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Analysis of Biochemical Composition of Honey Samples from North-East Nigeria

Fatimah Buba*, Abubakar Gidado and Aliyu Shugaba

Department of Biochemistry, Faculty of Science, University of Maiduguri, Nigeria

Abstract

Natural honey is one of the most widely sought products due to its unique properties, which are attributed to the influence of the different groups of substances it contains. Honey is used for nutritional, medicinal and industrial purposes and it is an important commodity in the international market; serving as foreign exchange earner for many countries. In Nigeria, honey production (beekeeping) has the potential to develop as a prime agro-horticultural and forest-based industry which can be a major foreign exchange earner if international standards are met. The precise chemical composition and physical properties of natural honeys differ according to the plant species on which the bees forage. Differences in climatic conditions and vegetations are also important factors that can affect the various properties of honey. North-eastern Nigeria consists of humid, semi arid and arid climates with varying agricultural activities and blossoms from different types of vegetations, which can influence the natural composition and properties of honey. Thus, analysis of the biochemical composition of 18 honey samples obtained from different locations in the northeast sub-region of Nigeria was carried out to ascertain their qualities. Moisture and ash contents of the samples had average values of 16.00 ± 2.19 g/100 g and 0.47 ± 0.09 g/100 g, respectively. The protein contents ranged between 0.35 and 1.08g/100 g with a mean of 0.67 ± 0.25 g/100 g while fat content lied between 0.10 and 0.50 g/100 g with a mean of 0.29 ± 0.11 g/100 g. Total carbohydrate contents and Energy values showed average values of 82.30 ± 2.03 g/100 g and 1,401.33 ± 33.71 KJ/100 g, respectively. Fructose contents gave an average of 38.94 ± 0.90 g/100 g, while glucose contents had a mean value of 31.65 ± 2.79 g/100 g. The sucrose contents of the honey samples had a mean value of 1.84 ± 0.79 g/100 g. Total polyphenol and vitamin C contents showed mean values of 65.31 ± 19.50 mg Gallic Acid Equivalent (GAE)/100 g and 21.15 ± 3.99 mg/100 g, respectively. The results of this study indicate that the samples compare favorably with samples in many parts of the world and also fall within the limits of international standards.

Keywords: Honey; Proximate composition; Sugars; Polyphenol; Vitamin c; International Standards

Introduction

Natural honey is one of the most widely sought products due to its unique nutritional and medicinal properties, which are attributed to the influence of the different groups of substances it contains. Codex Alimentarius Commission defined honey as the natural sweet substance produced by honey bees, *Apis mellifera*, from the nectar of plants (blossoms) or from the secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which honey bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature [1,2]. The bees are said to produce honey in order to serve as their source of food in times of scarcity or during harsh weather conditions [3].

Essentially, natural honey is a sticky and viscous solution with a content of 80–85% carbohydrate (mainly glucose and fructose), 15–17% water, 0.1–0.4% protein, 0.2% ash and minor quantities of amino acids, enzymes and vitamins as well as other substances like phenolic antioxidants [3-7]. Each of these minor constituents is known to have distinctive nutritional or medicinal properties and the unique blend accounts for the varied and different applications of natural honeys [3]. Although the major constituents of honey are nearly the same in all honey samples, the precise chemical composition and physical properties of natural honeys differ according to the plant species on which the bees forage [3,8-11]. Furthermore, differences in climatic conditions and vegetations are important factors that can affect the various properties of honey.

Honey is used for nutritional, medicinal and industrial purposes and it is an important commodity in the international market; serving as foreign exchange earner for many countries. Beekeeping is an age-old tradition in Nigeria but it is not considered as a profit making venture in most parts of the country. Thus, while beekeeping has been part of normal agricultural enterprise among some communities in the country [12,13], honey production has largely been at a subsistence level [12-15]. However, honey is found in beehives in large quantities in Nigeria [16] and it has been recognized that honey production (beekeeping) has the potential to develop as a prime agro-horticultural and forest-based industry which can well become a major foreign exchange earner if international standards are met. For instance, it was shown that in Adamawa State, a beekeeper with an average number of 27 beehives made an average of $1,119.29 per annum from the sales of honey and beeswax [17]. Similarly, it was reported that in Ekiti State, a beekeeper with an average of 20 beehives made average revenue from sales of honey, bees wax and propolis amounting to about $2,148.42 per annum and $1,027.29 per annum for langstroth and topbar hives users, respectively [18,19] had reported that in Adamawa State, only a small percentage (5.62%) of the farming population who were already in the practice of beekeeping actually perceived apiculture as a profitable enterprise and know of its profitability; majority (56.25%) of the rural

***Corresponding author:** Fatimah Buba, Faculty of Science, Department of Biochemistry, University of Maiduguri, Nigeria, E-mail: fatimahbuba@gmail.com

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farming community and about 36.25%of the urban farming community considered apiculture only as a sideline economic activity.

