PHYSICOCHEMICAL PROPERTIES OF DIFFERENT UNIFLORAL BLOSSOM HONEY PRODUCED IN DIFFERENT REGIONS OF BANGLADESH

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Abstract

This study assessed some physicochemical properties of seven unifloral blossom honey samples collected from different regions of Bangladesh. The samples were analyzed for several parameters including pH, ash, moisture, total soluble solids (TSS), and carbohydrate, protein and fat contents. The values for the physical properties varied among the collected samples. The values obtained for pH, ash, moisture and TSS were ranged from 3.4 to 4.7, 0.0536% to 0.6995%, 9.68% to 23.00% and 63.0% to 78.0%, respectively. The total sugar content and reducing sugar of the analyzed honey samples were between 74.53% and 88.99% and between 45.60% and 68.23%, respectively. The protein contents ranged between 0.315% and 1.06% while fat content lied between 0.37% and 1.87%. The energy content of the tested honey samples 200 ml in each sample was mainly between 351.62 and 362.43 Kcal; however, one sample that was collected naturally showed relatively lower energy (316.22 Kcal). Although the results showed variation in different honey samples according to the floral origin, the physicochemical values of the samples, however, were in the range of approved limits.

Keywords: Unifloral blossom honey, physicochemical properties, box system, international standards.

INTRODUCTION

Honey is produced by honey bees from the nectars extracted from the nectarines of flower (Adebiyi *et al.* 2004). White and Doner (1980) reported that it (honey) comes from the secretion of living parts of plants or from excretion of plant sucking insects when live on plants. According to Ligia *et al.* (2013) honey's elaboration starts with the nectar collected from many plants, which honeybees transform and combine with their own specific substances, store and leave to mature in honey combs. Al-Mamary *et al.* (2002) reported that the chemical composition of honey is complex, containing approximately 181 substances including sugars, proteins, moisture, vitamins, minerals, 5-Hydroxy Methyl Furfural (HMF), enzymes, flavonoids, phenolic acids and volatile compounds. White (2001), Whitmyre (2007) and National Honey Board (2011) reported that the main constituents of honey are moisture, glucose, fructose, sucrose, minerals and proteins. The composition of honey is rather variable and primarily depends on the floral source; however, certain external factors also play a role, such as seasonal and environmental factors and processing steps and conditions (Vit *et al.* 2004). Physico-chemical investigation and analysis of biochemical composition of natural and industrial honey samples were studied by Krishna *et al.* (2015). Linkon *et al.* (2015) assessed the comparative analysis of the physico-chemical and antioxidant properties of nigella honey available in Tangail, Bangladesh.

The aim of the study was to make a comparative analysis of the physicochemical characteristics of local honey in Bangladesh to assess the different types of honey quality. In the question of bioresource and bioresources management fields, honeybee is involved in carrying two important roles in nature. Honeybee itself is a primary category of biotic resource that produces honey by taking nectar from plants directly. Secondly, it maintains vital dynamism in the gene-flow mechanism of the plant kingdom which ensures environmental soundness in an ecosystem, especially in the forest system (Bashar and

Khan 2015). These polyramous vital role of the honeybee in nature need a high quality of management system. The system should not be only mechanical in approach, but also biochemical. By keeping the important points in front, present research attempt was undertaken.

MATERIAL AND METHODS

Sample collection and preparation

Seven selected honey samples were collected from different places of Bangladesh. Naturally produced kholisha honey was collected naturally from the Sundarbans and the other samples collected by box system were litchi (Jessore), coriander (Gopalganj), nigella (Rajbari), mustard (Pabna), jujube (Khulna) and drumstick (Khulna). The collected samples were practically uniform in colour and also were free from unwanted materials, such as presence of particles of seeds, skins, dark specks or other hard and coarse extraneous materials. The selected honey samples were directly analyzed and analytical data were presented on a raw weight basis. Each sample was divided into two parts. One part was used for the determination of pH, TSS, protein, fat, moisture etc.; the other part was for drying to determine minerals and ash.

Physicochemical analysis

pH: The pH was determined with a pH meter (Hanna instruments-ORPP) by the conventional procedure. A 10% (w/v) solution of each honey samples was prepared to measure the pH. Calibration of the pH meter was done according to the manufacture's instruction.

