EVALUATION OF PHYSIOCHEMICAL PROPERTIES OF NIGELLA HONEY FROM SHARIATPUR, BANGLADESH: A COMPARISON WITH INTERNATIONAL REGULATORY STANDARDS OF HONEY

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Abstract: *Honey is a complex mixture of a variety of ingredients. The intent of the current study is to characterize the physical, chemical and biochemical properties of Nigella honey obtained from Shariatpur district of Bangladesh and also compare those features with the international standards set up by Codex, EU and International Honey Commission (IHC) in prospect of export to global market. The selected physiochemical parameters included, moisture, color, pH, total acidity, free lactone, electrical conductivity, total dissolved solids, protein, total sugar and hydroxymethylfurfural (HMF) content all of which were analyzed using standard laboratory methods. The moisture content was 18.35%, color identified as dark brown/amber (131mm), pH was 4, total acidity was 42.5 meq/kg, total protein content was 2%, total sugar content was 70%, HMF content was 32.48 mg/kg, electrical conductivity was found to be 1.2mS/cm and total dissolved solids was 826 ppm. The overall quality of Nigella honey sample appeared acceptable and matched with international standards.*

Keywords: *Nigella honey, physicochemical, hydroxymethylfurfural, honey quality, international standards.*

Introduction

Honey is a sweet liquid produced either by honeybees that collect the material from the nectar of flowers (blossom honey) or by the secretions of plants or plant sucking insects (honeydew honey)[1]. In the hives, the honeybees store the nectar, which they then consume and regurgitate in a synchronized manner. This results in partial digestion of saccharides and with further evaporation a sweet, thick and golden liquid is formed which is honey. The color and texture of honey diverges with the former ranging from dark amber to completely colorless while the latter varying from liquid, thick and partly or entirely crystallized. Extracted honey is characterized as the one that has been parted from the comb with aid of some external force such as centrifugal, gravitational or straining[2]. Honey has a long history in human consumption, and is used in various food and beverages as a sweetener and flavoring. Besides imparting natural sweetness, it is due to the many health-promoting properties of honey that has made it a desirable product within developed countries, especially the European Union[3].

Honey has an acidic pH and low moisture content, hence well-preserved against fermentation and granulation, although it is still susceptible to spoilage by osmophyllic yeasts and molds[4]. The carbohydrates of honey are produced when specific enzymes work on nectar sugars. Therefore, honey is a complex blend mainly composed of carbohydrates (70-80 % w/w), water (10-20 % w/w), while the rest are minor constituents[5]. The latter group include almost 180 substances comprising of

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enzymes, amino acids, protein, vitamin, minerals, ash, organic acids and phenol compounds[6]. The distribution of carbohydrates in honey is related to the botanical origin, type and physiological state of bees and strength of bee colonies[7]. It is the relative quantity of each of these constitutional ingredients along with pollen activity that renders a plethora of functional properties and is a determinant of the quality and sensory characteristics such as color, aroma and taste[8].

Often the main criteria of quality judgment and botanical origin identification for honey are its physical and biochemical properties. Primarily it is the floral origin, which determines the composition of honey although conditions such as climate, rock, soil and vegetation along with beekeeping practices and processing steps may have an impact on the physiochemical and sensory characteristics as well[8]. Adulteration of honey is made possible through avenues such as addition of sugars, syrups or compounds into honey to change its flavor, viscosity, make it cheaper to produce or increase fructose content to prevent crystallization. Therefore, governments of different countries and international agencies have laid down strict regulations related to composition and quality requirements of honey for marketing and labeling purposes so that consumers are not misled regarding the quality product. The regulations such as by Codex, Council Directive of European union and International honey commission (IHC) have set up admissible values for selected physical, chemical and biochemical parameters that have significant impact on honey quality such as: the sum of fructose and glucose content, hydroxymethylfurfural, the content of water, electrical conductivity, acidity etc.