The ecology of Nigeria varies from tropical forest in the south to dry savanna in the far north, yielding a diverse mix of plant and animal life. The northeast sub-region, which lies within 9º–14ºN and 8o–15oE is about one fourth (¼) of the land mass of Nigeria [20] and comprises of six states (Adamawa, Bauchi, Borno, Gombe, Taraba and Yobe States), most of which share boundaries with international communities like Cameroun, Chad and Niger Republics. This sub-region of Nigeria consists of humid, semi arid and arid climates with varying agricultural activities and blossoms from different types of vegetations, which can influence the natural composition and properties of honey in the area; thereby making it very suitable for apicultural practice.

Available literature on the properties and qualities of Nigerian honey have largely focused on samples obtained in the southern parts of the country [10,21,22]; with very scarce information on samples obtained in the northern parts, especially the northeast sub-region where commercial beekeeping practice has been documented [17,19]. This paper reports on the biochemical properties of honey from northeast Nigeria.

Materials and Methods

Sample Collection and Preparation

Eighteen (18) honey samples harvested from different locations in the northeast sub-region were obtained and used for the study. All the samples were collected freshly in sterile containers (labeled with numbers, place and date of collection) and stored at ambient temperature until analyzed. Unwanted material such as wax sticks, dead bees and particles of combs were removed by straining the samples through cheesecloth before analysis.

Biochemical Analysis

Determination of proximate composition: Proximate compositions of the honey samples were determined using the methods of AOAC (1990; 2000). For moisture content, 2.0g of each sample was dried to constant weight in hot air oven at 70°C and the moisture was calculated on dry basis. Ash content was determined by drying 5.0g of honey samples in porcelain crucibles at 105°C for 3 hrs in hot air oven. The dried samples were ignited in a furnace at 550–600°C to constant weight, cooled and weighed. Protein content was determined using the micro-kjeldhal procedure to estimate the total nitrogen content and the protein content was calculated using the 6.25 conversion factor for protein nitrogen.

Crude fat content was determined following extraction using rob ring tube or Majonnier fat extraction apparatus [23]. Five grams (5.0 g) of the honey sample was weighed in the extraction apparatus and mixed thoroughly with 2.0 mL of 99%ethyl alcohol. Then 10.0 mL of dilute HCl (prepared by adding 11 volumes of water to 25 volumes of concentrated HCl) was added and mixed well. The tube was then set in a water bath held at 70–80°C and shaken frequently at intervals for 30–40 minutes. The fat extraction apparatus was then filled to half its volume capacity with alcohol and cooled. Twenty five mililitres (25.0 mL) of ethyl ether was then added, shaken vigorously and allowed to stand until the upper liquid was practically clear. The ether extract was then drawn off by passing through a filter (using a plug of cotton in the stem of the funnel just enough to allow free passage of ether extract) into a pre-weighed 125 mL beaker, and was then dried on a water bath. The liquid remaining in the tube was re-extracted twice each with only 1.0 mL of ether. A similar pre-weighed beaker was then used as counter poise at 100°C. The beakers were then cooled in desiccators to constant weight and the fat content calculated.

Carbohydrate contents of the honey samples were determined by calculation (by difference) as follows:

%Carbohydrate=100%–(%Moisture+%Crude Fat+%Crude Protein+%Ash).

The energy values of the samples were determined by calculation as follows:

Energy (KJ/100 g)=4.186 [(%Crude Protein x 4)+(%Crude Fat x 9)+(%Carbohydrate x 4)]

Determination of reducing sugars and sucrose contents: The estimation of reducing sugars was carried out using the Layne-Enyon method as described in AOAC [23]. About 2.6 g of honey was weighed and transferred to a 500 mL volumetric flask. Five milliliters (5 mL) of standardized Fehling's solutions A and B were transferred to a 250 mL Erlenmeyer flask containing 7.0 mL of water and 15.0 mL of honey solution. The Erlenmeyer flask was heated and 1.0 mL of methylene blue (0.2%) was added. Titration was carried out by adding the diluted honey solution until the indicator decolorizes.

