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Physicochemical and Microbiological Properties of Honey from North East Nigeria

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Abstract

Honey is used for nutritional, medicinal and industrial purposes and it is an important commodity in the international market. Honey production has the potential to become a major foreign exchange earner for Nigeria if qualities of the products are able to meet international standards. Commercial beekeeping practice has been known to exist in the north east sub-region of Nigeria for a long time but scientific information about the qualities of the products is scare. Therefore, some physicochemical parameters (including pH, electrical conductivity, acidities, and hydroxymethylfurfural and diastase activities) of eighteen honey samples obtained from different locations in the six States comprising the northeast sub-region of Nigeria were investigated to evaluate their quality and compliance with international regulatory standards. The pH and Electrical Conductivity (EC) values ranged from 3.5 to 4.9 and 0.05 to 0.41 with mean values of 4.9 ± 0.41 and 0.15 ± 0.09, respectively. The free, lactone and total acidities of the samples varied from 13.0 to 33.6 meq/kg; 1.16 to 4.63 meq/kg and 14.25 to 36.67 meq/kg with average values of 23.00 ± 6.20 meq/kg; 2.28 ± 0.89 meq/kg and 25.17 ± 6.86 meq/kg, respectively. Hydroxymethylfurfural (HMF) contents and diastase activities varied from 5.99 to 17.22 mg/kg and 8.00 to 13.00 (Schades units) with mean values of 11.73 ± 3.97 mq/kg and 9.37 ± 1.38 (Schades units), respectively. Significant differences ($P<0.05$) were observed in free acidities and HMF contents of samples from the different States in the sub-region. The results are comparable with reports from many parts of the world and are also within the limits of international standards. However, most of the samples showed bacterial and mould growth, suggesting poor sanitary procedure during harvest or storage.

Keywords: Honey; Acidity; Hydroxymethylfurfural; Diastase; Microorganisms

Introduction

Honey is the natural sweet substance produced by honeybees from the nectar of blossoms or from the secretion of living parts of plants or excretions of plant sucking insects on the living parts of plants, which honeybees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature [1,2]. It 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]. The minor constituents are known to have distinctive nutritional or medicinal properties [7] while the major constituents 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 [7-11] and also according to climatic conditions and vegetations which are important factors that can affect the various properties of honey. The northeast sub-region of Nigeria, which lies within 9°–14°N and 8°–15°E, consists of approximately one quarter of the land mass of Nigeria [12] and comprises of six states (Adamawa, Bauchi, Borno, Gombe, Taraba and Yobe States) characterized by humid, semi-arid and arid climates. The production and marketing of bee products, especially honey is a well known business in the sub-region [13] but information about the qualities of the products is scare.

Honey is used for nutritional, medicinal and industrial purposes and it is an important commodity in the international market. Honey production and processing has the potential to become a major foreign exchange earner for Nigeria if qualities of the products are able to meet international standards. The development of a viable honey production and processing industry in the northeast sub-region of Nigeria may benefit from biochemical analysis of natural honey samples obtained from different locations of the environment in order to ascertain product quality vis-à-vis international standards. Therefore this paper reports on the physicochemical and microbiological properties of honey from Northeast Nigeria.

Materials and Methods

Sample collection and preparation

Eighteen (18) honey samples harvested from different locations in the six States of the northeast sub-region (3 from each state) were obtained and used for the study. Samples from Adamawa State were obtained from Ganye, Hong and Tongo towns while those from Bauchi State were obtained from Alkaleri and two locations within Bauchi metropolis (designated as Bauchi 1 and Bauchi 2). The samples from Borno State were from Askira/Uba, Kwajaffa and Shani while those from Gombe State were collected from Billiri, Gombe and Shongom. In Taraba State the honey samples were collected from Bali, Takum and Yerro and in Yobe State samples were collected from the villages of Degubi, Dumbulwa and Gaji. All the samples were collected fresh in sterile containers (dully labeled with numbers, place and date of collection) and stored at ambient temperature until analysed. Unwanted material such as wax sticks, dead bees and particles of combs were removed by straining the samples through cheesecloth before analysis.

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Physicochemical analysis

Determination of pH and electrical conductivity: The pH values and electrical conductivities of the honey samples were measured at 28 ± 2°C using pH and conductivity meters. The pH of the honey samples were determined in a 10% aqueous honey solution using a digital pH meter after it was calibrated at pH 4.0 and 7.0 using standard buffer solutions.

