# MELISSOPALYNOLOGICAL AND BIOCHEMICAL EVALUATION OF THE AUTHENTICITY OF *APIS MELIFERA ADANSONIA* HONEYS OBTAINED FROM FIVE STATES OF NIGERIA

**NJOKUOCHA, Reginald Chukwuemeka**
Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu State, Nigeria
**Email:** reginald.njokuocha@unn.edu.ng **Phone:** +234 8160202317

***Received*** *August 15, 2019;* ***Revised*** *September 19, 2019;* ***Accepted*** *September 26, 2019*

**ABSTRACT**

***The study was conducted to determine the pollen composition, diversity and biochemical constituents of honey samples from five states of Nigeria. A total of 104 pollen types belonging to 57 plant families were recorded. The honey samples were classified into three botanical groups on basis of major contributing plants; Unifloral, bifloral and polyfloral honeys. Shannon-Wiener Diversity and Pielou's Evenness Indices ranged from 1.987 – 2.676 and 0.580 – 0.705 respectively indicating high plant heterogeneity foraged by the honeybees. The common plant sources of nectar and pollen include Combretaceae-Melastomataceae, Phyllanthus muelerianus, Elaeis guineensis, Alchornea cordifolia and Syzygium guineense. The biochemical analyses conformed to the Codex and EU Council standard for all parameters tested except moisture content from Akpanya and Anyigba samples that exceeded the limit. The highest crude protein content was recorded in Calabar sample (1.71 ± 0.01 %) while ash content was highest in Anyigba (0.29 ± 0.02 %). Calabar and Akpanya samples had the highest fibre value (0.80 ± 0.01 %). The hydroxymethylfurfural value was highest in Abakaliki (4.46 ± 0.02 mg/kg). Sample from Calabar had the highest free acidity (0.12 ± 0.01 meg/kg), while electrical conductivity was highest in Akpanya (17.70 ± 0.01 µS/cm) sample. The pH and refractive index values, 4.72 ± 0.01 and 1.50 ± 0.01 respectively were highest in Abakaliki sample. The sum of fructose and glucose ranged from 68.8 ± 0.01 – 82.62 ± 0.01 % in the samples and had ratios greater than one in the samples making them subject to low crystallization.***

**Keywords:** Pollen analysis, Biochemical constituents, Honey, Pollen diversity

## INTRODUCTION

Honey is a natural food product relished for its important source of simple sugars and other complementary nutritional components such as protein, mineral salts, vitamins, flavonoids as well as other antioxidants and organic acids that play vital roles in sustaining life. It is one of those naturally produced products that has high calorie and easily assimilated. As a natural product with abundant variable benefits and uses, it commands high commercial value in both local and international markets. This makes the product vulnerable to adulteration especially with sugar syrups, molasses and wax either at the bee farm or by commercial honey dealers. This anomaly informed the establishment of screening standards by various countries and organizations such as the International Honey Commission, Codex Alimentarius Commission (2001) and EU Commission (2002) on honey quality. Therefore, to assure honey safety, it must be free of any unwanted contaminant. Consequently, the determination of the physico-chemical properties of most natural products such as honey is an important criterion for the assessment of the purity of the product.

The characteristic of honey is influenced by multiple factors such purity and sources of nectar, pollen composition, climatic conditions,

method of harvest and extraction, storage conditions as well as geographical origin which influences the floral sources of nectar and pollen foraged by the honeybees (Agwu and Akanbi, 1985; Dafni *et al*., 1988; Abu-Tarbousch *et al*., 1993; Buba *et al*., 2013; Lullah-Deh *et al*., 2018). These conditions significantly determine the relative composition of the physicochemical components such as simple-complex sugar ratio, moisture, acidity, hydroxymethylfurfural (HMF), viscosity, aroma, vitamins, ash, organic acids, amino acids and electrical conductivity.

The pollen composition of honey has served efficiently in the determination of the botanical, geographical and major season of honey production in most regions (Agwu and Akanbi, 1985; Njokuocha and Ekweozor, 2007; Njokuocha and Nnamani, 2009; Song *et al*., 2012; Kayode and Oyeyeme, 2014). Although, quantitatively, pollen is regarded as a minor component of honey, it has remained valuable in assessing the premium and commercial value of honey in the international market, and its contribution to the nutritional quality of honeys as important sources of vitamins, proteins and amino acids has been reported (Hassan, 2011).

