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# Qualitative evaluation of honey available in Bangladeshi markets

**Mohammad Shaokat Ali[1]*, Md. Fahad-Bin-Quader[1], Md. Ashraful Islam[1], Shamima Ahmed[2], Afifa Siddiqua[1], Tanjina Akter[1]**

[1]Department of Applied Chemistry and Chemical Technology, Faculty of Food Science and Technology, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong-4225, Bangladesh
[2]Department of Food Processing and Engineering, Faculty of Food Science and Technology, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong-4225, Bangladesh

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***Corresponding Author**

M Shaokat Ali
shaokat.fst@gmail.com

**ABSTRACT**

The aim of the present study was to characterize the physicochemical properties of Bangladeshi honey samples. A total of twelve samples from four branded honey were examined to evaluate eight physicochemical properties such as, pH, moisture content, ash content, diastase activity, total solids (TS), acidity, HMF (hydroxymethyl-fufural) and specific gravity. The mean levels of the properties significantly ($p<0.05$) varied between 2.93 to 4.29 for $P^H$, 0.02 to 0.07% for acidity, 2.57 to 12.58% for moisture content, 88.4 to 97.53% for total solid, 0.05 to 0.118% for ash content, 1.36 to 1.42 for specific gravity, 5.7 to 38.66 mg/kg for HMF and 1.93° to 12.97° Gothe for diastase activity. All honey samples were found to meet Codex Alimentarius Commission and European Legislation (EC Directive 2001/110) standards for all properties, except for diastase activity. Other attributes such as reducing sugar, carbohydrates and ketose sugar were also evaluated. Reducing sugar, carbohydrate and ketose sugar showed positive results for all honey samples. It was also found that all the honey samples were adulterated with water but no honey samples were adulterated with sugar. Although honey sold in Bangladesh is generally of good quality, efforts need to be made to reduce the adulteration of honey.

## INTRODUCTION

According to the Council of European Union (2002), "honey is the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature." Honey is a sugar solution of high osmolarity that possesses valuable nourishing, healing and prophylactic properties (Pereira et al., 1995). Honey contains iron and copper which are responsible for the redox properties of honey and potassium, being the most abundant (Kumar et al., 2010). The chemical composition of honey is complex, containing approximately 181 substances including sugars, proteins, moisture, vitamins, minerals, 5-hydroxymethylfurfural (HMF), enzymes, flavnoids, phenolic acids and volatile compounds (White, 1975; Kirk and Sawyer, 1991). It is a high-energy carbohydrate food (80–85%) and the honey sugars are easily digestible as those in many fruits (White and Doner, 1980). Bogdanov et al., (2004) found more than 22 sugars in honey; however, fructose and glucose are the major sugar content.

In Bangladesh, honey is produced and consumed on a large scale. Sundarban which is the largest mangrove forest in the world produces about 50% honey produced in Bangladesh and is ideal for giant honey bees (*Apis dorsata*) and honey collectors (Gani, 2001). Since honey types differ from one country to another and in different regions in the same country due to floral origin, soil composition, season, environmental factors and treatment of beekeepers and other factors consequently, quality criteria differ from one honey type to another (Da Costa Leite et al., 2000; El-Metwally, 2015).

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The quality of honey is mainly determined by its sensorial, chemical and physical characteristics. The criteria for ensuring quality honey have been specified by the Codex Alimentarius Commission (2001) and the EC Directive 2001/110. The major characteristics are moisture content, total solids, pH, ash content, reducing and non-reducing sugars, free acidity, diastase activity and HMF content (Al et al., 2009; Kirk and Sawyer, 1991).

Hydroxymethylfurfural (HMF) is a cyclic aldehyde ($C_6H_6O_3$) that is produced by degradation of sugars (Cervantes et al., 2000). HMF has been widely used both to predict honey freshness and to evaluate its quality upon treatments such as overheating, storage abuse, and adulteration with syrup (Karabournioti and Zervalaki, 2001). Several factors influence the levels of HMF content, such as temperature and time of heating, storage conditions, pH and floral source (Khalil et al., 2010).