Sucrose content was determined by inversion, adding 10 mL of dilute HCl, 50 mL of diluted honey solution and water in a 100 mL volumetric flask. The solution was then heated in a water bath, cooled and diluted to the mark. Finally, the Layne-Enyon method was applied and the sucrose content was obtained by difference.

Determination of glucose content: Glucose content of the honey samples was determined by enzymatic oxidation with glucose oxidase reagent (Randox Laboratories Ltd., UK). Twenty microlitres (20 µL) of the sample or standard was allowed to react with 2.0 mL of the reagent, mixed well and incubated for 10 min at 37°C. The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was read against a reagent blank within 60 min. Glucose concentration was calculated as follows:

$$\text{Glucose content (mg/dL)} = (A_{sample}/A_{standard}) \times \text{Conc. of standard}$$

$$= (A_{sample}/A_{standard}) \times 100 \text{ (mg/dL)}$$

Determination of fructose content: Fructose content was determined using the resorcinol reagent method [24]. To a solution of the honey sample, 1.0 mL resorcinol reagent was added and mixed thoroughly, and then 1.0 mL of dilute HCl was added. Standard solutions containing 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ mL and made up to 2 mL with distilled water was also treated with 1.0 mL of the resorcinol reagent and 1.0 mL of diluted HCl as above. A blank solution was also prepared along with the standard and treated in the same manner. The test solution, the standard and blank were then heated in a water bath at 80°C for about 10min, the solution was then removed from the water bath, cooled by immersing in tap water for 5min and then the absorbance of both the test and standard solution were read against the blank solution at 520 nm within 30 min. The fructose contents of the honey samples were then extrapolated from a standard curve prepared using the absorbance of the standard.

Determination of total phenolic content: The phenolic compounds (flavonoids and phenolic acids) were extracted from the honey samples according to the method described by Kacaniova [25]. Ten grams (10 g) of the honey sample was dissolved in 50 mL of acidified deionised water (acidified to pH 2 with HCl). The solution was then filtered with a cotton filter to remove solid particles and the filtrate was used

for the estimation of total phenolic compounds. The total phenolic content was estimated using the Folin-Cioucalteu colorimetric method. Appropriately diluted honey sample, 0.2 mL of 10% aqueous extract of the honey sample was treated with 0.8 mL of the Folin-Cioucalteu reagent and 2.0 mL of 7.5% Na_2CO_3. The mixture was diluted using 7.0 mL distilled water and the absorbance was read after 2hrs at 765nm; the result was calculated as gallic acid equivalent [26].

Determination of Vitamin C (Ascorbic Acid): Vitamin C (Ascorbic acid) contents of the samples were determined by the 2,6-dicholorophenolindophenol titrimetric method as described by AOAC (1990). Two grams (2 g) of the honey sample was weighed and extracted in 5 mL of 20% metaphosphoric acid. A standard solution containing 50 mg L-ascorbic acid dissolved in 90 mL of 20%metaphosphoric acid and made up to 100 mL with water was also prepared. Two milliliters (2 mL) each of the standard and sample were titrated with the 2,6-dicholorophenolindophenol solution until a faint pink end point lasting at least 10 to 15 seconds was observed. The vitamin C content was calculated as follows:

$$\text{Vitamin C } (\text{mg}/100\text{ g}) = \frac{\text{Titer value x dye factor x } 100}{\text{Weight of sample}}$$

$$\text{Dye factor } (\text{DF}) = \frac{0.5}{\text{Standard Titer}}$$

Statistical Analysis

The data obtained in the study were analysed statistically using ANOVA and student t–test (using GraphPad Instat Statistical Program). Differences between mean values were considered significant at values of $P<0.05$.

Results

Proximate composition

The results of the proximate analysis of honey samples obtained from different location in the six states within the north east sub-region of Nigeria are presented in Table 1. The results showed no significant differences ($P>0.05$) between the samples for moisture, ash, fat and carbohydrate contents as well as the energy values of the honey samples from all the states. However, significant differences ($P<0.05$) in protein contents were observed between the honey samples. Samples from Bauchi State showed significantly ($P<0.05$) higher protein contents (1.04 ± 0.04 g/100 g) when compared to samples from Adamawa (0.50 ± 0.10 g/100 g), Borno (0.46 ± 0.09 g/100 g) and Yobe (0.55 ± 0.22 g/100 g) States, but showed no significant difference ($P>0.05$) from the values obtained for samples from Gombe (0.72 ± 0.14 g/100 g) and Taraba States (0.76 ± 0.29 g/100 g). The protein contents of the honey samples from Gombe and Taraba States did not, however, differ significantly ($P>0.05$) from the values obtain for samples from Adamawa, Borno and Yobe States.