Studies on the physicochemical properties of honey have been conducted in Nigeria and some other parts of the world (Adebiyi *et al*., 2004; Anyansola and Banjo, 2011; Yadata, 2014; Njokuocha and Osayi, 2015; Subbiah *et al*., 2015; Nweze *et al*., 2017; Azonwade *et al*., 2018; Bako *et al*., 2019). In south eastern, Nigeria where honey is produced in commercial quantity, studies on the physicochemical properties of honey are vast and represents most of the honey producing areas within the the region (Agwu and Akanbi, 1985; Agwu and Uwakwe, 1992; Njokuocha and Ekweozor, 2007; Njokuocha and Nnamani, 2009; Njokuocha and Osayi, 2015; Nweze *et al*., 2017; Njokuocha *et al*., 2019). There is need for continuous study of physicochemical and melissopalynological properties of honey in this region arising from changes in vegetation types due to climate change and anthropogenic factors. It has also been argued that the use of either physicochemical or pollen analysis alone in assessing the quality of honey has some limitations (Oddo *et al*., 1995). Only few authors have attempted to under study both the use of physicochemical and pollen analysis of honeys in this region as a quality assessment boost (Njokuocha and Osayi, 2015; Aina, 2016; Ebigwai *et al.,* 2017). Therefore, this study was design to undertake the assessment of honey qualities soured from five states of Nigeria: Ebonyi, Enugu, Anambra, Cross River and Kogi States) from the physicochemical and melissopalynological standpoint. This study will contribute additional data towards harmonization and characterization of Nigerian honeys aim at improved production and better marketing strategy.

## MATERIALS AND METHODS

Six honey samples from *Apis mellifera adansoni* were collected from authentic honey vendors from Akpanya and Anyigba (Kogi State), Nsukka (Enugu State), Abakaliki (Ebony State) Aguata (Anambra State) and Calabar (Cross River State). Physicochemical analyses were conducted at the National Centre for Energy Research and Analysis, while pollen analysis was done in the Environment and Palynology Research Unit, Department of Plant Science and Biotechnology, both in University of Nigeria, Nsukka. All the reagents used were of analytical grades.

**Pollen Analysis:** Pollen analysis was conducted with modification according to Moore and Webb (1978) and Njokuocha *et al*. (2019). Ten grams of the agitated honey samples was weighed (INNSYS Weighing Balance Model WBK005E6) and each diluted with 40 ml of warm (40$^{o}$C) acidified water (3 ml of conc. $H_2SO_4$ and 997 ml of distilled water) in order to dissolve the colloidal matters and sugars. The honey colour was recorded by comparison with munsel colour chart. The sample was centrifuged at 2000 g for ten minutes to concentrate the palyniferous residue which was subsequently acetolysed (Moore and Webb, 1978; Njokuocha and Nnamani, 2009). The palyniferous residue was washed with 5 ml of glacial acetic acid and 10 ml of distilled water following centrifugation at 2000 g. The final polliniferous residue was suspended in 3 ml of glycerol-alcohol (3:1) in

vial from where samples were taken for routine pollen grain counts and identification under the microscope (WESO) at x400 magnification. Pollen record was based on routine count on the total surface area (484 $cm^2$) of the cover slip, while identification of pollen grains was aided by pollen atlas of APLF (1974), Y'bert (1979) and Bonnefille and Riolett (1980). The number of the counted pollen types were converted to percentage frequency (Louveaux *et al.*, 1978) as predominant (P ≥ 45 %), secondary (S = 16 – 44 %), important minor (I = 3 – 15 %) and minor pollen (M = ≤3 %). The diversity of pollen and evenness were calculated using Shannon-Wiener Diversity Index and Pielou's Evenness Index. Shannon-Wiener Diversity Index (H) = $\sum_{i=1}^{s} Pi\ In\ Pi$ where Pi is the proportion of characters belonging to the ith type of letter in the string of interest. ∑ is the sum of the calculations. S is number of species. In is the natural log. Pielou's Evenness Index (J') = $H'/H'max$, where H' is the calculated Shannon-Wiener diversity index and H'max is the natural logarithm of the total member of plant species found in the honey sample.