Moisture content: Moisture was analyzed by a moisture analyzer (ANDMX–50) with 2-3ml samples.

Ash: The ash content was determined as described previously (AOAC 1990). About 1.5-2.0 gm sample was taken in a crucible, placed on a burner and heated first over a low flame. Then the crucible was put in a Muffle furnace at about 600°C for 3-5 hours. The crucible was then cooled in a desiccator and weighed. To assure the completion of ashing, the crucible was again heated in the Muffle furnace for 0.5 hour and weighed. The ash was calculated as follows-

Percentage of ash= (Weight of ash/Weight of sample taken)×100

TSS: The Brix is defined as a unit of measurement of Total Soluble Solids (TSS) present in any sugary solution either prepared or in natural state. It is the measurement of the refractive indices of the said substances at 20°C. The Brix of all the tropical honey samples was determined by a hand refractometer (ATAGO 9099, Japan) ranging from 0° to 99°.

Carbohydrate content: For the determination of carbohydrate content we need to determine the total carbohydrate content (TC), total sugar (TS) and reducing sugar (RS) content. The procedures of determining them are given below-

Total carbohydrate content: Carbohydrate content was calculated by difference rather than direct analysis.

Carbohydrate (%) =100−{Moisture (%)+Protein (%)+Fat (%)+Ash (%)}

Total sugar: Lane and Eyanon method 1, 2 was used to determine total sugar content. Invert sugar reduces the copper solution of red, insoluble, cuprous oxide. The sugar content in a food sample is estimated by determining the volume of the unknown sugar solution required to completely reduce a measured volume of Fehling's solution. An amount of 25 ml of the standard invert solution was pipetted into a 100 ml volumetric flask and about 50 ml of water was added. A few drops of phenolphthalein indicator was added and neutralized with 20% NaOH until the solution turned pink. Then acidity with 1N HCI was added dropwise until one drop caused the pink to mark with water (1 ml=25 mg of invert sugar). The following equations were determined to calculate the total sugar content-

Percentage of total sugar=mg of total sugar (after invasion)×dilution×100/titrate×weight or volume of sample×100

Percentage of reducing sugar =mg of invert sugar×dilution×100/titrate×weight or volume of sample×100

Protein content: Estimation of total protein was made by Kjeldahl method following AOAC (1990). Protein content can be measured by estimating the nitrogen content of the material and then multiplying the nitrogen value by 6.25. This is referred to as crude protein content, since the non-protein (NPN) present in the materials was taken into consideration in the present investigation. The estimation of nitrogen was made by modified Kjeldahl method, which depends on the fact that organic nitrogen, when digested with concentrated sulphuric acid (H_2SO_4), which in the presence of a catalyst, is converted into ammonium sulphate $(NH_4)_2SO_4$. Alkali is added to the sample to convert ammonium (NH_4^+) to ammonia (NH_3). The ammonia is steam distilled into a receiver flask containing boric acid and titrated with a standard acid solution. This determines % of N that is multiplied by 6.25 to give the value of crude protein. Protein content of the sample on the percentage basis was calculated by the following formula-

$$\text{Percentage of protein(g)} = \{(c-b) \times 14 \times d \times 6.25/a\} \times 100$$

Where, a= sample weight (g), b= volume of the sodium hydroxide required for the back titration, c= volume of sodium hydroxide required for the back and to neutralize 20ml of 0.1N H_2SO_4 (for blank), d= Normality of NaOH used for titration and the conversion factor of nitrogen to protein is 6.25 and atomic weight of nitrogen is 14.

Fat: 10 ml sample was taken in a separating funnel. The sample was refluxed with petroleum ether and chloroform for one day. Then the sample was distillated and weight of a small conical flask was taken. The sample was poured into a conical flask and petroleum ether was evaporated from the sample with a water bath. Finally, the sample was cooled in desiccators and then the weight of the sample was taken. The formula is-

$$\text{Percentage of fat (g)} = \text{weight of container with fat-weight of empty container/weight of sample taken} \times 100$$

Total energy content: Total energy content per 200 ml of honey of the blossom of seven plants was calculated by Atwater's conversion factor rather than direct analysis.

$$\text{Energy Content (kcal)} = \{(\text{Carbohydrate} \times 4) + (\text{Fat} \times 9) + (\text{Protein} \times 4)\}$$