Determination of free, total and lactone acidities

The free acidities and lactones were determined by equivalence point titration according to IHC [14]. Briefly, five grams (5 g) of honey (M) was accurately weighed and dissolved in a few milliliters of water and then transferred quantitatively into a 50 ml volumetric flask and filled to the mark with distilled water. The solution was mixed thoroughly and 25 ml was pipetted into a 250 ml beaker and the initial pH of the solution (pH_i) was noted, followed by gentle stirring and titration first with the NaOH solution (up to 10 ml), then into the same beaker with sulphuric acid solution up to the second equivalence point. From the titration curve the free acidity neutralization volume in ml (v) and the NaOH excess neutralization volume (corresponding to pH 7.0) in ml (v^1) were noted. The free acidity was expressed in miliequivalent of NaOH required to neutralize 1 kg of honey. The lactone acidity and total acidity are also expressed in the same units. These parameters were calculated using the following formulae [14].

F.A=V×T×(50/25)×(1000/M)

L.A=[(10-V)×T−0.05×V^1]×(50/25)×(1000/M)

T.A=F.A+L.A

Determination of Hydroxymethylfurfural (HMF)

HMF was determined spectrophotometrically according to the method of White [15] as described in IHC [14]. Briefly, five grams (5.0g) of honey was dissolved in 25 ml of water, transferred quantitatively into a 50 ml volumetric flask, followed by the addition of 0.5 ml of Carrez solution I and 0.5 ml of Carrez II and made up to 50 ml mark with water. The solution was filtered through filter paper, rejecting the first 10 ml of the filtrate. Aliquots of 5.0 ml were placed in two test tubes; to one tube was added 5.0 ml of distilled water (sample solution); to the second was added 5.0 ml of sodium bisulphate solution 0.2% (reference solution). The absorbance of the solutions at 284 and 336 nm was determined using a UV–visible spectrophotometer. The quantitative value of HMF was calculated using the formula:

HMF (mg/kg)=(A_{284}−A_{336}) ×149.7×5×D/W

Where: A_{284}=Absorbance at 284 nm

$A_{336=}$Absorbance at 336 nm

$$149.7=\frac{126\times1000\times1000}{16830\times10\times5}=\text{Constant}$$

126=molecular weight of HMF

16830=molar absorptivity ε of HMF at λ= 284 nm

1000=conversion g into mg

10=conversion 5 into 50 ml

1000=conversion g of honey into kg

5=theoretical nominal sample weight

D=dilution factor, in case dilution is necessary

W=Weight in g of the honey sample

Determination of diastase activity

The diastase activity was also determined in accordance with the method of IHC [14]. Five grams of honey was dissolved in 15 ml water; then 2.5 ml of acetate buffer (pH 5.3) was added and transferred to a 25 ml volumetric flask. Ten milliliters (10 ml) of this solution was then mixed with 5 ml of 2% starch solution in a test tube and incubated at 40°C for 15 minutes. After 5 minutes, 1 ml of the solution was taken and 7.0×10^{-4} meq/l of iodine solution was added. The absorbance was then read in a spectrophotometer at 660 nm until readings obtained were less than 0.235 absorbance units. The unit of Diastase Activity, the Gothe unit, is defined as that amount of enzyme which will convert 0.01 gram of starch to the prescribed end-point in one hour at 40°C under the conditions of test. Results are expressed in Gothe units (or Schade units) per gram of honey. The diastase activity was calculated and expressed as Diastase Number (DN) as follows:

$$DN=\frac{60\,minutes}{tx}\times\frac{0.10}{0.01}\times\frac{1.0}{2.0}=\frac{300}{tx}$$

t_x=reaction time in minutes obtained as follows:-

The absorbance values of the test sample solutions were plotted against the corresponding reaction times in minutes on graph paper after subtracting the absorbance of the blank value. The regression line was drawn through the measuring points in the range of A=0.155 to 0.456 in order to determine the time t_x for A=0.235. There should be at least three points in the absorbance range 0.155 to 0.456.