**Physicochemical Analysis**

The physicochemical properties were analyzed according to the methods of Association of Analytical Chemist (AOAC, 2005) and analysis of each sample was replicated three times for each parameter tested.

**Determination of pH:** The pH meter (Jen Way pH model 3510) was standardized using standard buffer solutions of pH 4, 7 and 10. Ten percent of honey solution was prepared by dissolving 10 g of honey sample in 100 ml of distilled water. The cleaned electrode of the pH meter was immersed into the honey solution and left for a short pending stabilization of the reading, and was recorded.

**Determination of protein:** This was determined using Kjeldhl method according to AOAC (2005). Two grams of the honey sample was weighed into micro-kjedhl flask and 25 ml of concentrated $H_2SO_4$ and a digestion mixture consisting of a speak of selenium, 1 g of anhydrous $K_2SO_4$ and 7 g of $CuSO_4$ were added into the flask. The flask was slightly agitated to mix the content and then heated for 2 hours to digest until the colour changed from green to a clear solution. It was allowed to cool and transferred to 100 ml distillation flask and made up to the mark with distilled water. Ten milliliter of the digest was transferred to a distillation flask and 10 ml of 40 % NaOH was added to it. The ammonia ($NH_3$) released during the distillation was collected in form of NaOH in a 50 ml conical flask containing 20 ml of 4 % Boric acid solution and drops of methyl indicator. 35 ml of the distillate was titrated against standard 0.1 N HCl solution until the colour turned pink. A blank was also run through the above steps. Percentage crude protein of each honey sample was calculated thus: % crude protein = (A-B) x N x 0.014 x C x 100 x 6.25/weight of honey sample x V. Where; A = honey sample titrated; B = blank titration; N = normality; C = dilution sample after digestion; V = volume taken for distillation; 0.014 = millilitre equivalent weight of nitrogen; 6.25 = conversion factor.

**Determination of hydroxymethylfurfural (HMF):** Ten grams of the honey sample was dissolved in 20 ml of oxygen free cold water and transferred to 50 ml of volumetric flask and made up to 50 ml with distilled water. Two mills of the solution were introduced into two test tubes and 5 ml of solution of p-toluidine was added to each tube. Thereafter, 1 ml of barbituric acid was added into one test tube and 1 ml of water to the other test tube (blank). The absorbance of the test sample was read against the blank at 5550 nm using spectrophotometer (Spectro 20D, PecMedical, USA). HMF (mg/100 g) = Absorbance/cell path length x 19.2.

**Determination of free acidity:** Ten grams of the honey sample was dissolved in 75 ml of distilled water in a beaker and 0.3 ml of phenolphthalein indicator was added. The solution was then titrated against 0.01 M of NaOH until a pink colour appeared. The relative amount of titrable acid was determined thus: Free Acidity = Titre value x molarity of NaOH x 4.6/ weight of sample.

**Determination of moisture:** Ten grams of honey sample was weighed in a crucible and heated in an electric heater (Metter, B & TBS 2648) at 75$^{o}$C for 2 hours and at 100$^{o}$C for the next four hours until constant weight was attained. The percentage moisture content was determined as follows: Percentage Moisture = W1 – W2/ W1 x 100, while percentage weight of dry sample (solid) = W1 – W0 /W2 – W0 x 100, where; W1 = weight of fresh honey sample + crucible, W2 = weight of dry sample (solid + crucible) and W0 = weight of crucible

**Determination of fat content: T**wo grams of the honey sample was weighed into an extraction thimble lined with filter paper. The thimble and its content were put in the soxhelt apparatus. A weighed flat bottom flask was filled with three quarter of its volume with petroleum ether at 80$^{o}$C boiling point. The apparatus was then set up and heated for 4 hours after which the petroleum ether was recovered. The residue collected in the flask was dried at 105$^{o}$C in an oven (Gallen Kamp, USA), cooled and weighed. The percentage fat content was calculated as follows: % fat = C – A/B x 100, where; A = weight of empty flask; B = weight of initial sample; C = weight of flask + fat.