Another important parameter is diastase activity. Diastase (α-amylase) is one of the predominant enzymes in honey, next to invertase and glucose oxidase, which is added to honey by the bee during the collection and ripening of flower nectar (Oddo et al., 1990). Its level also depends upon geographic and floral origins of the product. Along with HMF, diastase activity can be used as indicative of aging and temperature abuse (Fallico et al., 2006). Diastase activity may be decreased at the time of storage or the product subjected to overheating (Yücel and Sultanoglu, 2013).

Nevertheless, there is no sufficient work on quality determination as well as effect and extent of adulteration for locally produced natural honey. Honey because of its relatively high prices, it has been targeted to be adulterated with other low prices products (Cotte et al., 2007). The most common adulteration practiced with honey is the addition of sucrose, corn syrup, molasses, water or other harmless or harmful materials. The act of honey adulteration is causing severe impact on the domestic and international market opportunities of the product and may result nutritional and health problems on consumers (Ayansola and Banjo, 2011). Authentication of honey by its natural physicochemical properties, and other attributes is important to protect genuine honeys from fraud products and to create consumers trusts.

## MATERIALS AND METHODS

### Collection of sample

A total twelve samples from four different honey brands (Brand A, B, C and D) was bought from the local market of Chittagong, Bangladesh. The collected samples were immediately transported to the laboratory for analysis. All samples were stored at (28 ± 2°C) till further analysis to avoid the effect of laboratory conditions on the chemical composition and physical properties of honey samples (El-Metwally, 2015).

### Quantitative analysis of physicochemical properties

All honey samples were examined for physicochemical properties such as pH, moisture, ash, acidity, total solids (TS), specific gravity, HMF (Hydroxymethyl-furfural), diastase activity. Ash, pH, acidity, total solids, HMF, diastase activity and moisture were determined according to the standard methods of the Association of the Official Analytical Chemists (AOAC, 1990). While specific gravity of the study samples were investigated by using the method of (AOAC, 2000).

### Qualitative phyto-chemical analysis

#### *Detection of carbohydrate (Molisch's test)*

Two ml of each of the honey sample was placed into individual test tubes. Few drops of molisch reagent and 1 ml of concentrated sulfuric acid were added to the test tube. The development of a red-violet color ring at the junction of the two liquids indicated the presence of carbohydrates in the honey samples (Sadasivam and Manickam, 2005, p.2).

#### *Detection of reducing sugar (Fehling's test)*

To the each honey samples, equal quantities of Fehling's solution A and B were added. It was then kept in a boiling water bath for few minutes. Formation of a brownish-red precipitate indicated

the presence of reducing sugar (Sadasivam and Manickam, 2005, p.3).

***Detection of reducing sugar (Benedict's test)***

Two ml of Benedict's reagent was added to few drops of each of honey samples and was heated in a boiling water bath for 5 minutes and then cooled under tap water. Development of green, yellow or red color indicated the presence of reducing sugar in honey samples (Sadasivam and Manickam, 2005, p.3).

***Detection of ketose sugar like fructose (Seliwanoff's test)***

Two drops of each sample solution was heated with 2ml seliwanoff's reagent in boiling water bath. Formation of a deep red color indicated the presence of ketose sugar (Sadasivam and Manickam, 2005, p.3). Fructose gave red color within half minute.

***Adulteration Confirmation Test***

The collected honey samples were analyzed for adulterants. Following physical tests were carried out to identify the purity and adulterants added to the sample.

***Detection of added water (Flame test)***

The presence of added water in each honey sample was determined by putting a drop of honey on a laboratory Bunsen burner by using cotton wick. The presence of added water was confirmed by observation of cracking sound without flame. Pure honey gave smokeless flame (Patil, 2016, pp 12).

***Detection of added sugar (Fiehe's test)***

This test was performed according to the methods followed by Instituto Adolfo Lutz (IAL, 2008). Two grams of each honey sample was dissolved in 10 ml of water, extracted with 30 ml of diethyl ether in a separating funnel, and the layer was concentrated to 5 ml. 2 ml of freshly prepared resorcinol solution (1 g of resublimed resorcinol in 100 g ml of hydrochloric acid) was added. The solution was shaken. A cherry red color appearing in a minute indicates the presence of commercial added sugar. Yellow and other shades had no significance.

