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Antioxidant and Physico-Chemical Properties of Litchi Honey Procured from Gazipur and Tangail District, Bangladesh

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Abstract
Among the panoply of food products, honey has been used since prehistoric times as an effective healing, antiseptic, antioxidant and antibacterial therapy. The present research aimed to investigate erstwhile undocumented litchi honey samples from Bangladesh, for its antioxidant and physico-chemical parameters that may increase knowledge of product quality in a given geographic area, as well as its commercial value. Three litchi honey samples were collected from different regions of Gazipur and Tangail district, Bangladesh. The major physicochemical properties such as- moisture, ash, protein, fat, carbohydrate, energy content, p^H and TSS were measured. Although the physicochemical parameters of the honey samples varied, most of the honey samples were in the acceptable range of international standards. Results indicated that total phenolic content ranged from 33.241 to 34.824 mg Gallic acid/ 100g, flavonoid content varied between 4.024 and 4.954 mg Catechin/100 g and vitamin C was found in the range 13.612 to14.636 mg/100g, indicating a high antioxidant potential. The study revealed that Bangladeshi litchi honey samples maintain the international honey standards and contain a good source of antioxidants.

Keywords: Bangladeshi litchi honey, flavonoid, physico-chemical properties, polyphenol, vitamin C.

1. Introduction
Honey is a natural global food product, well known for thousands of years for its high nutritive value and healing properties. Honey, derived either from the nectar of flowers or from honeydew, is produced by *Apis mellifera* bees [1,2] following enrichment with secretion, transformation, storage and maturation in combs [2]. It is made up of many components: 70% saccharides, the main sugars being fructose and glucose, 10% water, organic acids, mineral salts, vitamins, proteins and phenolic compounds [3]. The composition of honey varies depending on the floral source, seasonal and environmental factors. However, with basic nutrient components e.g. sugars, proteins, fat, vitamins (vitamin C and B complex mainly), iron, calcium; honey has been found to contain significant antioxidant compounds including glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives, organic acids and Maillard reaction products [4].
Among the oldest books, 1432 years ago the Holy Quran clearly defines honey in a simple and understandable language. It was already mentioned it this Holy book that, *"And thy LORD taught the bee to build its cells in hills, on tree and in men's habitations, then to eat of all the produce of the earth and find with skill the spacious path of its LORD, there issues from within their bodies a drink of varying colours, wherein is a healing for men, verily in this a sign for those who give thought"*. Blessed by these varying colourful colloids, it has set templates since prehistoric times and is still being exploited for its tremendous benefits and has been used in folk medicine since ancient times. In the modern era, the different biological, chemical and physical properties of honey have revealed several beneficial claims through different techniques. The multi facet properties of honey anchored in the scientific world is regarded as a sweetener, functional food, antioxidant, antimicrobial, antibacterial, bacteriostatic, antiseptic, prebiotics, pro-biotics, immune modulatory, anti-inflammatory, anti-tumour and anti-cancer effect amongst others [5].

Consumption of honey alone or in combination with other beverages significantly increases antioxidant capacity of human serum [6]. It has the capacity to prevent or reduce oxidative damage to erythrocytes and lipid peroxidation associated inflammatory diseases in which oxidative stress is involved [7]. Malaysian Gelam honey has anti-inflammatory effect by decreasing the immune response against inflammation and by having protective effects against organ failure [8]. It is also effective in preventing deteriorative enzymatic browning of fruits and vegetables [9] and lipid oxidation in meat [10].

However, to the best of our knowledge, there is invariably insignificant information in literature performed on quality assessment and antioxidant properties of Bangladeshi Litchi Honey. Hence, the study is essential to meet a void in research. The present study was, therefore, carried out to investigate the antioxidant and physico-chemical properties of honey procured from Gazipur and Tangail District, Bangladesh.

2. Materials and methods

The experiment was conducted in the laboratory of the Department of Food Technology and Nutritional Science, Mawlana Bhashani Science and Technology University and Department of Food Processing and Engineering, Chittagong Veterinary and Animal Sciences University, Bangladesh. The study was conducted from March 2013 to November 2013.