**Determination of ash:** Two grams of honey sample were weighed in a pre-weighed crucible and ashed at 600$^{o}$C for 3 hours in a muffle furnace (Gallen Kamp, Hotpot, USA) (AOAC, 2005). The percentage ash was calculated thus; weight of crucible + ash – weight of crucible ÷ weight of the honey sample (2 g) x 100.

**Determination of fibre content:** Two grams of the honey sample was weighed (W1) into a 600 ml beaker and 150 ml of preheated 0.128 M $H_2SO_4$ was added to it. The mixture was heated for 30 minutes and filtered under suction and washed with hot water to remove acid content. The residue was boiled again for 30 minutes with 150 ml of preheated KOH (0.223 M) and washed to remove alkaline content. The residue was washed in acetone and dried in an oven at 105$^{o}$C for 2 hours, cooled and reweighed (W2). It was then ashed in a muffle furnace (Gallen Kamp, Hotpot, USA) at 500$^{o}$C for 4 hours and reweighed (W3). % crude fibre = W2 – W3/W1 x 100, where W1 = weight of sample; W2 = weight of dry residue; W3 = weight of ash.

**Determination of electrical conductivity:** The conductivity meter (WTN model: LF 90) was standardized with 0.01 M KCl solution. The electrode was rinsed with deionized water, wiped and dipped into the honey sample and left to stabilize. The reading was taken in Siemens per centimeter (µS/cm).

**Determination of refractive index:** Abbe refractometer-bench type was used. The illuminating light display was set at zero. A drop of the agitated honey sample was put on the surface of the lower refracting prism. The rotating arm and the collecting lens cone of the light illuminating units were positioned such that the light-intake surface of the upper light – intake prism illuminated evenly. By means of the adjustable hand wheel the line dividing the dark and light area were to fall in the cross line. The dispersion correction hand wheel was rotated as to get good contrast between the light and dark area and minimum dispersion. The refractive index was read by pressing the red button.

**Determination of sucrose:** Ten grams of the honey sample were weighed and dissolved in 100 ml of distilled water and 5 ml of 20 % oscinol was added to 1 ml of the solution, boiled at 100$^{o}$C for 12 minutes. The cooled solution was made up to 25 ml with distilled water and the absorbance read at 620 nm.

**Determination of fructose:** Ten grams of the honey sample were weighed and dissolved in 100 ml of distilled water. 1 ml of the solution was taken and 1 ml of reagent A (50 mg resorcinol dissolved in ethanol) and 1 ml of reagent B (50 ml of conc. HCl + 10 ml of $H_2SO_4$), and heated at 50$^{o}$C for 18 minutes, cooled at room temperature and the absorbance read at 530 nm.

**Determination of glucose:** Ten grams of the honey sample were weighed and macerated

with 20 ml of distilled water and filtered. 1 ml of the filtrate was pipetted into a test tube and 1 ml of alkaline copper reagent was added, boiled for 5 minutes and cooled. 1 ml of phosphomolybic reagent and 7 ml of distilled water were added and the absorbance read at 490 nm.

**Data Analysis:** The pollen data collected were analysed using descriptive statistics for their central tendencies and percentage composition. The pollen spectrum table was constructed using percentages. The diversity of plants recorded were analysed using Shannon-Wiener Diversity and Pielou's Evenness indices. The biochemical data were analysed descriptively and the results presented as mean ± standard deviation. For all the analyses, 95 % probability was accepted as significant.

## RESULTS

**Melissopalynological Composition:** The honey samples yielded a total of 104 pollen types belonging to 57 families with percentages ≥ 0.01 (Table 1). Plant species/families whose pollen types occurred in more than 50 % of the honey samples include *Nauclea latifolia, Lannea* sp., *Syzygium guineense,* Poaceae, *Alchornea cordifolia, Elaeis guineensis,* Combretaceae-Melastomataceae, *Irvingia gabonensis, Bombax buonopozense, Senna* sp., *Prosopis africana, Parkia biglobosa, Pentaclethra macrophylla, Hymenocardia acida*, Moraceae, *Phyllanthus muellerianus, Lophira lanceolate*, and Asteraceae, *Khaya senegalensis*. The most diverse family in the honey samples were Fabaceae with 10 taxa, Rubiaceae (4 taxa), Euphorbiaceae and Meliaceae with three taxa each. One honey sample was characterized as monofloral, three bifloral and two polyfloral (Table 1).

