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EVALUATION OF THE PHYSICOCHEMICAL PROPERTIES AND POLLEN CONTENT OF HONEYSAMPLES FROM EBONYI STATE, NIGERIA

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Abstract: The present study was carried out to determine the pollen content and quality of honey samples sourced from different localities (Onicha, Ivo and Ohaozara) in Ebonyi State in 2017. Physicochemical analyses of the samples were carried out using the official methods of analysis of the Association of Analytical Chemists and the pollen analysis was done using the acetolysis method. The ranges of different parameters are 10.80 - 11.72% moisture; 0.00 - 0.50% fat; 0.37 - 0.52% protein; 0.23 - 0.40% ash; 5.00 - 10.00% polyphenol; 56.00 - 81.20 meq/kg free acidity; 4.0 - 4.3 pH; 12.80 - 42.67 mg/kg hydroxymethylfurfural (HMF); 47.70 - 49.40 mg/100 g fructose; 23.80 - 36.90 mg/100 g glucose and 4.18 - 5.50% sucrose. The ranges of mineral contents are 3100 - 5350 mg/100 g zinc; 48.70 - 68.50 mg/100 g calcium; 3.90 - 5.50 mg/100 g magnesium; 2.06 - 2.97 mg/100 g potassium and 0.30 - 0.38 mg/100 g sodium. Evaluation of the physicochemical composition of the honey samples indicated that most of the tested parameters meet international standard, with the exception of free acidity, which recorded values beyond the recommended standard. Palynological study revealed that the honey samples were both monofloral and multifloral. A total of 30 pollen types belonging to 24 families of plants were recorded in the study. Important honey plants that were identified in this study include *Elaeis guineensis, Syzygium guineense, Alchornea cordifolia and Nauclea latifolia* etc.

Keywords: Pollen types, Physicochemical properties, palynological study, honey plant, mineral content.

Introduction

Honey, a natural sweetener known all over the world, is produced by honey bees from the nectars of plant flowers (Codex Alimentarius, 2001). Honey is the most common and economically important product of the honey bee colony. It is a complex mixture of minerals, sugars, vitamins and other phytochemicals. Its composition and colour is dependent on the type of flower that supplies the nectar. The percentage compositions of the physicochemical properties, such as hydroxymethylfurfural (HMF), simple sugars, moisture, free acidity and ash content present in honey, are used in accessing the quality of honey. These properties also influence the demand and commercial value of such honey (Kayode & Oyeyemi, 2014). Previous studies on the pollen content of honey samples from various parts of Nigeria and outside Nigeria have been conducted to determine the important floral sources utilised by bees in honey production. Agwu *et al*. (2013) used pollen in honey to evaluate the ecological origin, floral sources and production season of four honey samples obtained from four localities in Kogi State, Nigeria. Also, Wahizatul *et al*. (2015) identified the pollen grains in honey samples from a Besut apiary in Terengganu from November 2012 until February, 2013 in order to determine the foraging activity of the stingless bee, *Lepidotrigona terminata*. Similarly, the pollen spectra of seven honey samples sourced from Nsukka, Enugu State, Nigeria investigated by Njokuocha and Osayi (2015) revealed various pollen types from different families of plants, which include Arecaceae, Euphorbiaceae, Myrtaceae, Rubiaceae and Combretaceae/Melastomataceae. Oyeyemi and Kayode (2017) identified pollen grains in the families of Fabaceae, Asteraceae, Arecaceae, Hymenocardiaceae and phyllantaceae from the

palynological characteriaation of honey samples from Kwara State, Nigeria. Thus, pollen analysis can provide significant information on honey characterisation with reference to the occurrence of honey plants, botanical origin and season of honey collection.

Likewise, scientific reports on the physicochemical constituents (Njokuocha & Osayi, 2015; Njokuocha *et al.*, 2019) and antimicrobial properties (Eleazu *et al*., 2013; Azonwade *et al*., 2018; Hocine *et al*., 2018; Pajor *et al*., 2018) of some honey produced in Nigeria and outside Nigeria have shown that honey contains some important nutritive materials in addition to its antimicrobial potential. Adenekan *et al.* (2010) revealed that honey has antimicrobial properties that can delay or inhibit growth of many microbes through the analysis of the chemical constituents and microbial qualities of honey samples collected from Ibadan.