**Statistical Analyses**

All analyses were carried out in triplicates and the results were expressed as mean values with standard deviations (SD). The significant differences represented by letters were obtained by a one-way analysis of variance (ANOVA) along with the least significant difference (LSD) test using SPSS software (SPSS 16.0). The level of significance of the mean values was assigned at $P<0.05$.

## RESULTS AND DISCUSSION

**Physicochemical analysis**

The pH values of honey samples were measured and the obtained results confirmed that, all tested samples were acidic (pH 2.93 to 4.29) (Table 1) and within the standard limit (pH 3.40–6.10), (Codex Alimentarius, 2001) confirming the fressness of the honey samples. Among the honey samples, Brand C had the highest pH value (4.29) followed by Brand B (3.91), Brand D (3.6). The lowest pH was detected in Brand A (2.93) (Table 1). There was a significant difference ($P< 0.05$) in pH values recorded for the four studied honey Brands. The pH values obtained for the honey samples of this study were close to those previously reported in Brazilian, Spanish and Turkish honeys (Azeredo et al., 2003; Kayacier and Karaman, 2008). Low pH has significance effect on microorganisms which inhibits the presence and growth of microorganisms and increases the shelf life (Terrab et al., 2002). All pH values in this study were low enough to slow down or prevent the growth of many species of bacteria.

The acidity of different honey had increased significantly ($P<0.05$) from 0.02% to 0.07% (Table 1). Similar observation was made by Nagai et al., (2006) who reported 0.08% acidity in honey. 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: flavor and stability against microbial spoilage (Bogdanov et al., 2008). Furthermore, the pH of the honey is not

directly related to free acidity because of the buffering action of the various acids and minerals present (Abou-Tarboush et al., 1993).

There was significant difference (P>0.05) in the moisture content between Brand A and C. The moisture content in the investigated honey samples (Table1) was between 2.57 (Brand C) to 12.58% (Brand B), which are within the limit (≤20%) recommended by the international quality regulations (Council Directive of the European Union, 2002). The average values recorded for moisture content in this study were lower than those found in Northwest Moroccan honeys (between 14% and 24.1%) (Terrab et al., 2002). Water content is very important for the shelf life of honey during storage (Terrab et al., 2003) and can lead to undesirable honey fermentation due to osmotolerant yeasts, which form ethyl alcohol and carbon dioxide (Bogdanov et al., 1997). However, all of the tested Bangladeshi honey samples were of good quality, as indicated by the low moisture content.

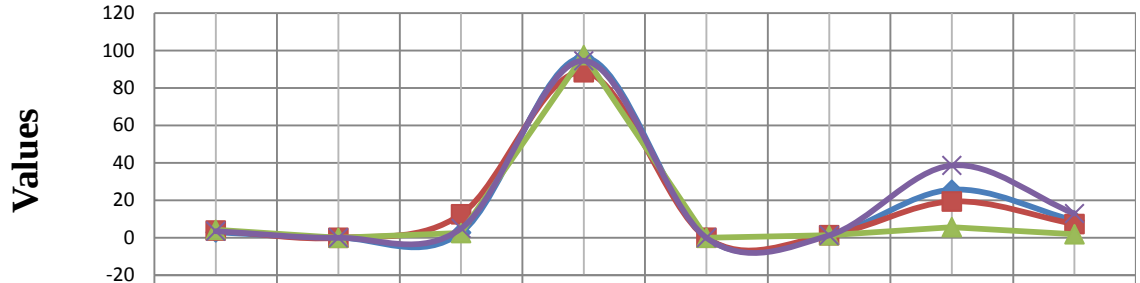
Certain nitrogen compounds, minerals, vitamins, pigments and aromatic substances contribute to the ash content of honey. The ash content of Bangladeshi honey samples ranged between 0.05 (Brand B) to 0.118% (Brand A) (Table 1). The Codex Alimentarius (2001) standard specified an ash content of not more than 0.6% for normal honey. Ash content of all samples was within the acceptable range (<0.6%). These results for ash content of honey samples were not significantly different from one sample to another, except samples of Brand A. These results are in accordance with those of Cranel (1976) who reported 0.1-1.0% ash content of honey.