In agriculture-based Bangladesh, where a subtropical climate with a dominance of humidity, hot temperatures and unpredictable monsoon exists, the configuration of honey cultivated in various parts of the country are bound to be different from each other and from samples found in other parts of the world. Though honey has always been inherent in the Bangladeshi food habit pattern, there is a lack of data on physical parameters of different honey according to regional and floral origin collected from across the country in relation to the internationally accepted standards. These standards need to be met in case of product export to global market and earn foreign exchange. For this study, our first objective is focusing on the characterization of physiochemical properties of Kalijira (*Nigella sativa*) honey collected from the beekeepers of Shariatpur, which is a district in the Dhaka division of central Bangladesh. The second objective is to compare the quality of the collected sample with the compositional criteria by European Union, IHC and Codex Standards relating to honey.

Materials and Methods

Sample collection

Sample of honey was collected from beekeepers of Shariatpur who are responsible for cultivating honey from flora of *Nigella sativa*. The honey was gathered by the process of extraction in which caps were detached from honeycombs and the honey was removed by centrifugation. The honey sample was stored in airtight container until further analysis. The study was carried out in the Food Processing and Analytical Laboratory of Daffodil International University.

Physiochemical and quality analysis

Moisture analysis

It was determined by the refractometric method in which at an ambient temperature the refractive index of the honey sample was obtained using a refractometer (Atago refractometer). The device was first standardized using deionized water and then a drop of honey was placed on the prism to obtain a reading using the scale. The refractive indices of the honey samples were measured at ambient

temperature and the measurements were further corrected for the standard temperature of 20°C by adding a correction factor of 0.00023/°C. The percentage of moisture content corresponding to the corrected refractive index was calculated using a Wedmore's table[7].

Total sugar estimation

Honey was suspended in MilliQ water to make a 25% (w/v) solution. The total sugar content of each honey sample was then determined using a refractometric method (Atago handheld refractometer, ATAGO, N-1α, Tokyo, Japan).

Total protein content analysis

Total protein substance of the honey sample was estimated using the Kjheldahl method[3], based on the principle of converting the organic nitrogen in sample to ammonium sulfate. The sample was first prepared by drying to make it devoid of water. It then underwent two consecutive steps: at first digestion and then next neutralization and distillation. In the first step sample was mixed with a catalyst and sulfuric acid for complete breakdown of organic material and thus liberated the nitrogen from protein to form ammonium sulfate. The digest was then diluted, neutralized with alkali and the distillate then collected in a flask with boric acid. Lastly the mixture was titrated with Hydrochloric acid using a conversion factor of 6.25, the % nitrogen obtained was converted to % crude protein.

Color analysis

Honey color characterization was done using spectrophotometer as described by White *et al*.[9]. Honey was first warmed to 50°C to clear off presence of any crystals and then allowed to stand to stop forming of bubbles. The honey was diluted by 50% and then absorbance reading was taken at 635 nm. Using the Pfund scale the color of honey was classified after converting the absorbance reading as stated by Ferreira *et al*.[10].

Free lactone and total acidity in honey (Electrometric method)

Total acidity in honey is based on the principle of titrating sample into sodium hydroxide to obtain the free acidity[11]. Sodium hydroxide was added to hydrolyze any lactone present and the excess immediately back titrated with hydrochloric acid. The total acidity was calculated as free acidity plus lactone in milliequivalents per kilogram. Honey was accurately weighed (10g) and then dissolved. Calibrated pH/reference electrodes and a stir bar were placed into the solution for mixing and pH was recorded, then titrated with 0.05N NaOH (rate of 5 ml/minute) until the pH reached to 8.5. The burette reading was recorded and immediately using a pipette, 10ml of 0.05N NaOH was added to the solution which was then back titrated to pH 8.3 using 0.05N HCL. A blank titration was also done without the analyte to check for possible sources of error. The free acidity (F.A.) is the acidity titratable with sodium hydroxide up to the equivalence point.

The lactone acidity corresponds to the combined acidity which is not directly titrable:

$$L.A.\left(\frac{meq}{Kg}\right) = [10 - (ml\ of\ 0.05\ HCl\ titrated)] \times 0.05 \times \left(\frac{1000}{10}\right)$$

The total acidity (T. A.) is the sum of the free acidity (F. A.) and the lactone acidity (L. A.):

$$T.A. = F.A.\left(\frac{meq}{Kg}\right) + L.A.\left(\frac{meq}{Kg}\right)$$

All results are expressed as milliequivalent of acid per kilogram of honey.