Similar studies on the physicochemical composition. as well as the pollen content, of honey samples from Ebonyi State have been conducted by some authors to ascertain the quality and floral sources of the honey samples (Nnamani & Uguru, 2013; Nwoko *et al.*, 2017). Despite the aforementioned studies, there is yet limited knowledge of the physicochemical properties, bee foraged plants and nectar flow from this area. Hence, the present study is a further contribution to the already existing information on honey samples from this area. This study, therefore, evaluated the physicochemical composition, the mineral constituents and the pollen content of honey samples from Ebonyi State in order to determine their nutritional quality and the floral sources utilised by the bees in the honey production.

Materials and Methods

Collection of Samples

The honey samples used for this study were collected during the month of August 2017 from a villager who gathered them from the wild from the different local governments (Ivo, Ohaozara and Onicha) in Ebonyi State. All the honey samples were stored in containers with tight-fitting lids during the period of analysis.

Physicochemical Analyses

Physicochemical analyses, including polyphenol, free acidity, pH level, moisture, ash, fat, protein and sugar types, were determined according to the protocols as detailed in A.O.A.C (2012). whereas hydroxymethylfurfural (HMF) was determined according to the methods of White (1979).

Determination of Moisture

Ten grammes (10 g) of each honey sample were obtained. The samples were placed in the silica dish and dried in an oven (Heraeus 064054, Hanau, Germany) at 100^0C until the weight was constant.

The percentage moisture content was calculated as follows:

$$\text{Percentage (\%) moisture content} = B\text{-}C/A \times 100$$

where A = sample weight before drying; B = weight of silica dish + sample prior to drying; C = weight of silica dish + sample after drying.

Determination of Protein

The total nitrogen (TN) was determined using the Kjeldahl method, from which the crude protein could be calculated as % N x 6.25. Two grammes (2 g) of the honey sample were weighed using a weighing balance (Mettler Toledo PL 303, Colmworth, UK). Five grammes (5 g) of anhydrous sodium sulphate, 1 g of copper sulphate and a speck of selenium were added to it. Twenty-five millilitres (25 mL) of concentrated sulphuric acid was added into the mixture. The ammonia liberated during digestion was collected with 10 mL of a boric acid indicator mixture. When the boric acid indicator mixture turned green, the distillation was allowed to stay for five minutes. Then, the flask was removed and titrated with 0.01N hydrochloric acid.

Determination of Fat

Two grammes (2 g) of the samples were weighed using a weighing balance (Mettler Toledo PL 303, Colmworth, UK) and transferred into a rolled filter paper and placed inside the extractor thimble, which was put inside the Soxhlet extractor. Two hundred and fifty millilitres (250 mL) of petroleum ether was added to the flask. The apparatus was set up, heated to 80°C and allowed to run for 4 hours. The ether was recovered at the end of the extraction before the thimble was removed. The oil was collected and dried in the oven (Heraeus 064054, Hanau, Germany), cooled and finally weighed. The fat content was expressed as a percentage of the raw material.

The percentage fat was calculated using the following formula:

$$\text{Percentage (\%) fat} = C\text{-}A/B \times 100$$

where A = weight of empty flask; B = weight of sample; C = weight of flask + oil.

Determination of Ash

Five grammes (5 g) of the honey samples were transferred into a crucible and weighed. Pre-ashing was done using an electric heater in a Fume Hood (Labe 1286, Delhi, India). The crucible was placed in a muffle furnace (Bentex Rx, Ambala Haryana, India) at 600^0C. This temperature was maintained until a whitish-grey colour was obtained.

The percentage ash was calculated as:

$$\text{Percentage (\%) ash content} = C - A/B \times 100$$

where A = Weight of dish and ashed sample; B = Weight of the fresh sample; C = Weight of dish and fresh sample.

Determination of Free acidity

Ten grammes (10 g) of the honey samples were weighed using a weighing balance (Mettler Toledo PL 303, Colmworth, UK) and titrated with 0.1 N NaOH using one millilitre (1 mL) of phenolphthalein indicator. The volume of alkali used was recorded from the burette reading and this was used in determining the free acidity.

$$\frac{0.1\times (\text{Final Titre value} - \text{Initial Titre value}) \times 100}{\text{Vol. of sample used}}$$

Determination of Hydroxymethylfurfural (HMF)

Five grammes (5 g) of the honey samples were measured and dissolved in twenty-five millilitres (25 mL) f deionised water. 0.5 mL of Carrez solution 1 (150 mg/ mL potassiumferrocyanide) as well as 0.5 mL of Carrez solution 11 (300 mg/mL zinc acetate) were added to the sample and mixed well. The samples were brought to a final volume of 50 mL with deionised water and a drop of alcohol was used to suppress the surface foam. A reference sample was prepared by adding 5 mL of 0.20% sodium bisulfate to one test tube of filtrate and the test samples were prepared by adding 5 mL of deionised water to the other test tube of filtrate. The absorbance of the test samples was measured against the reference sample at 284 nm and 336 nm using the spectrophotometric method (White, 1979).