2.1 Sampling

Litchi honey samples were sourced mainly from the Sripur of Gazipur district (coded as S_1), Sakhipur and Gopalpur of Tangail district (coded as S_2 and S_3 respectively), Bangladesh during March 2013. Sufficient unifloral natural raw honey samples were collected directly from specific beekeepers as all experiments during the study were performed from litchi floral origin. After collection, honey samples were stored in air tight glass jars at ambient temperature (20-25 ˚C) in the dark for further analysis. The specification and source of honey used are illustrated in Table 1.

2.2 Preparation of honey samples

All honey samples were prepared according to Association of Official Analytical Chemists (AOAC) standard [11]. Honey samples used were free from granulation, suspended solids and from any form of crystallization. The samples were thoroughly mixed using a clean sterile glass rod to ensure homogeneity prior to use.

Table 1: Specification of honey samples.

Honey Sample	Melliferous plant Scientific Name	Sample Code	Region (Source)
Litchi	*Litchi chinensis*	S_1	Sripur (Gazipur)
		S_2	Gopal (Tangail)
		S_3	Shakipur (Tangail)

2.3 Physico-chemical properties

All litchi honey samples were analyzed for moisture, ash, protein, fat, TSS (Total Soluble Solid), pH, vitamin-C as per the methods of AOAC [12]. The total carbohydrate content was determined by difference of total moisture, ash, protein and fat from hundred [13]. All the estimations were done in triplicate and the results expressed as average value. Energy value was calculated using modified Atwater general factors by multiplying the portions of protein, fat and carbohydrate by their physiological fuel value of 4.0, 9.0 and 4.0 Kcal/g respectively and taking the sum of the products [14].

2.4 Antioxidant content

Polyphenol compounds from Litchi honey samples were detected by a modified spectrophotometric Folin-Ciocalteu method [15]. The total flavonoid concentration of each honey sample was determined according to the colorimetric assay developed by Zhishen *et al*. [16].

2.5 Statistical Analysis

The data obtained were statistically analyzed for analysis of variance (ANOVA) and consequently Duncan's Multiple Range Test (DMRT) was used to determine significant difference. Data were analyzed using the software, IBM SPSS Statistics, version 20 at the 5% level of significance (P= 0.05) [17].

3. Results and discussion

3.1 Physico-chemical analysis

The results on physico-chemical analysis of Bangladeshi litchi honey samples are given in Table 2.

The moisture in the investigated samples ranged from 14.42% to 15.01% and all of them were below the maximum limit of moisture content (≤20%) for honey recommended by the international quality regulations [18]. In contrast to our results, Wanjai *et al.* (2012) reported 19.97% moisture content of litchi honey in Thailand [19]. The moisture content of honey has been reported to depend on various factors such as the harvesting season, source plant's floral type, and the moisture content of the original plant nectar [20]. As the moisture content present in litchi honey samples is important and contributes to its ability to resist fermentation and granulation during storage, low moisture content in the litchi honey samples indicates its good storage ability and can lead to undesirable honey fermentation due to osmotolerant yeasts, which form ethyl alcohol and carbon dioxide [21]. To guarantee the quality of honey, the proper timing of honey extraction and degree of maturity of honey in the hives should be taken into account for each floral type [19].

Ash content was considered to be an indicator of the cleanliness of honey samples [22]. The S_3 Litchi honey sample had higher ash content (0.32%) where S_1 Litchi honey sample had lower ash content (0.27%) and all samples of honey were below 0.6%, the maximum value allowed in the international standards. Wanjai *et al*. (2012) analyzed the ash content of litchi honey as 0.16% [19], being less than that obtained in the present study. This depends on the geographical locations, environmental conditions of the producing regions, and the material collected by the bees during the foraging on the flora [19]. An increase in ash content was found to be accompanied by an increase in the electrical conductivity [19, 23]. The high electrical conductivity generally indicates the presence of minerals in honey. These two parameters therefore could reflect possible environmental pollution and geographical origin of honey [19].