Electrical conductivity (EC) and total dissolved solids (TDS)

The EC and TDS were measured using a portable conductivity meter with a single probe (HI9813, Hanna Instruments). The probe was at first calibrated using a standard solution and then immersed in the test solution of honey. The latter was prepared by dissolving 20g of anhydrous honey into 100ml of deionized water to produce 20% of test solution (w/v) for measuring of conductivity[12]. The EC reading was obtained in mS/cm and the TDS reading was expressed as ppm.

Results and Discussion

Moisture

In the current study, the moisture obtained was 18.35%, which is within the international limit set by Codex Alimentarius i.e. 20%[13]. Previous moisture analysis of honey samples from Bangladesh have yielded moisture content within similar nearby range (12.79%-22.32%) as in found in this study[14]. The value of moisture content in honey is dependent on the level of honey maturity and climate condition of the geographical area where honey is harvested. The higher the moisture content is the higher probability of honey fermentation and granulation during storage. Thus, low moisture content (<20%) is imperative in extending shelf-life during storage of commercial honeys. Honey quality and water content can vary widely from hive to hive and even from cell to cell. Moisture content also depends on the temperature and relative humidity within the ecological region during production of honeys in honey colonies[13]. Additionally, honey that has been stored for some time will have a moisture gradient with lesser moisture at the top than at the extremity[4].

Color

The color designation of honey extracted is based on the standards as approved by USDA[2] according to the range of score on the Pfund scale. In the present study, the Pfund score was found to be 131mm, which falls in the range for the designation of dark amber color. Darker honey is found to have greater phenolic content followed by amber colored varieties while the lighter colored ones exhibit lowest antioxidant property[14]. Honey samples of Bangladesh[14] and other countries such as Italy[5], Slovenia[15] and Algeria[16] when examined spectrophotometrically at 450 nm, indicated the presence of pigments such as carotenoids and flavonoids that exert antioxidant activity. The intensity of honey color can also be regarded as an identifier for the floral origin of honey as well as its anti-oxidant properties. Botanical origin is one of the prime factors that determine the color of unprocessed honey. Besides this, it is the manner of handling the combs of honey by the beekeeper, maturity of honey or exposure to environmental factors such light, temperature and even metals that contribute in any changes in color.

Electrical conductivity (EC) & TDS

Electrical conductivity is proportional to the amount of dissolved solids in the mixture since they contribute to the formation of ions, which helps in the conduction of current. EC is a useful parameter to determine the quality of honey sample especially in ensuring its floral origin for correct labeling purpose. According to the Codex standard[17] and the directive by European Union[18] relating to honey, the conductivity value of honey especially from nectar is restricted to not more than 0.8 mS/cm whereas honey from honeydew should not have EC less than 0.8 mS/cm. In the current study, EC was found to be 1.2 mS/cm and TDS appeared to be 826 ppm which is very high compared to the standard. Even previous studies on honey of Bangladeshi origin have reported EC for nigella honey to be 0.3[19]. But heterogeneity of conductivity among several samples and varieties of honey is plausible due to varying ecological and botanical conditions such as source of honey (floral or honeydew), season, acidity, moisture, viscosity and salt content[19]. The high EC value may also reflect on the

occurrence of honeydew particles in the present honey sample and it is up to further melissopalynological analysis that can quantify the proportion of honeydew in the sample from seemingly monofloral origin[14].