The calculation of HMF was done using the following formula:

$$\text{HMF (mg/100 g of honey)} = \frac{(A_{284} - A_{336}) \times \text{Factor}}{W}$$

Determination of Polyphenol

Five millilitres (5 mL) of the samples were measured. 1 mL of 1% $FeCl_3$ (Iron II Chloride) was added to it. This was followed by the addition of 1mL of 1% potassium iron cyanide 1% $K(Fe(CN)_6$. It was allowed to stand for 30 minutes, and the absorbance was read at 490 nm with a spectrophotometer (AE - IID, Ningbo, Zhejiang, China).

$$\frac{\text{Concentration of sample} = \text{Abs. of sample} - \text{Conc. of Std.}}{\text{Abs. Std.}}$$

pH Determination

The pH of the samples was determined with a pH meter (Jenway, model 3510, Staffordshire, Uk). Ten grammes (10 g) of the honey samples were measured and dissolved in 75 mlL of distilled water. It was allowed to stand for 30 minutes. The pH meter was inserted into the

honey samples and retained for a while until the reading was steady. The reading was recorded from the display.

Colour Determination

Ten grammes (10 g) of each of the honey samples was weighed with a Fuzhou Furi electronic balance (FEJ-600, Fujian, China) and diluted with 35 mL of warm acidified water (3 mL of concentrated sulphuric acid plus 997 mL of warm distilled water). The colour of the honey samples was determined by comparing the samples with the Munsel colour chart (Agwu and Akanbi, 1985).

Determination of Sugar

The fructose, glucose and sucrose contents of the honey samples were analysed according to the methods of A.O.A.C (2012).

Determination of Fructose

This test was carried out by measuring 0.1 mL of the samples into a test tube. 5.9 m: of distilled water was added to dissolve it. This was followed by the addition of 2 mL of 2% $ZnSO_4$ solution and 4% NaOH. The tube was placed in near boiling water in a water bath at 90°C for two minutes and it was filtered hot with filter paper. 2 mL of the solution of (0.1% resorcinol solution in 95% ethanol) and 6 mL of 30% HCl solution were added into 2 mL of the filtrate. This was mixed and placed in a water bath at 80°C for 15 minutes and the absorbance was read using a spectrophotometer (AE - IID, Ningbo, Zhejiang, China) at 520 nm.

Determination of Glucose

Two millilitres (2 mL) of the samples were measured and transferred into a beaker. Two millilitres (2 mL) of phenol were added followed by 1 mL of concentrated H_2SO_4. Two millilitres (2 mL) of water were added to dilute it and the absorbance was read at 549 nm with a spectrophotometer (AE - IID, Ningbo, Zhejiang, China).

Determination of Sucrose

One gramme (1 g) of each of the honey samples was weighed using a weighing balance (Mettler Toledo PL 303, Colmworth, UK) and dissolved in 50 mL of distilled water. Into 1ml of each of the filtrate, 5 mL of 2% Oscinol was added and boiled for 12 minutes. It was made up to 25 mL with distilled water and the absorbance was read with a spectrophotometer (AE - IID, Ningbo, Zhejiang, China) at 620 nm.

Determination of Mineral Composition of Honey

The mineral compositions of the various honey samples were determined following the methods of A.O.A.C. (2010) and Pearson (1976).

Determination of Calcium

A pinch of calcine indicator was added into twenty-five millilitres (25 ml) of the digested sample. Two millilitres (2 ml) of NaOH solution was also added and the mixture was titrated with 0.01N Ethylenediaminetetraacetic acid (EDTA) solution.

$$(Ca/L)/PPM\ ca = \frac{T \times M \times E \times 1000}{\text{Volume of sample used}}$$

Where T = Titre value; M = Molarity of EDTA; E = Equivalent weight of calcium

Determination of Magnesium

A pinch of EBT (Eriochrome Black - T) was added into twenty-five millilitres (25 mL) of the digested sample and shaken. This was followed by the addition of 2 mL of ammonium buffer solution. The mixture was titrated using 0.01N EDTA.

$$Mg\ /PPM = \frac{T \times M \times E \times 1000}{\text{Volume of sample used}}$$

Where T = Titre value; M = Molarity of the standard EDTA; E = Equivalent weight of magnesium.