The results of pollen analysis of honey sampled from Akpanya, Kogi State showed that the honey yielded a total of 50 pollen types and one fern spore type belonging to 30 families. Of these, the commonest families with values ≥0.01 % were Combretaceae – Melastomataceae, *Alchornea cordifolia, Hymenocardia acida, Syzygium guineense, Nauclea latifolia*, Poaceae, *Lannea* sp., *Crossopteryx febrifuga*, and Moraceae, *Citrus sinensis*. The honey sample was characterized as polyfloral because the pollen type with the highest frequency was not predominant and majority of the most common pollen types were of important minor. The source floral diversity as indicated by Shannon-Weiner Diversity Index value of 2.676 and Pielou's Evenness Index value of 0.681 indicated heterogeneous foraging habit of the honey bees. In honey sampled from Anyigba, twenty-seven pollen types belonging to 20 families were recorded. Of these, the most common pollen types ≥2.03 % were those of *Phyllanthus muellerianus, Senna* sp., Combretaceae – Melastomataceae, *Elaeis guineensis, Syzygium guineense, Lannea* sp. and *Crossopteryx febrifuga*. The honey sample was classified as polyfloral because four pollen types had secondary frequency values. Although the Shannon-Weiner Diversity Index did not indicate high diversity value (1.987), the Pielou's Evenness Index of 0.603 still justified the heterogeneity in resource exploitation by the honeybees.

The results of pollen analysis of honey sampled from Nsukka showed a total of 28 pollen types belonging to 20 families. The most commonly recorded pollen types with values ≥ 2.41 % were *Elaeis guineensis, Irvingia gabonensis, Alchornea cordifolia, Euginea uniflora, Prosopis africana*, Poaceae and *Senna* sp. Two pollen types had secondary values, the honey sample was classified as bifloral. The value of Shannon-Wiener Diversity Index of 2.017 and Pielou's Evenness Index value of 0.605 showed the heterogeneous foraging behaviour of the honeybees. Honey sampled from Aguata had a total of 41 pollen types and one fern spore belonging to 26 families. The most important plant pollen sources ≥2.0 % were *Pterocarpus* sp., *Psorospermum* sp., Combretaceae – Melastomataceae, *Hymenocardia acida, Lannea* sp., *Afzelia africana, Elaeis guineensis, Mangifera indica* and *Piliostigma thonningii*. The honey sample was classified as bifloral because only two pollen types had secondary percentage values.