RESULTS AND DISCUSSION

Estimation of pH, ash, moisture and TSS

pH: The pH of different honey samples, viz. litchi (*Litchi chinensis*), coriander (*Coriandrum Sativum),* nigella (*Nigella sativa*), mustard (*Brassica indica*), jujube (*Ziziphus manuritiana),* drumstick (*Moringa oleifera)* and kholisha (*Aeqicerus corniculatum*) is shown in Table 1. The range of pH in seven samples varied from 3.4 to 4.7. The highest pH (4.7) was observed in jujube and the lowest (3.4) in kholisha, which were comparable with the international standards (3.2-4.5) as reported by Kinati *et al.* (2011). A report published by Bogdanov *et al.* (1995) indicated that some honeys, such as chestnut and fir honey might have high pH values, viz. 5-6 and 4.6-5.9, respectively. The pH values of the tasted honey samples were similar to those Malaysian, Algerian, Brazilian, Indian and Spanish honeys (between pH 3.6-4.7) (Moniruzzaman *et al.* 2013, Ouchemoukh *et al.* 2007, Saxena *et al.* 2010, Khalil *et al.* 2012). Overall, the pH values of the studied honey samples were within the limit that indicated the freshness of the honey samples (pH between 3.6 and 4.7) as described by Khalil *et al.* (2012).

Ash: The ash content of litchi, coriander, nigella, mustard, jujube, drumstick and kholisha were 0.16%, 0.18%, 0.05%, 0.69%, 0.21%, 0.27% and 0.29%, respectively (Table 1). In our study the ash content varied between 0.05% (nigella) and 0.69% (mustard). Concerning the *Apis* honey, the ash content was slightly inferior to the amount obtained by Estevinho *et al.* (2012), who analysed 75 samples. Cano *et al.* (2001) studied Brazilian honey samples and found similar results. The honey samples from Ghana were found to be high in ash, being 0.8% with a range value of 0.3-1.4% compared with a value of not more than 0.6% stated in the Codex Alimentarius (1994). The ash contents of honey obtained in this study were all within the limits of 0.05-0.69 specified by international norms (Codex Alimentarius

Commission 2001a, b). The results of the ash contents are similar to those reported for honey samples from southern part of Nigeria (Omafuvbe and Akanbi 2009) as well as the values reported for the samples from Argentina, Spain and Turkey (Cantarelli *et al.* 2008), different areas of Pakistan (Kamal *et al.* 2002, Iftikhar *et al.* 2011).

Moisture: Table 1 shows the moisture content of the honey samples, such as litchi (13.20%), coriander (12.05%), nigella (12.52%), mustard (12.68%), jujube (9.68%), drumstick (12.76%) and kholisha (23.00%). According to the Codex Alimentarius Comission (2001a) the international standard for moisture content of honey is <20%. All examined samples fulfilled the standard limit except kholisha (23.00%). Moisture content plays an important role in the preservation of honey. If the moisture content exceeds 22%, honey is likely to ferment (Marvin 1933); so, for preservation, the honey of higher moisture content requires lowering of the moisture content. The average moisture content of honey extracted from *Apis ceranaindica* and *Apis mellifera* as reported by different authors was 19.98% and 17.14%, respectively in the honey samples of north India (Phadke 1967) and according to Lin *et al.* (1969), it varied from 16.60% to 26.40% which were higher than the samples we observed. The stingless bees' honey presented a mean moisture content of 24.80±1.01% (ranged from 23.86% to 25.88%), while the mean content of the other type of honey was 18.27±0.40% (ranged from 17.66% to18.86%) reported by Ligia *et al.* (2013). The moisture content of the analyzed samples was consistent with the previously reported values of some Malaysian honeys for which the corresponding values ranged from 12.79% to 22.32% (Khalil *et al.* 2010) and 14.86% to 17.53% (Moniruzzaman *et al.* 2013).

TSS: Table 1 shows the TSS content of the tested honey samples. The TSS content of litchi, coriander, nigella, mustard, jujube, drumstick and kholisha were 77.0%, 78.0%, 74.0%, 77.3%, 73.5%, 74.5% and 63.0%, respectively which varied between 74.0% and 78.0%. The highest value obtained was for coriander (78.0%) and the lowest was for kholisha (63.0%). From Table 1 we concluded that the TSS content of the honey samples was almost similar. They differ very slightly. Singh (1994) determined the TSS of five different floral sources of honey in Punjab which ranged between 81.30% and 83.95%; these were quite higher compared to the seven honey samples we observed in the present study.