In general, the results for the eighteen honey samples from the sub-region (Table 2) showed that moisture contents ranged between 12.50 and 21.00 g/100 g with an average value of 16.00 ± 2.19 g/00g. Ash contents varied from 0.28 to 0.60 g/100 g with an average of 0.47 ± 0.09 g/100 g. The protein contents ranged between 0.35 and 1.08g/100 g with a mean of 0.67 ± 0.25 g/100 g while fat content lied between 0.10 and 0.50 g/100 g with a mean of 0.29 ± 0.11 g/100 g. Total carbohydrate contents and Energy values ranged from 77.60 to 86.20 g/100 g and 1,323.10 to 1,470.50 KJ/100 g, with average values of 82.30 ± 2.03 g/100 g and 1,401.33 ± 33.71 KJ/100 g, respectively.

Sugar contents

The results of sugar analysis of the honey samples are presented in Table 3. No significant differences ($P>0.05$) in the fructose, glucose, fructose+glucose and reducing sugar contents were observed in samples from the six States of the sub-region. Similarly, no significant difference, ($P>0.05$) in both fructose/glucose ratio and glucose/water ratio were observed between samples from all the six States. However, the apparent sucrose contents of samples from Bauchi (2.57 ± 0.26 g/100 g) Gombe (2.54 ± 0.97 g/100 g) and Yobe (2.46 ± 0.27 g/100 g) were statistically similar and significantly ($P<0.05$) higher than those for Adamawa (1.62 ± 0.40 g/100 g), Borno (1.01 ± 0.42 g/100 g) and Taraba (1.01 ± 0.33 g/100 g) States.

The results of the sugar analysis of all the eighteen (18) honey samples (Table 4) showed that the fructose contents varied between 37.68 and 40.31 g/100 g with an average of 38.94 ± 0.89 g/100 g. The glucose contents of the samples were within a range of 27.25 to 39.56 g/100 g with a mean value of 31.65 ± 2.27 g/100 g. The fructose contents of the samples were significantly ($P<0.001$) higher than the glucose contents. The fructose/glucose ratio and glucose/water ratio were within the range of 1.00 to 1.45 and 1.59 to 2.75 with mean values

Parameter	Adamawa	Bauchi	Borno	Gombe	Taraba	Yobe
Moisture (g/100g)	15.83 ± 0.58	15.83 ± 1.26	16.67 ± 4.25	17.33 ± 2.56	15.00 ± 2.78	15.33 ± 1.53
Ash (g/100g)	0.37 ± 0.008	0.54 ± 0.11	0.41 ± 0.09	0.48 ± 0.05	0.47 ± 0.11	0.52 ± 0.02
Protein (g/100g)	0.50a ± 0.10	1.04b,c ± 0.04	0.46a ± 0.09	0.72a,c ± 0.14	0.76a,c ± 0.29	0.55a ± 0.22
Fats (g/100g)	0.20 ± 0.10	0.22 ± 0.13	0.40 ± 0.10	0.35 ± 0.09	0.32 ± 0.08	0.30 ± 0.10
Carbohydrate (g/100g)	83.09 ± 0.54	82.20 ± 1.22	82.10 ± 4.31	81.10 ± 2.40	82.33 ± 1.76	83.00 ± 1.31
Energy (KJ/100g)	1,407.11 ± 10.94	1,405.06 ± 18.04	1,397.40 ± 73.72	1,383.23 ± 39.09	1,404.97 ± 30.09	1,410.20 ± 24.43

Values presented are mean ± SD of three determinations. Mean values with different superscript along a row are significantly different ($P<0.05$)

Table 1: Proximate Composition and Energy Values of Honey Samples from the Six States in Northeastern.