Microbiological analysis

Microbiological examination of the honey samples was carried out as described by Omafuvbe and Akanbi [10]. To determine total aerobic mesophilic bacteria, total coliforms, and aerobic endospore-forming bacteria, moulds and yeasts, ten grams of sample was mixed with 90 ml of sterile maximum recovery diluent (MRD). Subsequent decimal dilutions were prepared in sterile MRD and appropriately diluted suspension of sample (100 µl) was cultured in duplicate by the spread plate method. Standard Plate Count (SPC) was enumerated on plate count agar incubated at 30 ± 2°C for 48 hrs. Total coliforms were counted on violet red bile glucose agar (VRBG) incubated at 35°C for 24- 48 hrs. Moulds and yeasts were enumerated on Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (100 mg/l) incubated aerobically at 25°C for 3-5 days. For aerobic endospore bacterial counts, the diluted suspension of the honey sample was heated for 2 min in continuously boiling water to eliminate any microbial vegetative forms. Bacterial endospores were enumerated on nutrient agar (NA) incubated at 30°C for 48 h. All colonies appearing at the end of incubation were counted and the results expressed as colony forming units per gram (cfu/g). Colonies of endospore - forming bacteria were further observed, isolated and purified by repeated streaking on fresh agar plates of the isolation media. After successive transfers, the resulting pure isolates were Gram stained and identified based on standard morphological and biochemical methods.

Statistical analysis

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

Results

Physicochemical parameters

Table 1 shows the results of some physicochemical parameters of honey samples from the different states in the northeast sub-region. No significant differences (P>0.05) were observed between the pH values, Electrical Conductivities (EC) and diastase numbers of samples from all the six States in the sub-region. Significant (P<0.05) differences were, however, observed in the mean values of free acidity, lactone acidity, total acidity and Hydroxymethylfurfural (HMF) for some of the samples. Honey samples from Yobe and Gombe States had significantly (P<0.05) higher values of free acidity, lactone acidity and total acidity than those from the other North-Eastern States. Although the free acidities of samples from Bauchi (24.33 ± 0.76 meq/kg), Borno (18.40 ± 1.83 meq/kg) and Taraba (21.93 ± 1.14 meg/kg) did not differ significantly (P>0.05) they were all significantly (P<0.05) higher than the values for samples from Adamawa State (13.73 ± 0.70 meq/kg) and lower than values obtained for samples from Gombe State (27.73 ± 1.22 meq/kg) and Yobe State (31.87 ± 1.80 meq/kg). The mean Hydroxymethylfurfural (HMF) contents of honey samples from Bauchi (14.47 ± 2.29 mg/kg), Gombe (13.21 ± 2.63 mg/kg) Taraba (11.98 ± 1.98 mg/kg) and Yobe (16.47 ± 0.75 mg/kg) States were not significantly (P>0.05) different from each other. The samples from Adamawa and Borno States had mean HMF values of 5.99 ± 0.75 mg/kg and 8.23 ± 0.75 mg/kg, respectively; both of which were significantly (P<0.05) lower than those for the other States.

In the results of the physicochemical parameters for eighteen (18) samples analysed from the sub-region (Table 2), the pH and Electrical Conductivity (EC) values ranged from 3.5 to 4.9 and 0.05 to 0.41 with mean values of 4.08 ± 0.41 and 0.15 ± 0.09, respectively. The free, lactone and total acidities of all the samples lie between 13.0 and 33.6 meq/kg; 1.16 and 4.63 meq/kg and 14.25 and 36.67 meq/kg with average values of 23.00 ± 6.20 meq/kg; 2.28 ± 0.89 meq/kg and 25.17 ± 6.86 meq/kg, respectively. The HMF contents and diastase activities of all the samples analysed were observed to be within the range of 5.99 to 17.22 mg/kg and 8.00 to 13.00 (Schades units) with mean values of 11.73 ± 3.97 mq/kg and 9.37 ± 1.38 (Schades units), respectively.

Microbiological analysis

Microbial examination of the honey samples obtained in the sub-region (Table 3) showed the presence of bacteria and mould, but no yeast was detected. Bacteria were detected in fifteen (15) of the eighteen (18) samples analysed; i.e., samples from Adamawa State (Ganye, Hong and Tongo), Bauchi State (Alkaleri, Bauchi 1 and Bauchi 2), Borno State (Askira/Uba, Kwajaffa and Shani), Gombe State (Billiri, Gombe town, and Shongom), Taraba State (Bali, Takum and Yerro) and Yobe State (Degubi, Dumbulwa and Gaji). Among the bacterial isolates *Bacillus spp* predominated in almost all the samples except in the samples from Tongo which contained *Kliebsiella spp* and the samples from Alkaleri which contained *Staphylococcus aureus*.