Total Carbohydrate (TC): The Total Carbohydrate (TC) content of litchi, coriander, nigella, mustard, jujube, drumstick and kholisha honey were 84.96%, 87.42%, 86.36%, 86.37%, 88.99%, 86.43% and 74.53%, respectively (Table 1) The total carbohydrate contents of the honey include monosaccharides, fructose and glucose which are the main sugars found in honey; these hexoses are the products of the hydrolysis of sucrose. In addition to these sugars, 25 other sugars were detected in honey samples (Doner 1977, Siddiqul 1970).

Reducing Sugar (RS): The reducing sugar (RS) content of the honey was obtained from litchi (50.52%), coriander (52.04%), nigella (50.42%), mustard (65.55%), jujube (58.27%), drumstick (68.23%) and kholisha (45.60%) (Table 1). Our results indicated that the reducing sugars were the primary soluble sugars present in honey samples (Moniruzzaman *et al.* 2013). The honey samples from Ghana contained low reducing sugars (calculated as invert sugar), being 57% with a range of 45.60-68.23% compared to the Codex requirement of not less than 65%. The reducing sugar contents of the samples used in the present study had an average value of 68.23%, the values obtained in the study were similar to the values reported for honeys from Bangladesh (Khalil *et al.* 2001), Pakistan (Kamal *et al.* 2002), Argentina and Turkey (Cantarelli *et al.* 2008).

Total Sugar (TS): Total sugar (TS) of litchi (66.97%), coriander (86.12%), nigella (66.97%), mustard (81.00%), jujube (72.53%) drumstick (77.79%) and kholisha (53.59%) honey are also shown in Table 1. The overall sugar content of the analyzed honey sample in the study was between 53.59% (kholisha) and 86.12% (coriander). In our study, the total sugar content of kholisha was lower (53.59%) and the sugar content of coriander was higher (86.12%); this findings are similar to those reported for the total sugar

content of sourwood honey (Moniruzzaman *et al.* 2013), TS content of Indian honey ranged from 43.3% to 66.7% (Saxena *et al.* 2010) and Bangladeshi honey, which ranged from 42.80% to 60.67% (Islam *et al.*, 2012). The lower total sugar content can be contributed by the conversion of sugar into inorganic acid (Moniruzzaman *et al.* 2013). It has also been reported that overheating of honey samples during processing or storage for very long periods can lead to the conversion of sugars to HMF (Saxena *et al.* 2010).

Glucose, fructose and sucrose content: The Table 1 also shows the glucose, fructose and sucrose content of Litchi (19.27%, 31.24% and 1.56%, respectively), coriander (19.27%, 32.77% and 3.24%, respectively), nigella (29.91%, 34.52% and 1.56%, respectively), mustard (22.08%, 43.46% and 1.47%, respectively), jujube (21.68%, 36.58% and 1.36%, respectively), drumstick (23.69%, 44.99% and 0.91%, respectively), and Kholisha (13.05%, 32.55% and 0.76%, respectively). The major sugars present in honey were found as fructose and glucose which is comparable to Malaysian honeys (Moniruzzaman *et al.* 2013). The sucrose (saccharose) contents of the tested Malaysian honey ranged from 1.66% to 3.17%. These values were within the maximum prescribed limit of sucrose content for honey recommended by the Codex standard. The variations in the sucrose levels may indicate that different regions may have compositional differences of honey. According to White and Doner (1980) the dominance of fructose over glucose is one way in which honey differs from commercial invert sugar. Fructose and glucose constitute the primary sugars in all honey samples, and in the honey of good quality the fructose content should exceed that of glucose (Zafar *et al.* 2008).