Parameters	Mean ± SD	Range of values (Min – Max)	Limits of Int'l Std.	Samples outside Limits of International Standard
Moisture (g/100g)	16.00 ± 2.19	12.50 – 21.00	Not > 20 g/100g	One (1) sample with 21g/100g
Ash (g/100g)	0.42 ± 0.09	0.28 – 0.60	≤ 0.6 g/100g	None
Protein (g/100g)	0.67 ± 0.25	0.35 – 1.08	No fixed limit	
Fat (g/100g)	0.29 ± 0.11	0.10 – 0.50	No fixed limit	
Carbohydrate (g/100g)	82.30 ± 2.03	77.60 – 86.20	No fixed limit	
Energy KJ/100g	1401.33 ± 33.71	1323.10 – 1470.50	No fixed limit	

Table 2: Proximate Composition and Energy Values of Eighteen (18) Honey Samples from Northeastern Nigeria

Parameter	Adamawa	Bauchi	Borno	Gombe	Taraba	Yobe
Fructose (g/100g)	38.58 ± 0.57	39.02 ± 0.82	39.21 ± 1.27	39.06 ± 1.13	38.60 ± 1.27	39.15 ± 0.86
Glucose (g/100g)	31.27 ± 6.30	31.81 ± 6.74	33.36 ± 1.99	30.27 ± 1.37	31.81 ± 1.11	31.37 ± 1.79
Fructose + Glucose (g/100g)	69.85 ± 2.18	70.83 ± 7.14	72.57 ± 1.86	69.34 ± 2.35	70.41 ± 1.27	70.53 ± 1.13
Reducing sugar (g/100g)	75.83 ± 9.36	75.26 ± 13.71	72.85 ± 4.75	69.56 ± 0.99	69.83 ± 3.82	71.05 ± 2.63
Sucrose (g/100g)	1.62^{a} ± 0.40	2.57^{b} ± 0.26	1.01^{a} ± 0.42	2.54^{b} ± 0.97	1.01^{a} ± 0.33	2.46^{b} ± 0.27
Fructose/Glucose Ratio	1.23 ± 0.05	1.26 ± 0.23	1.18 ± 0.10	1.29 ± 0.04	1.21 ± 0.07	1.25 ± 0.10
Glucose/water Ratio	1.98 ± 0.11	2.04 ± 0.61	2.09 ± 0.58	1.77 ± 0.26	2.16 ± 0.34	2.05 ± 0.09

values are presented as mean ± SD of three determination values with different superscripts are along a row arare significantly different from each other (P<0.05)

Table 3: Sugar Contents of Honey Samples from the Six States in Northeastern Nigeria.

Parameters	Mean ± SD	Range of values (Min-Max)	Limits of Int'l Std	Samples outside the Limits of International Standards
Fructose (g/100g)	38.94 ± 0.89	37.68 – 40.31	No fixed limit	
Glucose (g/100g)	31.65 ± 2.27	27.25 – 39.56	No fixed limit	
Fructose + Glucose (g/100g)	70.59 ± 3.01	66.70 – 79.08	Not < 60 g/100g	None
Sucrose (g/100g)	1.84 ± 0.79	0.53 – 3.29	Not > 5 g/100g	None
Fructose/Glucose Ratio	1.24 ± 0.10	1.00 – 1.45	No fixed limit	
Glucose/Water Ratio	2.01 ± 0.35	1.59 – 2.75	No fixed limit	

Table 4: Sugar Contents of Eighteen (18) Honey Samples from Northeastern Nigeria.

of 1.24 ± 0.10 and 2.01 ± 0.35, respectively. No significant difference (P>0.05) was observed between the fructose/glucose and the glucose/water ratios.

The sum of fructose and glucose (fructose+glucose) contents ranged between 66.70 and 79.08 g/100 g with an average of 70.59 ± 3.01 g/100 g while the reducing sugar contents varied between 65.53 and 91.05 g/100 g with an average of 72.40 ± 6.65 g/100 g. There was no significant (p>0.05) difference between the mean values of the fructose plus the glucose contents and the reducing sugar contents of the honey samples. The sucrose contents of the honey samples gave a range from 0.53 to 3.29 g/100 g with a mean value of 1.84 ± 0.79 g/100 g and is significantly (P<0.001) lower than the fructose contents as well as the glucose contents.

Vitamin C and Total Polyphenol Contents

Table 5 shows the vitamin C and total polyphenol contents of the honey samples from the various States. The results indicate that there are no significant differences (P>0.05) in the values of these two parameters when comparisons are made between the samples from all the States. For the eighteen (18) samples studied the polyphenol and vitamin C contents showed ranges from 36.26 to 102.80 mgGAE/100 g and 13.86 to 27.32 with mean values of 65.31 ± 19.50mg GAE/100 g and 21.15 ± 3.99 mg/100 g, respectively.

Discussion

The average moisture of the honey samples from all the States in Northeastern Nigeria were found to be within the limit of not more than 20.0 g/100 g as prescribed by Codex Alimentarius Commission [27-29]. Most of the samples showed low moisture contents (average value 16.00 ± 2.19 g/100 g), and only one sample exceeded the limit of 20.0 g/100 g established by international norms. The values fall within the range of moisture contents reported by White and Doner [4] for 490 samples of floral honey having a range from 13.4 to 22.9 g/100 g and an average value of 17.2 ± 1.46 g/100 g. These results are similar to results of other researchers [8,10,30,31].