**Table 1: Pollen spectrum of honey samples from six locations in Nigeria**

| S/N | Family | Taxon | Pollen spectrum (%) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Akpanya | Anyigba | Nsukka | Aguata | Abakaliki | Calabar |
| 1 | **Rubiaceae** | *Nauclea latifolia* | 7.81 | 2.88 | 1.03 | 1.58 | 6.54 | 2.27 |
| 2 | | *Crossopteryx febrifuga* | 3.38 | 2.03 | | 0.67 | | |
| 3 | | *Mitragyna stipulosa* | | | | | 0.01 | 0.08 |
| 4 | | *Kohautia* sp. | 0.72 | 0.01 | | | 0.01 | |
| 5 | **Anacardiaceae** | *Lannea* sp. | 5.45 | 2.15 | 1.38 | 5.88 | 3.25 | 2.92 |
| 6 | | *Mangifera indica* | | | 0.34 | 2.25 | 0.01 | |
| 7 | **Myrtaceae** | *Syzygium guineense* | 9.59 | | 1.38 | 1.38 | 5.31 | |
| 8 | | *Euginea uniflora* | | 0.01 | 3.1 | | | 1.69 |
| 9 | **Chrysobalanaceae** | *Cleome* sp. | | 0.02 | | | | |
| 10 | **Poaceae** | | 9.57 | 0.3 | 2.76 | 0.29 | | 1.42 |
| 11 | **Annonaceae** | *Uvaria chamae* | | | | 0.04 | 0.01 | 0.01 |
| 12 | **Euphorbiaceae** | *Alchornea cordifolia* | 10.73 | 0.84 | 4.14 | 0.96 | | 29.57 |
| 13 | | *Euphorbia* sp. | 0.05 | | | 1.33 | | 0.31 |
| 14 | | *Manihot esculentus* | 0.02 | | | | | 0.01 |
| 15 | **Clusiaceae** | *Psorospermum* sp. | 0.22 | 0.01 | | 18.27 | | |
| 16 | **Tiliaceae** | *Triumfetta rhomboidea* | 0.13 | | | 0.04 | | 0.35 |
| 17 | **Arecaceae** | *Cocos nucifera* | | 16.5 | 0.34 | | 0.01 | |
| 18 | | *Elaeis guineensis* | 2.58 | | 43.45 | 0.08 | | 17.33 |
| 19 | **Combretaceae-Melastomataceae** | | 17.25 | 17.1 | 2.76 | 10.92 | 11.35 | 9.43 |
| 20 | **Rutaceae** | *Citrus sinensis* | 3.25 | 0.03 | | | | 4.2 |
| 21 | **Irvingiaceae** | *Irvingia gabonensis* | 2.71 | | 22.76 | | 0.01 | 0.52 |
| 22 | **Bombacaceae** | *Bombax buonopozense* | 1.29 | 0.01 | 1.75 | | | 0.12 |
| 23 | **Dioscoreaceae** | *Dioscorea alata* | | | | | | 0.01 |
| 24 | **Fabaceae** | *Senna* sp. | 0.01 | 22.61 | 2.41 | 0.92 | 0.71 | 0.03 |
| 25 | | *Prosopis africana* | 0.63 | | 2.76 | 0.21 | 0.29 | |
| 26 | | *Parkia biglobosa* | 0.19 | 1.69 | 0.09 | | 0.47 | |
| 27 | | *Crudia* sp. | 0.02 | | | | 0.01 | |
| 28 | | *Afzelia africana* | 0.07 | | | 4.34 | | |
| 29 | | *Berlinia grandiflora* | 0.09 | | | | 0.01 | 0.13 |
| 30 | | *Brachystegia eurycoma* | 0.07 | | | | | 0.11 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **31** | | *Piliostigma thonningii* | 1.23 | | | 2.5 | | |
| **32** | | *Pseudarthria* sp. | 0.03 | 0.01 | | | | |
| **33** | | *Pentaclethra macrophylla* | 0.11 | | 0.34 | 0.08 | | 0.15 |
| **34** | **Hymenocardiaceae** | *Hymenocardia acida* | 10.27 | 1.14 | | 7.55 | 4.88 | 0.33 |
| **35** | **Moraceae** | | 3.35 | 0.57 | 1.38 | 0.92 | 4.86 | 12.17 |
| **36** | **Vitaceae** | *Cissus araloides* | 0.01 | | | | 0.01 | |
| **37** | **Phyllanthaceae** | *Phyllanthus muelerianus* | 4.5 | | 1.03 | 2.67 | 4.34 | 13.65 |
| **38** | | *Antidesma* sp. | 0.12 | | | 1.83 | | 0.01 |
| **39** | **Casuarinaceae** | *Casuarina equisetifolia* | | | | 0.46 | | 0.01 |
| **40** | **Burseraceae** | *Canarium sweinforthii* | 0.27 | | | | 0.01 | 0.03 |
| **41** | **Solanaceae** | *Physalis angulata* | | | 0.69 | 0.08 | 0.01 | |
| **42** | **Ochnaceae** | *Lophira lanceolata* | 0.4 | | 0.69 | 0.04 | 0.09 | |
| **43** | **Asteraceae** | | 0.41 | | 0.69 | | 0.01 | 0.57 |
| **44** | **Olacaceae** | *Olax* sp. | | | | 0.04 | | 0.09 |
| **45** | **Meliaceae** | *Khaya senegalensis* | 0.06 | 0.13 | | 1.88 | 3.62 | |
| **46** | | *Trichilia* sp. | | 1.02 | | | 3.57 | |
| **47** | | *Azadirachta indica* | 0.06 | | | | 24.99 | |
| **48** | **Malvaceae** | *Sida acuta* | | | 0.34 | | 0.01 | |
| **49** | **Rhamnaceae** | *Lasiodiscus* sp. | | | 0.34 | | 0.01 | |
| **50** | **Cyperaceae** | | 0.01 | 0.01 | | 0.25 | | |
| **51** | **Polygonaceae** | *Polygonium* sp. | 0.01 | | | | | 0.04 |
| **52** | | Pollen type 1 | | | | | 0.01 | 0.21 |
| **53** | | Pollen type 2 | | | | | 0.01 | 0.17 |
| **54** | | Indeterminate | 0.22 | 0.02 | 1.72 | 0.58 | 0.02 | |