Protein: The protein content of all the honey samples is also shown in Table 1. Total protein content of honey is known to dependent on the flower sources and can be subsidized by the enzymes introduced by either the bees or other substances derived from the nectar (Alvrez-Suarez *et al.* 2010). In the study, the Nigella honey had the highest concentration of protein (1.06%) compared to other honey samples. The protein content of honey is normally less than 5.00 g/kg (Saxena *et al.* 2010). A high protein concentration has also been reported in some Algerian honey (3.7-9.4 g/kg) (Ouchemoukh *et al.* 2007*).*

Fat: The fat content of litchi, coriander, nigella, mustard, jujube, drumstick and kholisha were 1.27%, 0.55%, 0.37%, 0.71%, 0.39%, 0.42% and 1.87%, respectively (Table 1). In the study, the percentage of fat was between 0.37% and 1.87% which was similar to *Apis mellifera*'s honey, the total fat being oscillated between 0.37% and 0.39% (Ligia *et al.* 2013). Reports indicating that honey contains little or no fat are available (Tan *et al.* 1988, Singh and Kumar 1997). In a biochemical analysis of five different brands of unifloral honey available in the northern region of Bangladesh reported total fat contents in the range of 0.134-0.146 g/100g; thus, indicating that honey contains very little amount of lipid and therefore not considered a good source of lipid (Khalil *et al.* 2001).

Total energy content: The total energy content of litchi, coriander, nigella, mustard, jujube, drumstick and kholisha per 200 ml of each of the samples were 352.92, 356.00, 353.07, 354.72, 362.43, 351.62 and 316.22 kcal., respectively (Table 1) where the highest energy content was observed in jujube (362.43) and lowest was in kholisha (316.22). The results showed that naturally collected honey (kholisha) had comparatively lower energy content than artificial honey collected from box system. The honey collected from box system had energy content ranged from 351.62 kcal to 362.43 kcal. The average value for the honey samples all the states ranged between 1383.23±39.09 and 1410.20±24.43 KJ/100g according to Doner 1977. Honey is primarily a high energy content food and the honey sugars are easily digestible similar to fruits (White and Doner 1980). For this reason honey is regarded as a good food for both infants and adults.

Table 1. Physical properties and proximate composition of seven honey samples obtained from seven plant sources.

Scientific Name	Local Name; English Name	Place of collection	Physical Properties				Proximate Composition									
			pH	Ash (%)	Moisture (%)	TSS (%)	Carbohydrate							Protein (%)	Fat (%)	Total Energy (kcal)
							TC (%)	RS (%)	TS (%)	Glucose (%)	Fructose (%)	F:G (%)	Sucrose (%)			
Litchi chinensis	Lichu; Litchi	Jessore	4.1	0.16	13.20	77.0 84.96		50.52	66.97	19.27	31.24	1.62	1.56	0.41	1.27	352.92
Coriandrum sativum	Dhonia; Coriander	Gopalganj	4.1	0.18	12.05	78.0	87.42	52.04	86.12	19.27	32.77	1.70	3.24	0.36	0.55	356.00
Nigella sativa	Kalozira; Nigella	Rajbari	4.2	0.05	12.52	74.0	86.36	50.42	66.97	29.91	34.52	1.16	1.56	1.06	0.37	353.07
Brassica indica	Shorisha; Mustard	Pabna	3.7	0.69	12.68	77.3	86.37	65.55	81.00	22.08	43.46	1.96	1.47	0.71	0.71	354.72
Ziziphus manuritana	Boroi; Jujube	Khulna	4.7	0.21	9.68	73.5	88.99	58.27	72.53	21.68	36.58	1.68	1.36	0.71	0.39	362.43
Moringa oleifera	Shojina; Drumstick	Khulna	3.6	0.27	12.76	74.5	86.43	68.23	77.79	23.69	44.99	1.89	0.91	0.53	0.42	351.62
Aeqicerus corniculatum	Kholisha; Kholisha	Sundarbans	3.4	0.29	23.00	63.0	74.53	45.60	53.59	13.05	32.55	2.49	0.76	0.32	1.87	316.22
International Standard			3.2-4.5**	<0.6*	<20%*	-	Not Fixed*	<65*	Not Fixed*	22.89-40.26***	30.91-44.26***	0.26-1.86***	<5*	Not Fixed*	0.134-0.146***	Not Fixed*

International Standard: *Codex Alimentarius 2001(a); **Kinati *et al.* 2011; ***National Honey Board 2011

Honey samples from seven different flower sources were collected from different part, of Bangladesh to analyze these physicochemical properties. The proximate composition like carbohydrate, protein, fat and total energy content of honey samples exhibited a significant variation compared to the international standards. The people widely consumed honey in Bangladesh. Thus, the honey of different flowers may be used in different purposes according to its chemical ingredients.

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