Determination of Sodium

Two grammes (2 g) of the samples were digested and dissolved with ten millilitres (10 mL) of 30% HCl and aliquots of the digest sample was taken for photometry using flame photometer (Jenway PFP 7, Staffordshire, UK). Sodium concentration was obtained from the calibration curves obtained from the standard.

Determination of Potassium

Two grammes (2 g) of the sample was digested and dissolved with ten mililitres (10 mL) of 30% HCl. Aliquot of the digest sample was taken for photometry using flame photometer (Jenway PFP 7, Staffordshire, UK). Potassium concentration was obtained from the calibration curves obtained from the standard.

Determination of Lead

Ten millilitres (10 mL) of the digested samples were pipetted into a flask. Five millilitres (5 mL) of deionised water were added and this was followed by the addition of 0.5 mL of phenolphthalein indicator. Twenty millilitres (20 mL) of hydrazine acetate and sodium tartrate solutions were also added. The solution was extracted with 10 mL of $CHCl_3$ (Chloroform) and the $CHCl_3$ layer was discarded. Ten millilitres (10 mL) of KCN (potassium cyanide) solution were added and the pH adjusted to 8.5. The lead was extracted with 5 mL dithizone solution (II). Absorbance was read with a spectrophotometer (AE - IID, Ningbo, Zhejiang, China) at 520 nm and the concentrations of the samples were extrapolated.

Determination of Cadmium

Twenty-five millilitres (25 mL) of the digested samples were pipetted into a flask. One millilitre (1 mL) of potassium tartrate solution was added followed by 5 mL of NaOH-KCN (sodium hydroxide potassium cyanide) solution (1) and one millilitre (1 mL) of hydroxylamine hydrochloride solution. To the filtrate, 2.5 mL of cold tartaric acid solution was added, followed by 10 mL of chloroform and then shaken for two minutes and the chloroform layer discarded. 0.25 mL hydroxylamine hydrochloride solution was added to the remaining solution, followed by 15 mL standard dithizone solution (II). The standard and the blank were prepared, and the absorbance was read at 518 nm with a spectrophotometer (AE - IID, Ningbo, Zhejiang, China).

Determination of Zinc

To ten millilitres (10 mL) of digested samples, 5 mL of acetate buffer and 10 mL of sodium thiosulphate solution were added and mixed. Ten millilitres (10 mL) of dithizone solution (II) was added and shaken vigorously for five minutes. The chloroform layer was decanted into a cuvette and the absorbance was read with a spectrophotometer (AE - IID, Ningbo, Zhejiang, China) at 535 nm. The standard and blank were prepared, and the absorbance was read at 535 nm (Pearson, 1976).

Data Analysis

Data from the physicochemical and mineral composition were subjected to one-way Analysis of Variance (A.N.O.V.A.), while significant means were separated using least significant differences (L.S.D.) at $p \leq 0.05$.

Pollen Analysis

Each of the honey samples was thoroughly shaken to ensure distribution of botanical elements. To dissolve the colloidal matters and sugars, ten grammes (10 g) of each sample was weighed using a Fuzhou Furi electronic balance (FEJ-600, Fujian, China) and diluted with 50 mL of warm acidified water (3 mL of concentrated sulpuric acid to 997 mL of distilled water). This was followed by centrifugation for 10 minutes at 2500 revolution per minute, after which the supernatant was decanted and the sediment retained (Agwu & Akanbi, 1985). Acetolysis mixture was freshly prepared from acetic anhydride and concentrated sulphuric acid in a ratio of 9:1 and the recovered sediments were acetolysed following the procedure of Erdtman (1969). The recovered pollen weight for each

of the samples was determined. Two drops of glycerol-alcohol was added to the polliniferous residue and stored in vial bottles, from where samples were taken for microscopy. The pollen frequency classes were determined using the classification of Jones and Bryant (2004); predominant (> 45%), secondary (16 - 45%), important minor (3 - 15%) and minor (< 3%).

Pollen grains identification was aided by photomicrographs in books and journals, like (Y'bert, 1979; Bonnefille & Riolett, 1980; Gosling *et al*., 2013), as well as some of the permanent pollen slides stored in the Palynology Research Unit, Department of Plant Science and Biotechnology, University of Nigeria Nsukka.