Moulds were also detected in all the honey samples, showing the predominance of *Aspergillus niger* in almost all the samples, except in samples from Shani in Borno State and Gombe town, which had *Erothium spp* and *Rhizopus stolinifer*, respectively. In addition to the *A. niger*, samples from Tongo in Adamawa State and Yerro in Taraba State also had *Mucor spp*, while samples from Hong, Bauchi 1, Gaji and Shongom had *Scopulariopsis fusca* and *R. stolinifer*.

The distribution of the microorganisms (isolates) in the samples examined is presented in Table 4. The frequency of occurrence of *Aspergillus spp* and *Bacillus spp* in the samples were 88.9% and 72.2%, respectively. Table 5 shows the microbial profile of the honey samples after about three (3) months of storage from the initial evaluation. Only the endospore forming bacterial species of the genus Bacillus were observed; other organisms were no longer detected in the samples. Total coliforms were also not detected.

Discussion

The pH values of the honey samples from the different States were not significantly different from each other; the values ranged between 3.5 and 4.9 with an average of 4.1. Values of pH within the range of 3.5 to 4.5 with an average of 3.9 were reported for American blossom honeys [16]. Similarly, pH range of 3.40 to 6.23 [17] and 3.67 to 4.70 [18] with average values of 3.94 and 4.30, respectively were reported for Algerian honeys. Also, average pH values ranging between 3.6 and 4.32 were reported for Argentine, Spanish, and Italian honeys [8,19]. In

Parameter	Adamawa	Bauchi	Borno	Gombe	Taraba	Yobe
pH	4.53 ± 0.12	4.07 ± 0.06	4.33 ± 0.35	4.17 ± 0.67	3.67 ± 0.21	3.77 ± 0.12
EC (Ms/cm)	0.14 ± 0.04	0.21 ± 0.13	0.12 ± 0.01	0.12 ± 0.02	0.26 ± 0.13	0.06 ± 0.01
Free acidity (meq/kg)	13.73^{a} ± 0.70	24.33^{b} ± 0.76	18.40^{b} ± 1.83	27.73^{c} ± 1.22	21.93^{b} ± 1.14	31.87^{d} ± 1.80
Lactone acidity(meq/kg)	1.58^{a} ± 0.35	2.51^{a} ± 0.19	1.85^{a} ± 0.09	3.64^{b} ± 0.87	1.36^{a} ± 0.34	2.70^{a} ± 0.38
Total acidity (meq/kg)	15.31^{a} ± 0.92	26.84^{b} ± 0.81	20.25^{c} ± 1.87	31.38^{d} ± 1.51	22.63^{c} ± 0.41	34.63^{d} ± 2.18
HMF (Mg/kg)	5.99^{a} ± 0.75	14.47^{b} ± 2.29	8.23^{a} ± 0.75	13.21^{b} ± 2.63	11.98^{b} ± 1.98	16.47^{b} ± 0.75
Diastase (Schade unit)	10.97 ± 0.61	8.50 ± 0.49	10.33 ± 2.52	8.67 ± 0.40	8.83 ± 0.73	8.92 ± 0.85

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

Table 1: Physicochemical parameters in honey samples from the six states in Northeastern Nigeria.

Parameters	Mean ± SD	Range of values (Min – Max)	Limits of Int'l Standards	Samples outside the Limits of International Std.
pH	4.08 ± 0.41	3.50 – 4.90	No fixed limit	–
Conductivity (mS/cm)	0.15 ± 0.09	0.05 – 0.41	Not > 0.8 mS/cm	–
Free acidity (meq/kg)	23.00 ± 6.20	13.00 – 33.60	Not > 50 meq/kg	–
Lactose acidity (mg/g)	2.28 ± 0.89	1.16 – 4.63	No fixed limit	–
Total acidity (mg/kg)	25.17 ± 6.86	14.25 – 36.67	No fixed limit	–
HMF (mg/kg)	11.73 ± 3.97	5.99 – 17.22	Not > 40 mg/kg	–
Diastase activity (Schade unit)	9.37 ± 1.38	8.00 – 13.00	Not < 8 Schade unit	–

Table 2: Physicochemical parameters of eighteen (18) honey samples from Northeastern Nigeria.

