and stability against microbial spoilage (Bogdanov *et al.*, 1999). Furthermore, it might also indicate that the honey samples have high content of minerals (Mohammed and Babiker (2009) and El-Metwally, 2015).

Moisture content

Moisture content of honey is a limiting factor in determination of its quality, stability and spoilage resistance against yeast fermentation. The higher value of the moisture content indicates higher probability of honey fermentation during storage. Lower moisture limits (<20%), elongates honey shelf life which would be met by a large majority of the commercial honeys. In present study, the moisture content of the honey was determined by using equation (1) which was followed from the measured value of the refractive index. The obtained values of refractive index were 1.481 ± 0.05 for sun sunflower, 1.478 ± 0.03 for mustard and 1.470 ± 0.02 for multifloral honey, respectively (see Table 2). The obtained value of the moisture contents was 19.61 ± 0.86 g/100 g for sunflower, 20.81 ± 0.94 g/100 g for mustard and 23.99 ± 1.79 g/100 g for multifloral honey, respectively (Table 2) and is in accordance with the maximum limit set by FSSAI (i.e., < 25%). A difference in the moisture content was also observed, which could be attributed to the variations in floral origins of samples, climatic conditions (temperature and relative humidity) prevailing at the time of honey production and the practices adopted by the beekeepers. These observations agree with the findings of various researchers (Acquarone *et al*., 2007; Belay *et al*., 2013; Bogdanov *et al*., 2004) that water content is strictly related to botanical origin of the honey sample, harvesting techniques and extraction from the comb in relation to the ripening process by bees and can vary from season to season and from year to year. Low moisture content also helps to promote longer shelf life during storage (Terrab *et al.*, 2003). BRIX represents the total soluble solids present in the honey and is directly related to sugar contents. The BRIX values obtained in the present study varied between 76.21% and 78.37% (Table 2), which suggests that the samples were most likely unadulterated.

Specific gravity

The selected samples have acceptable range of specific gravity at room temperature i.e., > 1.35 gm/cm^2. As shown in the Table 1, the specific gravity is highest for mustard honey (1.43 ± 0.03 gm/cm^2) followed by sunflower (1.39 ± 0.03 gm/cm^2) and then multifloral honey (1.35 ± 0.03 gm/cm^2). The values obtained are in conformity with the findings of Kamal *et al.* (2002), Ouchemoukh *et al.* (2007) that the specific gravity values of multifloral and unifloral honeys varies from 1.35 to 1.37 gm/cm^2 and 1.40 to 1.43 g/cm^2, respectively. Significant difference ($p < 0.05$) was observed among samples under study.

Conclusions

This study was planned to investigate and evaluate the physiochemical characteristics of different samples of honey of different origins collected in the Punjab state of India. All investigated types of honey were acidic and were within the standard limit that indicates freshness of all investigated samples and were also within the standard limit of moisture content (< 25%), which can elevate the honey ability to resist fermentation and granulation and promote longer shelf life during storage. The ash content of all samples was in

acceptable range. There is a linear relationship between the ash content and the electrical conductivity (EC), and significant differences between examined samples ($p < 0.05$) was observed. All the samples have acceptable level of specific gravity (i.e., > 1.35 gm/cm^2) and significant difference ($p < 0.05$) was observed among samples under study. The present study concludes that, the quality and physicochemical properties of honey were varied based on the botanical origins, handling, transportation and storage conditions.

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