Results

Physicochemical Composition

Table 1 shows the mean values of the physicochemical constituents of the honey samples from the three locations. Significant differences in moisture and ash content were observed among the honey samples. The mean moisture content of the honey sample from the Ivo Local Government (10.80±0.16%) differed significantly at $p \leq 0.05$ from that of the Ohaozara Local Government (11.50±0.11%) and the Onicha Local Government (11.72±0.13%). Likewise, the mean ash content of honey sample from Ivo (0.40±0.03%) was significantly different from that of the Onicha and Ohaozarra honey samples. Fat was not present in the honey sample from Ivo though it was recorded in trace amount in honey samples from the Onicha and Ohaozara Local Governments. The mean values of polyphenol recorded in Onicha (10.00±0.00%) and Ivo (9.25±0.4%) honey samples were the highest and significantly different at $P \leq 0.05$ from that of the Ohaozara honey sample, having the value of 5.00±0.00%. Significant differences in free acidity contents were observed amongst the three honey samples (Table 1).

Sugar and Mineral Composition

The result of sugar analysis showed that there is no significant difference in the mean values of glucose (23.80±0.00 mg/100g) recorded in the honey samples from Ohaozara and Onicha. However, they differed significantly at $P \leq 0.05$ from that of the Ivo honey sample (36.90±1.10

Table 1: Mean values of the physicochemical compositions of honey samples from the three locations

	Ivo	Ohaozara	Onicha	LSD
Moisture (%)	10.80 ± 0.16^{b}	11.50 ± 0.11^{a}	11.72 ± 0.13^{a}	0.47
Fat (%)	0.00 ± 0.00^{b}	0.10 ± 0.06^{b}	0.50 ± 0.06^{a}	0.16
Ash (%)	0.40 ± 0.03^{a}	0.30 ± 0.00^{b}	0.23 ± 0.01^{b}	0.07
Free acidity (meq/kg)	56.00 ± 6.47^{c}	81.20 ± 1.62^{a}	61.60 ± 3.23^{b}	14.80
Polyphenol (%)	9.25 ± 0.43^{a}	5.00 ± 0.00^{b}	10.00 ± 0.00^{a}	0.87
HMF	23.73 ± 0.41^{b}	42.67 ± 2.56^{a}	12.80 ± 1.59^{c}	6.08
pH	4.30 ± 0.00^{a}	4.00 ± 0.00^{a}	4.00 ± 0.00^{a}	NS
Glucose (mg/100g)	36.90 ± 1.10^{a}	23.80 ± 0.00^{b}	23.80 ± 0.00^{b}	0.08
Fructose (mg/ 100g)	47.70 ± 0.17^{b}	49.00 ± 0.35^{a}	49.40 ± 0.12^{a}	1.19
Sucrose (mg/100g)	5.50 ± 0.29^{a}	4.18 ± 0.10^{b}	4.50 ± 0.29^{b}	0.84

*means with different alphabet on the same horizontal array represents significant differences ($P \leq 0.05$)

mg/100 g). Also, the sucrose mean values in honey from Ohaozara and Onicha were not significantly different. The fructose contents of the Onicha and Ohaozara honey samples were statistically similar and significantly different at $p \leq 0.05$ from that of the Ivo sample (47.70±0.17 mg/100 g). The mean value of HMF recorded in honey sample from the Onicha Local Government was the lowest and this was significantly different from mean values of HMF in honey samples collected from the Ivo and Ohaozara Local Governments. There were no significant differences in the mean values of pH recorded in the samples (Table 1).

Among the minerals analysed, zinc, calcium and magnesium recorded the highest values. The values of potassium and sodium were low. Cadmium and lead were not present in the three samples (Table 2).

Pollen Content of the Honey Samples

A total pollen count of three thousand two hundred and two (3,202) was recorded in the study (Table 3). The highest pollen count was recorded in the Onicha honey sample, followed by the Ivo sample and lastly, the Ohaozara sample. Thirty (30) pollen types that belong to 24 families were recorded. Some pollen types were identified to the genus level and they include *Ludwigia stolonifera, Elaeis guineensis, Syzygium guineense, Bridelia ferruginea, Alchornea cordifolia, Nauclea latifolia and Hymenocardia acida*, while others were identified to the family level (Passifloraceae, Amaranthaceae, Sapindaceae, Asteraceae, Moraceae and Celastraceae) (Table 3). One of the honey samples was a monofloral honey (Onicha honey sample). whereas the other two were multifloral honey (Ivo and Ohaozara honey samples). From the frequency class, it was observed that only two pollen types occurred as the predominant types (*Irvingia* sp. and *Elaeis guineensis*), three occurred as secondary (*Elaeis guineensis, Nauclea latifolia* and *Irvingia* sp.) and six occurred as minor classes in the samples. The rest occurred as important minor types (Table 4). Also, the pollen weight of the honey collected from the Ivo Local Government was the highest, followed by the Ohaozara and Onicha samples (Table 5).