		ORGANISM				
Honey Samples	**Location**	**Bacteria**	**Total count x10²**	**Mould**	**Total Count x10²**	**Yeast**
Adamawa	Ganye	+(*Bacillus* spp)	<10	+(*Aspergillus niger*)	<10	N.D
	Hong	+(*Bacillus* spp)	<10	+(*A. niger*, *Scopulariopsis fusca*)	<10	N.D
	Tongo	+(*Kliebsiella* spp)	<10	+(*Mucor racemosus*, *A. niger*	<10	N.D
Bauchi	Alkaleri	+(*Staph aureus*)	<10	+(*A. niger*)	<10	N.D
	Bauchi 1	-		+(*A. niger*, *Rizopus. stolinifer*)	<10	N.D
	Bauchi 2	+(*Bacillus* spp)	<10	+(*A. niger*)	<10	N.D
Borno	Askira-Uba	+(*Bacillus* spp)	<10	+(*A. niger*)	<10	N.D
	Kwajaffa	+(*Bacillus* spp)	<10	+(*A. niger*)	<10	N.D
	Shani	-		+(*Erothium* spp)	<10	N.D
Gombe	Billiri	+(*Bacillus* spp)	<10	+(*A niger*)	<10	N.D
	Gombe Town	+(*Bacillus* spp)	<10	+(*Rhizopus stolinifer*)	<10	N.D
	Shongom	-		+(*A. niger*, A. *flavus*)	<10	N.D
Taraba	Bali	+(*Bacillus* spp)	<10	+(*A. niger*)	<10	N.D
	Takum	+(*Bacillus* spp)	<10	+(*A. niger*)	<10	N.D
	Yerro	+(*Bacillus* spp)	<10	+(*A. niger*, *Mucor* spp)	<10	N.D
Yobe	Degubi	+(*Bacillus* spp)	<10	+(*A. niger*)	<10	N.D
	Dumbulwa	+(*Bacillus* spp)	<10	+(*A. niger*)	<10	N.D
	Gaji	+(*Bacillus* spp)	<10	+(*A. niger*, *R. stolinifer*)	<10	N.D

Key: +indicates present, – indicates absent, N.D=Not-detected

Table 3: Microorganisms isolated from honey samples from State of Northern Nigeria.

Microorganisms	**Frequency of microbes in samples (n=18)**
Bacteria	
Bacillus spp.	13 (72.2%)
Kliebsiella spp.	1 (5.5%)
Staphylococcus aureus	1 (5.6%)
Mould	
Aspergillus spp.	16 (88.9%)
Rhizopus spp.	3 (16.7)
Scopulariopsis spp.	1 (5.6%)
Mucor spp.	2 (11.1)
Erothium spp.	1 (5.6%)

Figures in parentheses represent the percentage of samples contaminated with each species of microorganisms

Table 4: Distribution of microbes in the honey samples from Northeastern Nigeria.

a study of some commercial Nigerian honeys from the southern parts, Omafuvbe and Akanbi [10] reported a pH range of 3.61 to 4.05 with an average of 3.86.

Thus, with respect to pH, the honey samples analysed in this study compare favourably with honey samples from other geographical locations. Although there is no fixed limit of National and International standards for pH, this parameter is of significance in honey quality assessment. Most honeys are acidic with their pH values<7.0. The pH of blossom honeys generally varies between 3.2 and 4.6 [16,21], with chestnut honey being an exception in having relatively high pH values of 5.0 to 6.0. Honeydew honeys have high pH values varying between 4.5 and 6.5, due mainly to the fact that their mineral contents are higher than floral honey. Honey is a buffer and, therefore, its pH does not change by the addition of small quantities of acids or bases [21]. The buffer capacity of honey is due to the content of phosphate, carbonate and other mineral salts.

According to Leveen et al. [22], the acidic pH of honey is desirable because acidification promotes wound healing by causing oxygen release from haemoglobin. In addition, the pH of honey is low enough to prevent growth of many species of bacteria on wounds. Honey pH can provide a good indication of its botanical origin and it can also be used for the prediction of honey degradation during storage. Honeys with pH ranging from 3.5 to 4.5 are said to originate from nectar [18]. The electrical conductivities of the honey samples were not significantly different from each other. The conductivity of honey samples depends on their ash and acid contents, i.e., the higher the ash and acid contents, the higher the conductivity [20,21]. Indeed, Piazza et al. [23] had demonstrated a linear relationship between the ash content and electrical conductivity in Italian unifloral honeys described by the equation, C=0.14 + 74A, where C denotes the electrical conductivity in milliSiemens per cm and A denotes the ash content in g/100 g.