Table 2: Physico-chemical analysis of Bangladeshi litchi honey samples [1, 2]

Parameter	Sample code		
	S_1	S_2	S_3
Moisture (%)	15.01±0.006^a	14.88±0.006^b	14.42±0.006^c
Ash (%)	0.21±0.006^c	0.27±0.003^b	0.32±0.006^a
Protein (%)	0.53± 0.003^a	0.53 ± 0.003^a	0.52 ± 0.003^b
Fat (%)	0.003±0.000^a	0.003±0.000^a	0.002±0.001^a
Carbohydrate (%)	84.246±0.012^c	84.317±0.009^b	84.738±0.012^a
Energy Content (Kcal/100 g)	347.613±0.047^c	347.898±0.036^b	349.578±0.047^a
p^H content	4.69±0.003^a	4.68±0.003^a	4.67±0.003^b
TSS (°Brix)	80.84±0.168^b	81.33±0.167^{ab}	82.03±0.318^a

[1]Values are mean ± standard error of triplet determinations.
[2]Means with different superscript within the same row differ significantly (P < 0.05).

The protein content can be attributed to the presence of different types of enzymes and other derived products that were introduced by the bees from the flower nectar. The protein content of tested litchi honey samples was found in the range 0.52-0.53% (Table 2). Relatively higher protein levels have also been reported in Algerian honey samples [23]; whereas for honey samples from India, the content was reported to be lower [24]. The protein content can be attributed to the presence of different types of enzymes and other derived products that were introduced by the bees from the flower nectar. Protein levels in honey are dependent on the type of flora on which the bees forage [25].

Litchi honey samples content very low amount of fat (0.002-0.003%). The highest total carbohydrate content (84.738%) was obtained in S_3 followed by S_2 (84.317%) and S_1 (84.246%) (Table 2). Total carbohydrate content of litchi honey was recorded similar to that of honey samples from India [24]. The energy values of the Bangladeshi litchi honey samples were found to range between 347.613 to 349.578 kcal/100 g. According to USDA National Nutrient Database (2013), the energy value of honey was 304 kcal/100 g [26].

The pH of honey is due to presence of organic acids such as gluconic acid as well as inorganic ions such as phosphate and chloride [3]. All honey samples analyzed were acidic in nature. The pH of litchi honey samples investigated here diverse from 4.67 - 4.69, where S_3 was more acidic (pH=4.67) (Table 2). Published report tends to indicate that the pH of honey should lie within the range 3.3 - 5.6 [27]. Relatively higher pH content has been reported in Mauritius honey samples [5]; whereas for honey samples from Northern Thailand, the content was reported to be lower [19].

The highest TSS was found in S_3 (82.03°Brix) followed by S_2 (81.33°Brix) and S_1 (80.84°Brix) (Table 2). The TSS values of the samples were more or less similar to USDA Nutrient Database honey (82.12°Brix) [26]. Thus litchi honey samples are good source of TSS.

3.2 Antioxidant properties

3.2.1 Total polyphenol content

The total polyphenol content determined using Gallic acid as standard ranged between 33.241 and 34.824 mg Gallic acid/ 100 g (Figure 1) (r^2 = 0.989).

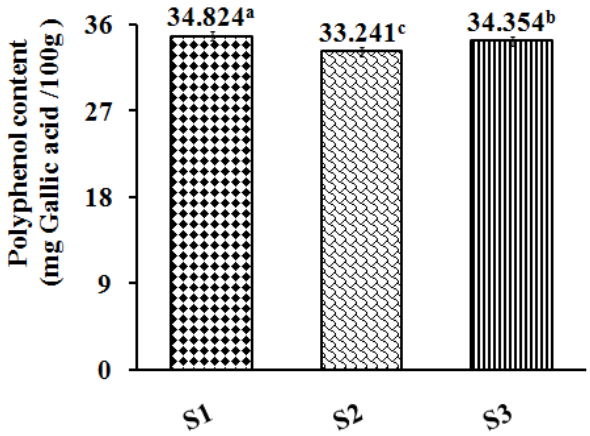

Fig 1: The mean value of polyphenol content of Bangladeshi litchi honey samples

S_1 had the highest polyphenol content (34.824 mg Gallic acid/ 100 g), unlike S_2 had the lowest polyphenol content (33. 241 mg Gallic acid/100 g) among the experimental samples (Figure 1). Similar amount of polyphenols was reported for litchi honey (35.4 mg Gallic acid/100 g) by Das *et al.* [28]. Relatively lower total polyphenol content of Thai honeys ranging from 10 and 14.4 mg Gallic acid/ 100 g have also been reported by Sangsrichan and Wanson [29]. Manuka honey from New Zealand was reported to have a total phenolic level of 43.4 mg Gallic acid/100 g [30]. The concentration and the type of polyphenol substances in honey are variable and are reported to be dependent on the floral origin of the honey samples [20].