The three honey samples differed in their colours. The Ivo sample had a yellow colour, the Ohaozara sample was golden yellow in colour, whereas the Onicha honey sample had an amber colour (Table 5).

Table 2: Mean values of the mineral content of the honey samples from the three locations

	Ivo	**Ohaozara**	**Onicha**	**LSD**
Zn (mg/100g)	3550 ± 0.30^{b}	3100 ± 0.60^{c}	5350 ± 0.90^{a}	215.80
Ca (mg/100g)	68.50 ± 0.14^{a}	52.70 ± 0.31^{b}	48.70 ± 0.08^{b}	6.96
Mg (mg/100g)	5.50 ± 0.12^{a}	4.20 ± 0.23^{b}	3.90 ± 0.06^{b}	0.53
K (mg/100g)	2.97 ± 0.02^{a}	2.19 ± 0.07^{b}	2.06 ± 0.03^{c}	0.02
Na (mg/100g)	0.30 ± 0.00^{b}	0.38 ± 0.00^{a}	0.33 ± 0.01^{b}	0.03
Cd	ND	ND	ND	-
Pb	ND	ND	ND	-

*means with different alphabet on the same horizontal array represents significant differences ($P \leq 0.05$)

Table 3: Pollen count/percentage contribution of different pollen types recorded in honey samples from different locations

S/N	Pollen types	Pollen count (%) Onicha	Pollen count (%) Ivo	Pollen count (%) Ohaozarra
1	Amaranthaceae	2(0.2)	0	0
2	Anacardiaceae	0	0	22(2.8)
	Lannea sp.	1(0.1)	2(0.18)	22(2.8)
	Spondias mombin		0	13(1.6)
3	Arecaceae			
	Elaeis guineensis	294(22.3)	960(86.0)	273(34.6)
	Hyphaene ventricosa	2(0.2)	1(0.1)	0
4	Asteraceae	43(3.3)	1(0.18)	0
5	Bursaraceae			
	Commiphora africana	1(0.1)	0	0
6	Bombacaceae			
	Bombax buonopozense	2(0.2)	0	0
7	Capparidaceae	0	3(0.18)	2(0.3)
8	Celastraceae	1(0.1)	0	3(0.4)
9	Euphorbiaceae	1(0.1)	0	0
	Alchornea cordifolia	1(0.1)	2(0.18)	80(10.1)
10	Fabaceae			
	Caesalpinioideae			
	Piliostigma sp.	1(0.1)	0	0
	Papilionioideae		95(8.7)	6(0.8)
	Indigofera sp.	4(0.3)	3(0.3)	0
11	Hymenocardiaceae			
	Hymenocardia acida	0	0	6(0.8)
	Irvingiaceae			
	Irvingia sp.	729(55.4)	8(0.7)	149(18.9)
12	Lamiaceae	0	0	1(0.1)
13	Liliaceae	7(0.5)	13(1.20	3(0.4)
14	Loranthaceae			
	Tapinanthus aurantiacus	0	0	2(0.3)
15	Moraceae	1(0.1)	0	6(0.8)
16	Myrtaceae			
	Syzygium guineense	132(10.0)	0	17(2.2)
17	Onagraceae			
	Ludwigia stolonifera	1(0.1)	0	0
18	Passifloraceae	51(3.9)	0	0

19	Phyllanthaceae			
	Bridelia ferruginea	9(0.7)	0	0
20	Poaceae	25(1.9)	4(0.4)	2(0.3)
22	Rubiaceae	0	5(0.5)	0
	Nauclea latifolia	0	0	149(18.9)
23	Sapindaceae	1(0.1)	0	33(4.2)
24	Solanaceae			
	Solanum sp.	7(0.5)	0	0
	Total (3,202)	1316	1097	789

Table 4: Frequency class of pollen types recorded in honey samples from different locations

S/N	Pollen types	Onicha	Ivo	Ohaozara
1	Amaranthaceae	Important minor	-	-
2	Anacardiaceae	-	-	Important minor
	Lannea sp.	Important minor	Important minor	Important minor
	Spondias mombin		-	Important minor
3	Arecaceae			
	Elaeis guineensis	Secondary	Predominant	Secondary
	Hyphaene ventricosa	Important minor	Important minor	-
4	Asteraceae	Minor	Important minor	-
5	Bursaraceae	-	-	-
	Commiphora africana	Important minor	-	-
6	Bombacaceae			
	Bombax buonopozense	Important minor	-	-
7	Capparidaceae	-	Important minor	Important minor
8	Celastraceae	Important minor	-	-
9	Euphorbiaceae	Important minor	-	Important minor
	Alchornea cordifolia	Important minor	Important minor	Minor
10	Fabaceae	-	-	-
	Caesalpinioideae	-	-	-
	Piliostigma sp.	Important minor	-	-
	Papilionioideae	-	Minor	Important minor
	Indigofera sp.	Important minor	Important minor	-
11	Hymenocardiaceae	-	-	-
	Hymenocardia acida	-	-	Important minor
12	Irvingiaceae			
	Irvingia sp.	Predominant	Important minor	Secondary
13	Lamiaceae	-	-	Important minor