Moisture content is an important quality parameter, important above all for honey shelf-life [32,33]. It is the only composition criterion which as a part of honey standard has to be fulfilled in world honey trade [34]. The significance of moisture in honey derives from the fact that there is a relationship between honey water content and yeast count; at 17.0 g/100 g moisture (humidity) there is very minimal fermentation danger due to very low yeast count [32]. Thus, honey having high water content is more likely to ferment [34]. A maximum value of 20.0 g/100 g was established by the Codex Alimentarius Commission and EU Commission as the international standard for honey moisture contents.

The ash contents of honey obtained in this study were all within the limits of<0.6 g/100 g specified by international norms [1,2]. There were no significant differences between the ash contents of the sample from all the States in the sub-region. The results of the ash contents are similar to those reported for honey samples from southern part of Nigeria [10] as well as values reported for samples from Argentina, Spain and Turkey [8]; northern region of Bangladesh [35]; different areas of Pakistan [36,37] and Algeria [30].

The Ash content of honey is also a parameter that is used in determining the floral origin of honeys. Thus, by reference to the Codex Alimentarius Standards, all the honeys analysed in this study correspond to nectar honey since their ash contents falls within the values of<0.6%. The ash contents of honeys represent their mineral and trace element contents. According to Bogdanov [33], blossom honeys have a mineral content mostly between 0.1 and 0.3%while that of honeydew honeys can reach 1.0%of the total. Several investigations have shown that the trace element content of honey depends mainly on the botanical origin of honey; i.e. light blossom honeys have low contents than dark honeys such as honeydew, chestnut and heather honeys [38,39].

The protein contents of honey samples from some of the States of the northeast were significantly (P<0.05) different. The values obtained in this study are similar to those reported by Khalil [35], for five different brands of unifloral honey from the northern region of Bangladesh, which ranged between 0.655 and 0.744 g/100 g. The amount of nitrogen in honey is generally low, in average of about 0.04% although it may reach up to 0.1% [4,40]. It was also reported that of the total amount of Nitrogen in honey only 40–65% is in protein, the remaining part of

Parameter	Adamawa	Bauchi	Borno	Gombe	Taraba	Yobe
Total Polyphenol (mg/100g)	72.06 ± 4.99	72.41 ± 26.45	64.08 ± 16.17	62.56 ± 27.35	60.94 ± 34.99	59.86 ± 6.41
Vitamin C (mg/100g)	20.68 ± 2.83	18.52 ± 5.46	19.76 ± 2.55	21.61 ± 4.82	20.84 ± 4.42	25.16 ± 2.33

Values are presented as Mean ± SD of three determinations. Values along rows are not significantly different (P>0.05) for all the States.

Table 5: Total Polyphenol and Vitamin C Contents of Honey Samples from States of Northeastern Nigeria.

total nitrogen resides in substances other than protein, such as amino acids. About 8 to 11 proteins have been found in various honeys but only four (4) proteins are common to all honeys and these four (4) proteins common to all appear to originate from the honey bee rather than from nectar. The honey proteins are mainly in the form of enzymes [40]. The honey bees add different enzymes during the process of honey ripening. The enzymes added include diastase (amylase), which digest starch to maltose and is relatively stable to heat and storage, and invertase (saccharase or α-glucosidase), which catalyses the conversion of sucrose to glucose and fructose. The invertase also catalyses many other sugar conversions and is mainly responsible for the sugar patterns of honey. Glucose oxidase and catalase are two other enzymes added by the honey bee, which regulate the production of hydrogen peroxide H2O2; the H2O2 serve as one of the anti-bacterial factor in honey.

The significant differences observed between the total protein contents of honey samples from some of the States within the sub-region may be ascribed to differences in the botanical origin of honey since it was reported that the diastase and the invertase enzymes varied in wide limits depending on the botanical origin of honey [41]. Bosi and Battalglini [42] had reported protein contents of honey varying between 0.01 to 0.04 g/100 g with proline, lysine, phenylalanine, aspartic acid and glutamic acid as the most widely detected amino acids.