The conductivities of the honey samples analysed in this study varied between 0.05 and 0.41 with an average value of 0.15 ± 0.02. The international norm specified by both Codex Alimentarius Commission and European Council (EU) established values of<0.8 mS/cm for blossom honeys or blends (mixtures) of blossom honeys and >0.8 mS/cm for honeydew honeys. The conductivity data in this study shows that all the samples fall within the range required by the international standard. The results also indicate that all the samples from the different locations are of floral botanical origin. Conductivity is good criterion for determining botanical origin of honey and today it is determined in routine honey quality control instead of the ash content [20]. On comparative basis the conductivity data obtained in this work are higher (P<0.05) than those reported for some commercial Nigerian

Sample source	Microbial count (x 10^2 cfu/g)				
	SPC	Bacillus spp.	Total coliforms	Yeast	Mould
Adamawa	1.7×10^2	1.7×10^2	ND	ND	ND
	1.2×10^2	1.2×10^2	✓	✓	✓
	1.3×10^2	1.6×10^2	✓	✓	✓
Bauchi	1.3×10^2	1.3×10^2	✓	✓	✓
	1.0×10^2	1.0×10^2	✓	✓	✓
	1.8×10^2	1.8×10^2	✓	✓	✓
Borno	1.2×10^2	1.2×10^2	✓	✓	✓
	2.0×10^2	2.0×10^2	✓	✓	✓
	3.8×10^2	3.8×10^2	✓	✓	✓
Gombe	1.3×10^2	1.3×10^2	✓	✓	✓
	1.1×10^2	1.1×10^2	✓	✓	✓
	0.5×10^2	0.5×10^2	✓	✓	✓
Taraba	1.9×10^2	1.9×10^2	✓	✓	✓
	0.3×10^2	0.3×10^2	✓	✓	✓
	1.6×10^2	1.6×10^2	✓	✓	✓
Yobe	1.1×10^2	1.1×10^2	✓	✓	✓
	0.8×10^2	0.8×10^2	✓	✓	✓
	1.4×10^2	1.4×10^2	✓	✓	✓

ND=Not Detectable

Table 5: Microbial profiles of honey samples from Northeastern Nigeria after some months of storage.

honeys; 0.02 to 0.06 mS/cm with an average of 0.03 ± 0.009 mS/cm [10], but consistent with values reported for Algerian honeys, 0.21 to 1.24 mS/cm with an average of 0.65 ± 0.21 mS/cm [17] and Tunisian honeys, 0.31 to 0.62 mS/cm, with an average of 0.43 ± 0.11 [24].

The results of free acidity, lactone, and total acidities of the samples analysed indicated significant variations among samples from some of the States in the sub-region. However, all the samples had their acidity values within the limits of<50 meq/kg as specified by the international standards [2]. Various researchers have reported varied values of acidities for different honeys from different locations around the world [8,17,19,24-31]. White and Doner [3] reported free acidity, lactone acidity, and total acidity in the average of 22.03 meq/kg; 7.11 meq/kg and 29.12 meq/kg, respectively for 490 honey samples. The average values of free acidity, lactone acidity and total acidity of some commercial Nigeria honeys from the south were reported to be 27.00 meq/kg, 10.55 meg/kg and 37.20 meq/kg, respectively [10]. In this study, the average values obtained for free acidity (23.00 meq/kg) are in line with the observation of White and Doner [3] and Omafuvbe and Akanbi [10]. This study also reveals that the free acidity predominates over the lactone acidity in all the samples analysed; similar observations were previously reported by other scientists [3,8,10,25]. The acid content of honey is relatively low, but it is an important factor for the honey taste [21]. Most of the honey acids are added by the bees [32]; the main honey acid is gluconic acid, which is a product of glucose oxidation by the enzyme glucose oxidase. The gluconic acid is, however, present as its own internal ester, which is a lactone and it does not contribute to honey's active acidity [21]. Several organic acids including formic acid, acetic acid, citric acid, lactic acid, maleic acid, malic acid, oxalic acid, pyeoyhtamic acid and succinic acid have been found in minor quantities in honey [33]. On the whole, the acids of honey account for less than 0.5 percent of the solids, but this level contributes to the flavour and is also partly responsible for the excellent stability of honey against microorganisms [3,34].