Total solid is an indication of dissolved solids in the collected honey samples. Meanwhile the highest value of TS (Total Solids) found in Brand C (97.53%), the lowest amount of TS found in sample B (88.4%) (Table1). Significant differences (p<0.05) were observed between different samples. Juszczak and Fortuna (2006) studied honey samples were found to contain lower solids compared to Bangladeshi honey. In all honey samples total solids were more than 82%. Honey with total solids greater or equal to 81.4% is considered to be of higher grade but ranging from 80% to 81.3% is considered to be of lower grade (USDA, 1985; Nyau et al., 2013). The variations in total solids might be dependent on climate, floral source and some other factors.

Table 1
Physicochemical properties of four different Bangladeshi honey samples.

| | Honey samples | | | |
|---|---|---|---|---|
| Parameters | Brand A | Brand B | Brand C | Brand D |
| pH | 2.93±0.042$^{a}$ | 3.91±0.04$^{b}$ | 4.29±0.064$^{c}$ | 3.6±0.30$^{d}$ |
| Acidity (%) | 0.036±0.008$^{a}$ | 0.02±0.001$^{b}$ | 0.04±0.001$^{a}$ | 0.07±0.002$^{c}$ |
| Moisture content (%) | 2.85±0.056$^{a}$ | 12.58±0.665$^{b}$ | 2.57±0.098$^{a}$ | 5.07±0.146$^{c}$ |
| Total solids (%) | 96.18±387.7$^{a}$ | 88.4±356.6$^{b}$ | 97.53±393.13$^{ac}$ | 94.5±381.13$^{ad}$ |
| Ash content (%) | 0.118±3.47$^{a}$ | 0.05±3.19$^{b}$ | 0.08±3.$^{b}$ | 0.09±3.362$^{b}$ |
| Specific gravity | 1.36±0.03$^{a}$ | 1.42±0.03$^{b}$ | 1.38±3.327$^{ab}$ | 1.36±0.083$^{ab}$ |
| HMF (mg/kg) | 25.65±0.390$^{a}$ | 19.4±0.186$^{b}$ | 5.7±1.170$^{c}$ | 38.66±1.03$^{d}$ |
| Diastase activity (Gothe units) | 9.18±0.246$^{a}$ | 7.37±0.096$^{b}$ | 1.93±0.127$^{c}$ | 12.97±0.686$^{d}$ |

Values are mean ± standard deviation (n=3). Mean values marked with different letters in a same raw are significantly different (P<0.05). Mean values marked with same letters in the same raw are not significantly different (P>0.05).




| | pH | Acidity (%) | Moisture (%) | Total solids (%) | Ash (%) | Specific gravity | HMF (mg/kg) | Diastase (Gothe °) |
|---|---|---|---|---|---|---|---|---|
| Brand A | 2.93 | 0.036 | 2.85 | 96.18 | 0.118 | 1.36 | 25.65 | 9.18 |
| Brand B | 3.91 | 0.02 | 12.58 | 88.4 | 0.05 | 1.42 | 19.4 | 7.37 |
| Brand C | 4.29 | 0.04 | 2.57 | 97.53 | 0.08 | 1.38 | 5.7 | 1.93 |
| Brand D | 3.6 | 0.07 | 5.07 | 94.5 | 0.09 | 1.36 | 38.66 | 12.97 |

Figure 1
Comparisons of physicochemical properties of different honey samples.

Table 2
Qualitative analysis for phyto-chemicals.

| Test | Test Method | Results | | | |
|---|---|---|---|---|---|
| | | Brand A | Brand B | Brand C | Brand D |
| Carbohydrate | Molicsh's Test | + | + | + | + |
| Reducing sugar | Fehling Test | + | + | + | + |
| Reducing sugar | Benedict Test | + | + | + | + |
| Ketose sugar | Seliwanoff's Test | + | + | + | + |

Note: + = Positive/Present; - = Negative/Absent;

Table 3
Detection of adulterants in honey samples.

| | Test Method | |
|---|---|---|
| Brand samples | Flame test | Fiehe's test |
| Brand A | Cracking sound | No color |
| Brand B | Cracking sound | No color |
| Brand C | Cracking sound | No color |
| Brand D | Cracking sound | No color |

The specific gravity of honey samples spanned between 1.36 (Brand A and D) to 1.42% (Brand B) (Table 1). The differences were not significant ($p>0.05$) among all the samples except for Brand B (Table 2). The maximum specific gravity of honey samples should be 1.38–1.45 (Codex Alimentarius Commission, 2001). Therefore, all the values obtained were within the standard limit. Ahmed et al., (2007) reported that the specific gravity of Indian honey samples ranged between 1.33–1.56. The variation observed in specific gravity may be due to differences in moisture contents.