***Note:*** *Pollen types that occurred only in one sample were not included in the table*

The high values of Shannon-Weiner Diversity Index (2.637) and Pielou's Evenness Index (0.705) indicated a high level of plant heterogeneity utilized by the honeybees as sources of pollen and nectar.

The results of the pollen analysis of honey from Abakaliki, Ebonyi State showed a total of 42 pollen types and one fern spore type belonging to 28 families in the honey sample. The most common pollen types with values ≥ 3.25 % were *Uapaca*, sp., *Azadirachta indica*, Combretaceae – Melastomataceae, *Nauclea latifolia, Syzygium guineense, Hymenocardia acida*, Moraceae, *Phyllanthus muellerianus, Khaya senegalensis, Trichilia* sp. and *Lannea* sp. the honey sample was characterized as biflora because the two secondary pollen types of *Uapaca* sp. and *Azadirachta indica*. Shannon-Weiner Diversity Index of 2.205 and 0.590 Pielou's Evenness Index are indication of the wide range of plant species foraged by the bees for honey production. Also, from the honey sample sourced from Calabar, Cross River State a total of 47 pollen types and two ferns spore types belonging to 29 families of plants were recorded. The plants with percentages pollen frequency ≥2.27 % were *Alchornea cordifolia, Elaeis guineensis, Phyllanthus muellerianus,* Moraceae, Combretaceae-Melastomataceae, *Citrus sinensis, Lannea* sp. and *Nauclea latifolia*. Although though *Elaeis guineensis* and *Alchornea cordifolia* formed the secondary pollen constituent, the honey was classified as monofloral and not bifloral because *Alchornea cordifolia* is not a source of flower nectar, it only supplied pollen grains. The Shannon-Weiner Diversity Index of 2.182 and Pielou's Evenness Index of 0.580 still demonstrated that tangible evidence of heterogeneity in the foraging activities of the bees.

**Biochemical composition:** The biochemical composition of the six honey samples from Calabar, Abakaliki, Nsukka, Aguata, Akpanya and Anyigba analyzed are shown in Tables 2 and 3. The moisture content varied from 10.96 ± 0.02 % in honey sampled from Abakaliki to 27.53 ± 0.01 % in honey from Akpanya. Evidently honey samples from Anyigba and Akpanya with values of 22.31 ± 0.01 and 27.53 ± 0.01 % respectively were above the limit (≤20 %) set by international regulations for good quality honey. The mean percentage protein content showed that all the samples had considerable level of crude protein with the highest value recorded in honey samples from Calabar (1.71 ± 0.01 %), followed by Abakaliki (1.13 ± 0.01 %) and the least percentage crude protein occurred in honey sample from Anyigba. For the ash content, the highest mean value was recorded in honey from Anyigba (0.29 ± 0.02 %) followed by honey from Akpanya (0.27 ± 0.01 %) and the least in honey from Calabar (0.01 ± 01 %). The fibre value ranged from 0.01 ± 0.01 % in honey from Abakaliki to 0.8 ± 0.01 % in honey sample from Calabar and Akpanya. The value of HMF ranged from 3.55 ± 0.01 mg/kg in honey sampled from Calabar to 4.46 ± 0.02 in honey sampled from Abakaliki. The mean value of free acidity also varied from 0.04 ± 00 meg/kg in honey sampled from Nsukka and Akpanya to 0.12 ± 0.01 meg/kg in honey sampled from Calabar. Electrical conductivity varied from 1.20 ± 0.01 µS/cm in honey sampled from Abakaliki to 17.7 ± 0.01 µS/cm in honey sampled from Akpanya. The pH ranged from 3.50 ± 0.01 in honey sampled from Aguata to 4.72 ± 0.01 in honey sampled from Abakaliki. The percentage fat content varied from 0.10 ± 0.01 % in honey sampled from Akpanya to 0.40 ± 0.01 % in honey sampled from Abakaliki, while the refractive index varied slightly from 1.48 ± 0.01 in honey sampled from Akpanya to 1.50 ± 0.01 in honey sampled from Abakaliki.