The contents of 5-Hydroxmethylfurfural (HMF) in the honey samples studied indicate significant ($P<0.05$) differences between samples of some States within the sub-region. The average HMF contents of the honey samples varied between 5.99 ± 0.75 mg/kg for samples from Adamawa State and 4.47 ± 2.29 for samples from Bauchi State. In general, the HMF content of all the samples varied between 5.24 mg/kg to 17.22 mg/kg with an average value of 11.73 ± 3.97 mg/kg. Vorlova and Pridal [35] had reported HMF contents ranging from 0 to 15.40 mg/kg, 1.60 to 49.30 mg/kg, 1.40 to 10.30 mg/kg and 0 to 11.30 mg/kg with average values of 3.89 mg/kg, 17.34 mg/kg, 4.55 mg/kg and 3.92 mg/kg, respectively for fresh, commercial, compound and honeydew honeys of Czech Province. The HMF contents obtained in this study are also similar to those reported for honeys produced in Tunisia [24], Pakistan [26], Argentina [8,9,19] and Algeria [17]. The results of this study also show that all the honey samples analysed had HMF contents within the specification of international standards; i.e., not more than 40 mg/kg [1].

HMF is a decomposition product of fructose and it is found only in trace amount in fresh honey [21]. However, its concentration increases with storage and prolonged heating of honey. Other factors including pH, storage temperature, moisture, acidity, metals, amino acid and simple sugars (glucose and fructose) are known to increase HMF

contents of honey [21,36]. The HMF content is used as an indicator for freshness and overheating of honey. The Codex Alimentarius Commission and European Union norm is a maximum of 40 mg/kg, while for honeys from the tropics and blends of honey with those from the tropics, the maximum values of HMF has been fixed at 80mg/kg. However, in some countries like Italy, Germany, Finland, Switzerland, etc, Beekeeping Organisations have set a maximum limit of 15 mg/kg for specially labeled 'quality' or 'virgin' honeys [21].

The diastase activity, calculated as Diastase Number (DN) ranged from 8.00 to 13.00 with an average value of 9.06 ± 0.39. The average values of DN obtained for samples from the different States showed slight but insignificant differences. Measurement of diastase and invertase activities in honey is important because these two enzymes play an important role in judging honey quality and are used as indicators of honey freshness [21]. A maximum value of 8 diastase units has been set by the Codex Alimentarius Commission and the European Honey Directive as the acceptable limit for honey in the international market. The activities of diastase and invertase are gradually eroded on storage and heating of honey. The invertase, being more susceptible to damage by storage and heat, is used in some countries as an indicator for honey virginity and freshness; i.e., fresh and virgin honeys are supposed to have at least 10 Hardon invertase units or 64 international units, while honeys with low enzyme activity should have at least 4 units [21]. However, the diastase and invertase activities in honey vary in wide limits depending on botanical origin of honey [37,38] and thus, have a limited freshness indicating power; HMF is regarded as better quality criterion in this respect.

Several authors have reported diastase activities of different types of honeys from different parts of the world. Diastase activities in honeys varying from 2.1 to 61.2 DN with average of 20.8 DN [3], 4.0 to 40.0 DN, with average of 17.4 DN [17]; 10.07 to 41.04 DN, with an average of 19.73 DN [8]; 3.0 to 39.6 DN, with average of 17.67 DN [24]; 8.54 to 13.04 DN, with average of 10.23 DN [25]; 14.50 to 24.25 DN, with average of 17.91 DN [29] have been reported for different samples around the world.