HMF content is an indicator of honey freshness (Schade et al., 1958). The HMF content increases depending on honey pH and storage temperature. Some European federations permit a maximum of 15 mg/kg HMF for ''quality honey''. On the other hand, an amount of 10 mg/kg HMF in honey is naturally present, but a large increase of content could be due to overheating or to adulteration

(Crane, 1980). Recently, the maximum proposed value of HMF by the Codex is 60 mg/ kg (Codex Alimentarius Commission, 1998). All the honey samples had low HMF values (between 5.7 and 38.66 mg/kg) (Table 2). Notably, all HMF concentrations were within the recommended range set by the Codex Alimentarius Commission (1998). We compared our results with honey samples from different countries. The HMF concentrations of some Australian honeys, such as rainforest, Homebrand and Mallee honey, were reported to be 2.2, 17.7 and 34.0 mg/kg, respectively (Fallico et al., 2004; Ajlouni and Sujirapinyokul, 2010). Bangladeshi honey samples with low pH levels and low moisture content may contribute to the low HMF formation.

Statistically there was a significant differences ($P<0.05$) within diastase activity of different samples. Sample of Brand D had significantly ($P< 0.05$) higher diastase (12.97°) followed by samples of Brand A (9.18°), Brand B (7.37°) and Brand C (1.93°) (Table 1). Two samples (Brand A and D) exceeded the limits of European Community Regulation (2002) with values less than 8° Gothe. However, similar results were also obtained by Oddo et al. (1995) where the honeys analyzed showing a range of 0° to 9.2° with a mean of 5.2° Gothe. Like HMF, diastase is sensitive to heat and affect the degree of preservation (Ahmed et al., 2007). Diastase activity of natural honey is rapidly reduced when honey is heated or stored at unfavorable temperature (Tosi et al., 2004).

**Test for adulteration**

The adulteration detection was done by Fiehe's and flame test. All the honey samples of this study were found to contain added water. It has been noticed that addition of water to normal honey was assumed to increase the quantity of honey. Adding,water not only reduces the nutritional value of honey but contaminated water may also pose a health risk. Fiehe's test confirmed that honey samples did not turn into either red or dark red in color.indicating honey samples were not adulterated by added sugar. Adulteration and dilution of honey has been a widespread problem in China, where test conducted in1999 indicated that almost one third of the brands were adulterated with sugar (Bowman, 1999).

**Qualitative analysis for phyto-chemicals**

Five identification tests were performed for the four honey Brands (Table 2). It was noted that, carbohydrates, reducing sugar, and ketose sugar were present in all the samples. First, molisch's test was used to detect any carbohydrate. A positive result for the molisch's test is indicated by the formation of a red to violet color. All the sample solutions changed to a red color. The second test was the Fehling test to detect the presence of reducing sugar. A yellow to brownish-red color is a positive indication of a reducing sugar. All the samples showed positive results for this test. The third was the Benedict's test which is commonly used to detect reducing sugar. A positive result for the Benedict's test is denoted by the formation of yellow, green or red precipitation. All the samples of this study showed color changes. The fourth was the seliwanoff's test which is used to detect the presence of ketose sugar. A positive result was indicated by the appearance of deep red color. All the honey samples also showed color changes for this test.

## CONCLUSION

All of the samples were found to be in acceptable range of international standards for all the tested parameters except for diastase activity which was lower in two samples. This study also showed that all the honey samples contain phyto-chemicals like carbohydrate, reducing sugar and ketose sugar. Further studies are needed to analyze all of the compounds present in market honey for assessment of their quality at international standards.

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