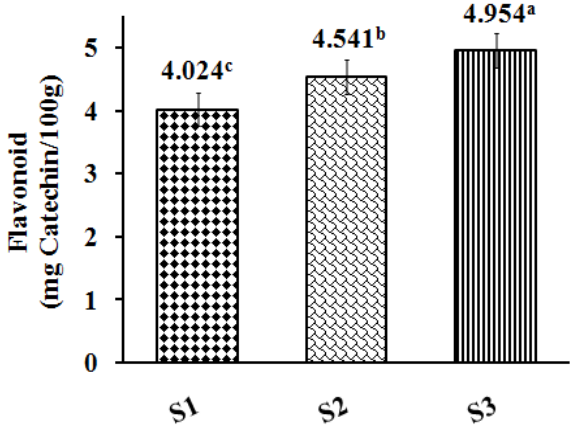

Fig 2: The mean value of flavonoid content of Bangladeshi litchi honey samples

3.2.2 Total flavonoid content

The flavonoid content determined using Gallic acid as standard was recorded between 4.024 to 4.954 mg Catechin/100 g (r^2 = 0.993). All the test honey samples had a lower content of flavonoids than polyphenols. The highest flavonoid content was obtained in sample S_3 (4.954 mg Catechin/100 g) (Figure 2). However, Khalil *et al.* (2011) expressed that Manuka honey contained 8.505 mg Catechin/100 g flavonoid content [30]. This variation of flavonoid content might be due to the geographical location and floral origin of honey.

3.2.3 Vitamin-C contents

In addition to polyphenols, honey contains a number of compounds known to act as antioxidants, including ascorbic acid and the enzymes glucose oxidase and catalase [31]. The vitamin C content of tested litchi honey samples was found in the range 13.612 to 14.636 mg/100 g where highest vitamin C content (14.636 mg/100 g) was found in sample S_3 (Figure 3). Ferreira *et al.* (2009) investigated the vitamin C content of Portuguese honey and reported the vitamin C content within the range 14.0 to 14.5 mg/100 g [32], in accordance with our findings. However, Algerian honey samples were reported to have higher ascorbic acid content (23.68 to 31.59 mg/100 g) when compared to tested honey samples [4].

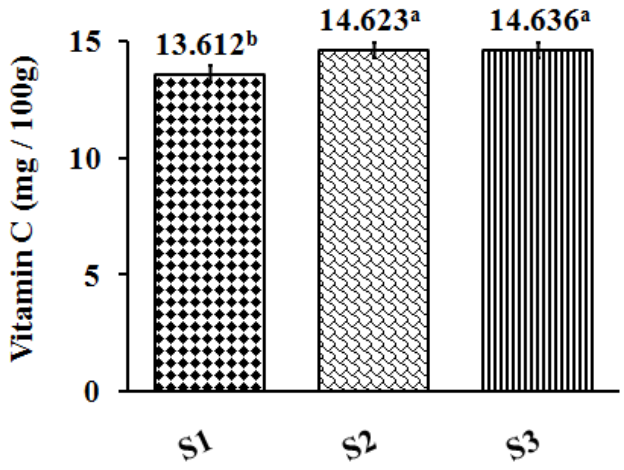

Fig 3: The mean value of vitamin C content of Bangladeshi litchi honey samples

4. Conclusion

The antioxidant properties of honey samples, procured from Gazipur and Tangail District, Bangladesh indicate that litchi honey samples was a good source of antioxidants, as indicated by their high polyphenol, ascorbic acid (Vitamin-C) and flavonoid contents. The physico-chemical properties of honey samples indicate that litchi honey have an acceptable quality, which fell in the accepted range for the international standards. All tested litchi honey samples were found to contain low moisture (<20%), low ash content (<0.6%), low pH and high TSS content indicating good honey quality. This work is a preliminary investigation of litchi honey; further research on a larger number of samples is needed for a statistical analytical approach.

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