The fat contents of the honey samples investigated in this study fall within the range of 0.1 to 0.5 g/100 g. Reports indicating that honey contains little or no fat are available in the literature [43,44], but the presence of free fatty acids like palmitic, oleic and linolenic acids have been reported in white clover honey. In a biochemical analysis of five different brands of unifloral honey available in the northern region of Bangladesh, Khalil [35] reported total fat contents in the range of 0.134 to 0.146 g/100 g; thus, indicating that honey contains very little amount of lipid and therefore not considered a good source of lipid.

The total carbohydrate contents of the honey samples from all the States were not significantly different from each other; this corresponds to the findings of others scientists [35-37]. Carbohydrates are the main constituents of honey comprising about 95% of honey dry weight. The monosaccharides, fructose and glucose, are the main sugars found in honey; these hexoses are products of the hydrolysis of sucrose. In addition to these sugars, 25 others have been detected in honey samples [45,46]. The principal oligosaccharides in blossom honey include the disaccharides sucrose, maltose, turanose, erlose, etc. On the other hand, honeydew honeys also contain the disaccharides melezitose and raffinose; with trace amounts of tetra and pentasaccharides also isolated [4].

The average energy value of the honey samples from all the States ranged between 1383.23 ± 39.09 and 1410.20 ± 24.43 KJ/100 g. Honey is primarily a high energy carbohydrate food and the honey sugars are easily digestible sugars similar to those found in many fruits (White and Doner, 1980). For this reason honey is regarded as a good food for both infants and adults. Blasa [47] had reported caloric value of about 303kcal/100 g of honey.

The reducing sugar contents of the samples used in this study had average value of 72.40 ± 6.65 g/100 g, the values obtained in this study are similar to the values reported for honeys from Bangladesh [35], Pakistan [36], Argentina and Turkey [8] and Venezuela [48].

The fructose contents of the honey samples analysed in this study varied between 37.68 to 40.31 g/100 g with an average of 38.94 ± 0.40 g/100 g. The average fructose contents for the samples from the different States within the sub-region were not significantly different from each other and they all fall within the range of values reported by other scientists [4,9,30,31,49].

In a similar manner, the glucose contents of the honey samples obtained from the various locations in the different States of the sub-region were not significantly different from each other. The glucose contents of the samples which varied from 27.25 to 39.56 g/100 g with an average of 31.65 ± 2.79g/100 g were significantly ($P<0.05$) lower than the fructose contents. This observation shows that fructose is the major sugar in all the samples analysed and, it is in agreement with the earlier observation of White and Doner [4]. Fructose and glucose are the dominant sugar types in honeys, which although no limits have been fixed for their individual values, their sum (Fructose+glucose) has been fixed at a value of ≥ 60 g/100 g as one of the requirements of the international standard for honey established by Codex Alimentarius Commission. The sum of fructose and glucose for the honey samples, used in this study, indicates that samples have their values corresponding to the limit required by the international norms; i.e., 60g/100 g and above. According to White and Doner [4] the dominance of fructose over glucose is one way in which honey differs from commercial invert sugar. Generally, the sugar spectrum of honey depends upon the sugars present in the nectar and the enzymes present in the bee and nectar [4,33,49]. Fructose and glucose constitute the primary sugars in all honey samples, and in honey of good quality the fructose content should exceed that of glucose [49].

In addition to the sum of fructose and glucose, other important factors that relate to honey quality include the fructose/glucose ratio and glucose/water ratio. In this study, the fructose/glucose ratio and glucose/water ratio fall in the range of 1.00 to 1.45 and 1.59 to 2.75 with average values of 1.24 ± 0.10 and 2.01 ± 0.35, respectively. Fructose/glucose ratio indicates the ability of honey to crystallize. White and Doner [4] stated that even though honey has less glucose than fructose, it is the glucose that crystallizes when honey granulates because it is less soluble in water than fructose. When the fructose/glucose ratio is high, honey remains liquid. Honey crystallization is slower when the fructose/glucose ratio is more than 1.3 and it is faster when the ratio is below 1.0 [30]. However, because honey contains others sugars (sucrose, maltose, turanose, etc) and insoluble substances (like dextrin, colloids, etc) which can influence the crystallization process, the glucose/water (G/W) ratio is considered more appropriate than the fructose/glucose (F/G) ratio for the prediction of honey crystallization. It has been stated that when the glucose/water ratio is <1.3 honey crystallization is very slow or even zero, and it is complete and rapid when the ratio is >2.0 [30]. Glucose, which is a major sugar in honey, can spontaneously crystallize from honey solutions in the form of its monohydrate [4]. This sometimes occurs when the moisture level in honey is allowed to drop below a certain level; i.e., when the moisture content is very low. It was stated earlier on that honey samples with (G/W) ratio of <1.7 are

considered non-granulating while samples with ratios of ≥ 2.1 predicts rapid granulation. Also, according to Manikis and Thrasivoulou [50], while glucose levels is a useful indicator of honey granulation, the G/W ratio appears to be one of the most effective indicator for predicting granulation tendencies in honey samples. Thus, G/W ratio may be used both to predict and control granulation tendencies in honeys.