Protein

The crude protein content of the investigated honey sample was found to be 2%. Previous reports state the average nitrogen content in honey not to be more than 0.5% along with large standard deviation. Floral origin and type of pollen also plays a role in variability of protein amount within different honey samples. A comparative analysis on physiochemical properties of different honey obtained from Tangail, Bangladesh reported the protein content to be 1.1% for mustard honey, 0.57% for litchi honey and 0.99% for Nigella honey, the latter value being much lower than the one obtained in our investigation[18]. This difference in result can be attributed to the disparity of pollen content of honey samples in the two studies caused by either dissimilarity in geographical position of plants or climatic conditions. The protein content in honey also adds up due to a combined endowment of enzymes such as glucose oxidase invertase and diastase introduced by the honey bees and of substances derived from the nectar[20].

pH and total acidity

Honey is naturally highly acidic. Its pH is extremely low, ranging between 3 and 4.5, which inhibits the growth of bacteria and other spoil-ready organisms. In the present investigated sample, pH obtained was 4 and total acidity amounted to 42.5mEq/kg. This aligns with the standard limit of pH imposed by Codex (pH: 3.4-6.1)[13]. The old standard fixed a maximum total acidity of 40 milliequivalents/kg, which has been increased to 50 milliequivalents/kg in the Codex draft, as there are some honeys, which have a higher natural acidity[21]. The result obtained is lower than pH value examined in nigella honey from Tangail, Bangladesh[21]. The high acidity of our present sample correlates with the greater fermentation of sugars present in the honey into organic acid, thus ensuring more flavor and strength against microbial spoilage.

Hydroxymethylfurfural

The EU directive have limited the HMF content to not more than 80mg/kg for honey from tropical climates. The studied sample had yielded HMF value of 32.48mg/kg which is acceptable according to all standards. A previous study on HMF analysis of different varieties of Bangladeshi honey yielded result from 0.0-200 mg/kg[22]. HMF is a cyclic aldehyde produced by the degradation of reducing sugars in presence of low pH of honey and the formation is accelerated by temperature. Low HMF (0-0.2mg/kg) indicates that honey has been freshly harvested whereas high value signifies inapt exposure to heating or improper storage of honey.

Sugar analysis

The total sugar content of the investigated sample was found to be 70% which aligns with the European Standard, where the content of reducing sugars (fructose, glucose and maltose) in pure honey sample is dictated to be not less than 60%[1]. The present result is similar to a previous study where the sugar content of Nigella honey sample from Bangladesh also was found to be 70%[22]. Yet in another study, the sugar content of Bangladeshi honey samples was found within the range of 42.8-60.67%[23]. The low sugar content can be attributed to processing, storage and even the floral source[22]. The measurement of reducing sugars mainly demonstrates botanical origin i.e. the distinction between blossom and honeydew honeys. Impure and pure honey do not significantly differ in their total proportion of reducing sugars. Thus, other trait in the sugar profile is needed to identify adulterated

honey. One example is that compared to pure honey, adulterated honey displays sucrose level surpassing 5%. According to the European Standard for pure honey, sucrose level of not more than 5% has been recognized[1].

Table 1: **Physiochemical properties of Nigella honey from Shariatpur in comparison to international guidelines**

Physiochemical Properties	Nigella Honey	Codex standard[13]	EU council directive[17]	IHC guideline
Appearance	Dark brown/ amber	NA	NA	Colorless to dark brown
Moisture	18.35%	Not more than 20	Not more than 20	Not more than 20
pH	4.2	3.4-6.1	NA	3.4-6.0
Total reducing sugar	70%	• Floral honey= >60% • Honey dew honey= >45%	>60%	>60%
Electrical conductivity	1.2 mS/cm	• Floral honey= not more than 0.8 • Honey dew honey= not less than 0.8	≤ 0.8	0.8-4.4
HMF (mg/kg)	32.48	• Blend honey= not more than 40 • Honey of known origin= 80	≤ 80	<80
Protein	2%	NA	NA	NA
Acidity (meq/kg)	42.5	≤ 50	≤40	NA

NA= not available

Conclusion

The present study can be concluded on the note that the investigated samples have shown good physiochemical properties with majority of the properties matching with different international guidelines, pointing the low moisture content, high pH, dark color intensity and which indicates towards high antioxidant property and HMF within required range indicating freshness and appropriate processing technique of the honey. For future investigation, the selected honey sample may be examined for it content of bio-active compounds to determine its anti-oxidant potential thus ensuring about its health-promoting features.

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