Also in Czech Province, Vorlova and Pridal [35] reported diastase activity ranging from 11.2 to 45.4 DN, with an average of 24.3 ± 1.5 DN for fresh honey samples; 10.9 to 17.8 DN, with an average of 13.6 ± 0.5 DN for commercial samples; 11.24 to 30.3 DN, with an average of 18.2 ± 1.6 DN for blossom (floral) honey; 15.9 to 40.3 DN, with an average of 29.4 ± 1.9 DN for compound honey; and 13.6 to 45.4 DN, with an average of 24.6 ± 3.8 DN for honeydew honey. These reports revealed that for the various types of fresh honeys, the lowest value of diastase activity was found in blossom honeys while honeydew honey had the highest diastase activity. Average diastase activity (DN) of some branded honeys obtained from Pakistani markets were reported as follows [26]: Langnese honey (15.58 DN), Salman honey (12.43 DN), Handard honey (13.04 DN), Marhaba honey (9.17 DN), and French honey (10.19 DN). More recently, Cornelia and Chis [39] reported that diastase activity (DN) varied between 12.3 and 24.6, with an average of 17.82 ± 4.63 DN for polyfloral honey; 8.3 and 10.8, with average of 9.48 ± 0.94 DN for Acacia honey and 6.4 to 20.2, with an average of 14.38 ± 6.95 DN for Linden honey. The values of diastase activity (DN) obtained in the present investigation are within the range reported for some honey samples from Pakistan [25,26] and for Acacia honey [39]. In addition, diastase activity obtained in this study for all the honey samples fall within the limits of>8.0 specified by international standards, suggesting that all the samples are of acceptable international quality, with respect to diastase activity.

The results of microbiological examination of the honey samples showed presence of both bacteria and mould at the initial stage, although their counts were less than 10 cfu/g. This observation is similar to those reported for samples from West Cameroon [40] and samples from Ibadan [41]. The low microbial counts of less than 10 cfu/g observed in this study correspond to values reported for some Moroccan honeys [42]. Yeasts and spore-forming bacteria are primarily the microorganisms of concern in honey; their total plate counts from honey samples may vary from zero to tens of thousands per gram for no apparent reason [43,44]. The presence of only *Bacillus spp*, in very low counts (Table 4), in the honey samples following some months of storage lends credence to suggestions that some fungi and spore forming bacteria may be present in honey for a limited period of time [40,43,44]. Bacteria do not replicate in honey and such high number of vegetative forms may indicate recent contaminations from a secondary source [43]. Honey also has antimicrobial properties that inhibit the growth or persistence of many microorganisms; therefore, it can be expected to contain low number and a limited variety of microorganisms

Bacterial spores particularly those in the genus *Bacillus* are regularly found in honey [43]; however, no vegetative forms of disease causing bacterial species have been found in honey. *Bacillus spp* are among the main spoilage organism in foods and food products due to their versatile metabolism and heat resistant spores, but they have not been associated with food poisoning [45]. The organism is generally not a hazard at the very low levels normally present in food; ingestion of more than 10^5 cells/g of food is required to produce illness [46]. The *Bacillus* counts observed in the present study are very low and comparable to values obtained for honey samples from southern Nigeria [10,41]. Honey is a very concentrated sugar solution with a high osmotic pressure making it less suitable for microbial growth; hence, it contains fewer microorganisms than other natural foods. The *Bacillus* bacteria contained in honeys are not toxic to human beings, but are rather toxic to the bees [21]. Thus, a number of bacteria are present in honey but most of them are harmless to man. The principal sources of microorganisms in honey are the nectar of the flowers and the honey bee. For instance, yeasts have been shown to come from the nectar as well as from intestinal contents of the honey bee [47].

The presence of moulds in food usually indicates microbial contamination due to lack of clean materials and hygiene at work and growth of the moulds in the food can lead to alteration in the nutritional values, thereby producing undesirable flavour and sensory characteristic. Some of the moulds (fungi) produce mycotoxins which diffuse in food matrix and cause acute or chronic poisoning to consumers. The presences of some moulds as earlier observed in this study may be due to lack of hygienic procedures during harvesting, packaging and/or storage of the honey samples. Moulds are generally known to thrive in samples with low water contents (between 16.2 and 17.0%) because these organisms are xerophiles [18]. The observation of the absence of yeast growth in this study may be attributed to the low moisture contents of the honey samples, since it was reported that the easiest way to control honey fermentation which results from the growth of yeast is to harvest honey with low humidity and also store it in air tight vessels [21].

Conclusion

Low pH values, conductivities, acidities, diastase activities and HMF contents, which indicate honey freshness and good conservation,

were observed in all the samples. In addition, all the samples generally conformed to quality standards specified by international honey regulation. It was thus concluded that honey samples from northeast Nigeria are of good quality and can meet international standards. The presence of mould and bacteria in some of the samples may be attributed mainly to contamination due to poor handling at harvest, packaging or storage.

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