The international norm established by the Codex Alimentarius Commission requires that a good quality honey should not contain more than 5 g/100 g sucrose. The apparent sucrose contents of the honey samples studied were in the range of 0.53 to 3.29 with an average of 1.84 ± 0.79 g/100 g. The values obtained for sucrose contents of the honey samples were all within the limits of international standards. According to White and Doner [4] even though honey contains an active sucrose splitting enzyme (sucrase, glucosidase), the sucrose level in honey never reaches zero. The sucrose contents obtained in this investigation are within the range of values reported for Argentine and Turkish [8], Venezuelan [48], American [4], Algerian [31], Pakistani [49] and Spanish [51] honeys.

The total polyphenol and vitamin C contents in the honey samples were not significantly different among the samples. The polyphenol contents of the honey samples from this sub-region varied between 36.26 and 102.80 mgGAE/100 g with an average of 65.31 ± 19.50 mgGAE/100 g, while vitamin C contents were observed to be within the range of 13.89 and 27.32 mg/100 g with an average of 21.15 ± 3.99 mg/100 g. A variety of phytochemicals, as well as other substances including organic acids, vitamins, and enzymes; some of which may serve as sources of dietary antioxidant [6,52] are known to occur in honeys.

The range and average values of total phenolic contents observed for the honey samples used in this study are similar to those reported by Vit [48] for Venezuelan *Apis Mellifera* honeys (38.15 to 182.10 mgGAE/100 g, with an average of 93.50 ± 51.62 mgGAE/100 g). The values of phenolic contents in this study are, however, higher ($P<0.05$) than those reported by Adetuyi [22] for *Apis Mellifera* honey samples in Owo community, Ondo State in southwest Nigeria (0.75 to 2.85 mgGAE/100 g). The phenolic contents obtained in this study are also higher ($P<0.05$) than those observed in selected Czech honey; 3.92 ± 0.13 to 16.71 mgGAE/100 g [53] and in some honeys from Poland, 7.17 ± 0.13 to 20.16 ± 1.68 mgGAE/100 g [54]. It was earlier reported that, in honey samples from Burkina Faso, total phenolic contents varied from 32.59 to 114.75 mgGAE/100 g with a mean of 74.38 ± 20.54 mgGAE/100 g [55]. Phenols are reported to have antioxidant capacities that are much stronger than those of vitamins C and E [56]. According to Blasa [47] raw honey contains copious amounts of compounds such as flavonoids and other polyphenols which may function as antioxidants. Honey polyphenols are said to originate from nectar, pollen or propolis [57].

The presence of vitamin C (L-ascobic acid in the honey samples used in this study is in agreement with earlier reports of other scientists [48,58,59]. It was reported that the honey of *Apis mellifera* has a low concentration of vitamin C, less than 5mg/100 g [40] and concentration of 2.5 mg vitamin C per 100 g honey is found in the literature [60]. However, higher concentrations of the vitamin have been reported in recent times. Matei [58] reported vitamin C contents ranging from 226 to 296 mg/100 g for floral honeys in Romania. In addition, vitamin C concentrations ranging between 37.22 to 378.30 mg/100 g were reported for various types of honeys from different locations in Bosnia Herzegovina, with the higher concentrations, in forest honeys [59]. In another report, Vit [48] found that vitamin C contents varied between 12.86 and 37.05 mg/100 g in Venezuelan honeys. The vitamin C contents obtained for the honey samples used in this study are within the range reported by Vit [48], but much lowers than the values reported by Matei [58,59]. Honey contains ascorbic acid because most flowers on which the bees forage contain this vitamin which serves as an antioxidant in addition to many other functions. Indeed, it has been shown that antioxidant activity of honey, which depends on its botanical origin, is related to its vitamin C contents; i.e., the content of vitamin C has a significant impact on total antioxidant activity of honey [59].

Conclusion

The values of quality parameters for all the honey samples studied coincide with those specified by the international honey regulations. The honey samples are also rich in phenolic and vitamin C contents which confer good antioxidant properties in honey.

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