14	Liliaceae	Important minor	Important minor	Important minor
15	Loranthaceae	-	-	-
	Tapinanthus aurantiacus	-	-	Important minor
16	Moraceae	Important minor	-	Important minor
17	Myrtaceae	-	-	-
	Syzygium guineense	Minor	-	Important minor
18	Onagraceae	-	-	-
	Ludwigia stolonifera	Important minor	-	-
19	Passifloraceae	Minor	-	-
20	Phyllanthaceae	-	-	-
	Bridelia ferruginea	Important minor	-	-
21	Poaceae	Important minor	Important minor	Important minor
22	Rubiaceae	-	Important minor	-
	Nauclea latifolia	-	-	Secondary
23	Sapindaceae	Important minor	-	Minor
24	Solanaceae	-	-	-
	Solanum sp.	Important minor	-	-

Predominant = (>45%); Secondary = (16-45%); Important minor = (15-3%); Minor = (<3%)

Table 5: The weight of pollen sediment and colour of the honey samples after dilution

Honey Sample	Colour of Diluted Honey	Weight of Honey	Weight of Residue
Ivo	yellow	10	3.93
Ohaozara	golden yellow	10	2.04
Onicha	amber	10	0.89

Discussion

The moisture content of all the honey samples fell within the acceptable limit of not more than 21%, which was stipulated for quality honey by Codex Alimentarius (2001). The values of the ash content recorded from the study were low. According to Nyau *et al*. (2013), low ash contents are typical of natural nectar honeys and not of honeydew honeys, which have high ash contents. This is also in agreement with the findings of Kayode and Oyeyemi (2014), who observed low values of ash in honey samples collected from different locations in Ekiti State. The mean pH values of samples from the studied areas were resolved at 4.10. From the observed values, it is evident that the honey samples were acidic in nature, which is a general model of assessing honey quality. Trace amounts of protein were observed in the samples. This result agrees with the values reported by Njokuocha and Osayi (2015) from the analysis of Nsukka honey. The protein content of honey is generally low when compared to sugar, though it is regarded as an important nutritional ingredient. The presence of trace amount of protein in honey samples is an indication that they are nutritionally rich and, therefore, suitable as dietary supplement according to Njokuocha and Osayi (2015). The polyphenol content of the studied samples was moderately high. Similarly, Eleazu *et al*. (2013) reported a significant quantity of this compound. The freshness and

degree of deterioration of honey is determined by the level of HMF present in the sample. All the honey samples were found to have values that were well within the limit recommended for quality honey by Codex Alimentarius (2001). According to Shapla *et al*. (2018), a standard limit of 80 mg/kg was recommended for quality honey originating from tropical regions by Codex Alimentarius (2001) to ensure that the product has not undergone extensive heating during processing and is safe for consumption. Free acidity is an important parameter that is used to determine the level of deterioration of a honey sample. A maximum limit of 50 meq/kg was recommended by Codex Alimentarius (2001) for free acidity. From the results obtained, all the samples had values above the maximum value of quality honey. The higher values may be indicative of fermentation of sugars into organic acids, though the acidity of honey can be affected by other factors, such as the presence of different organic acids, geographical origin and harvest season. Carbohydrates are the main elements in honey, equivalent to 95-99% of the dry matter (Olaitan *et al*., 2007). According to the standard limit set for quality honey by Codex Alimentarius (2001), the summation of fructose and glucose in honey should be greater than sixty ($\geq$ 60). From the results obtained, it was evident that glucose and fructose were the main sugar in all the samples analysed and this agrees with the earlier observation of Ayansola and Banjo (2011). The sucrose values recorded in the study were all within the stipulated range of not more than 5% recommended for quality honey by Codex Alimentarius (2001). All the honey samples were light in colour and as such are blossom honeys, which are of botanical origins. This is in line with the standard of quality honey set by Codex Alimentarius (2001), which states that honey should be nearly colourless to dark brown.

There were variations in the concentrations of the minerals found in the honey samples. These variations could be attributed to the nature of the soil of the source plants, which in turn is greatly affected by atmospheric precipitation, use of pesticides and fertiliser in crop field (Marcovecchio *et al*., 2007). The analysed honey samples showed high values of zinc across all the locations sampled and this result differed from the ones reported for Nigerian honey by Njokuocha *et al*. (2019), as well as the different sets of honeys from Al-Quassim Region, Saudi Arabia, by Khaled *et al.* (2007). Calcium (Ca) and magnesium (Mg) were also present in the honey samples. Calcium (Ca) is a macronutrient that is involved in the formation of strong bones and teeth. Magnesium, according to Vorman (2016), is the fourth most abundant cation and is essential for every cell. Magnesium is a co-factor in multiple enzymatic reactions, including the ones involving energy metabolism and DNA, and protein synthesis. Amongst all the honey samples investigated, cadmium (Cd) and lead (Pb) were at the undetected level. However, researchers have reported trace levels of these heavy metals in honey samples (Moniruzzaman *et al*., 2014).

The identified pollen types in the honey samples provided complete information on the botanical and geographical origins of the honey. They have also revealed the occurrence of pollen of diverse and wide range of plant species, which are visited by bees in the study areas. Most of the recorded pollen grains occurred as important minor and minor components in honey, while only a few were consistently predominant. Palynological results have shown dominance of pollen types from the families of Arecaceae, Irvingiaceae, Myrtaceae, Fabaceae, Rubiaceae and Euphorbiaceae. The common pollen types present in the samples were *Syzygium guineense*, *Irvingia* sp., *Nauclea latifolia*, *Elaeis guineensis*, *Lannea* sp. and *Alchornea cordifolia*. This is in line with the report of Nnamani and Uguru (2013), who identified these species as good floral sources for pollen and nectars. These honey plants (*Elaeis guineensis*, *Alchornea cordifolia*, *Anacardium occidentali* and *Ageratum conyzoides*) are the vestige of the tropical rainforest vegetation belt, as well as characteristic plant species of mosaic of lowland forest and secondary vegetation found in Southern Nigeria, according to Nnamani and Uguru (2013). Although *Elaeis guineensis*

(oil palm) showed continuous presence with a percentage predominance of 86.1% in Ivo honey, it was not labelled as a monofloral honey. According to Aino (2016), this is considered as over-representation since *Elaeis guineensis* is not nectariferous. The female inflorescence of *Elaeis guineensis* is heavily scented, though it is not clear if the bees are also rewarded by some access to the palm sap. Furthermore, Nnamani and Uguru (2013) reported that though *Elaeis guineensis* is not an entomophilous plant, it still stands out as a good source of pollen for honey bees.

The pollen of *Elaeis guineensis* was present in all the locations, constituting about 47% of the total pollen count. Similar results were reported by Nnamani and Uguru (2013). The weight of polliniferous residue in this present study compares favourably with the report of Aino *et al*. (2015). The pollen concentrations of honey are indicated by the weight of recovered pollen, likewise the foraging activities of the bees that produce them (Aino *et al*., 2015). An average undiluted Nigerian honey, according to Aino *et al*. (2015), contains 4.0 - 4.5 g of pollen in 100 g of honey. Based on this it can be deduced that the honey samples were undiluted as can be observed from the pollen weight, and they are of good quality.

Conclusion

Analyses of the honey samples have revealed that they possess some nutritional materials that can be used as supplement for the human needs. The results of this study showed that the honey samples contain minerals, such as calcium, magnesium, potassium and sodium, which are among the major elements required by the body for various metabolic functions. Also, high values of the trace element zinc were observed in the honey samples. This makes the honey samples good for health, since zinc is a nutritionally essential metal that plays a vital role in wound healing and disease resistance, though high intake of it can result in gastrointestinal distress and diarrhoea. Cadmium and lead were not detected in the honey samples. The sugar, moisture, protein and the ash content of the honey were all within the recommended standard of quality honey. Generally, this makes the honey essential for metabolic energy and also confirms their nutritional quality. The pollen contents of the honey samples not only showed that they were produced from different floral sources, but also are of botanical origins. The diversity of important honey plants identified in the study includes *Elaeis guineensis*, *Alchornea cordifolia*, *Syzygium guineense*, *Nauclea latifolia*, *Irvingia* sp., *Hymenocardia acida* etc. As a way of studying the vegetation of an area through pollen analysis of honey, the presence of these species in the honey samples also confirmed their geographical origins, which reflects the mosaic of lowland rainforest and secondary grassland vegetation.

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