Three major sugar constituents; glucose, fructose and sucrose were also analyzed as shown in Table 3. The percentage sum of glucose and fructose in the six honey samples varied from 69.80 % in honey sampled from Nsukka to 82.62 % in honey sample from Abakaliki which were above the minimum limit >60 % of the international acceptable standard of the sum of glucose and fructose for blossom honey. In all the samples the mean percentage value of fructose was greater than that of glucose. Equally the percentage value of sucrose ranged from 1.07 ± 0.01 % in honey sampled from Calabar to 2.10 ± 0.02 % in honey sampled from Nsukka.

**Table 2: Physicochemical composition of the honey samples from six locations in Nigeria**

| Honey source | Moisture (%) | Crude Protein (%) | Ash (%) | Fibre (%) | HMF (mg/kg) | Free Acidity (meg/kg) | Electrical Conductivity (µS/cm) | pH | Fat (%) | Refractive Index |
|---|---|---|---|---|---|---|---|---|---|---|
| **Calabar** | 19.37 ±0.01 | 1.71 ± 0.01 | 0.01 ± 0.01 | 0.80 ± 0.01 | 3.55 ± 0.01 | 0.12 ± 0.01 | 12.30 ± 0.01 | 4.53 ± 0.01 | 0.25 ± 0.01 | 1.49 ± 0.01 |
| **Abakaliki** | 10.96 ± 0.02 | 1.13 ± 0.01 | 0.10 ± 0.01 | 0.10 ± 0.01 | 4.46 ± 0.02 | 0.09 ± 0.01 | 1.20 ± 0.01 | 4.72 ± 0.01 | 0.40 ± 0.01 | 1.50 ± 0.01 |
| **Nsukka** | 16.09 ± 0.05 | 0.82 ± 0.02 | 0.16 ± 0.01 | 0.50 ± 0.01 | 4.00 ± 0.01 | 0.04 ± 0.00 | 3.60 ± 0.01 | 4.10 ± 0.01 | 0.30 ± 0.01 | 1.49 ± 0.01 |
| **Aguata** | 16.43 ± 0.01 | 0.76 ± 0.01 | 0.06 ± 0.01 | 0.45 ± 0.01 | 3.89 ± 0.01 | 0.05 ± 0.00 | 4.30 ± 0.02 | 3.50 ± 0.01 | 0.25 ± 0.01 | 1.49 ± 0.01 |
| **Akpanya** | 27.53 ± 0.01 | 0.98 ± 0.01 | 0.27 ± 0.01 | 0.80 ± 0.01 | 4.3 ± 0.02 | 0.04 ± 0.00 | 17.70 ± 0.01 | 4.20 ± 0.07 | 0.10 ± 0.01 | 1.48 ± 0.01 |
| **Anyigba** | 22.31 ± 0.01 | 0.49 ± 0.02 | 0.29 ± 0.02 | 0.4 ± 0.01 | 3.67 ± 0.01 | 0.06 ± 0.00 | 14.10 ± 0.01 | 3.90 ± 0.01 | 0.25 ± 0.01 | 1.49 ± 0.00 |

**Table 3: Major sugar composition of the honey samples from six locations in Nigeria**

| Honey source | Glucose | Fructose | Glucose + Fructose | Sucrose |
|---|---|---|---|---|
| **Calabar** | 35.31 ± 0.01 | 40.97 ± 0.02 | 76.28 ± 0.76 | 1.07 ± 0.01 |
| **Abakaliki** | 33.74 ± 0.01 | 48.88 ± 0.01 | 82.62 ± 2.02 | 1.67 ± 0.01 |
| **Nsukka** | 32.40 ± 0.01 | 37.40 ± 0.01 | 69.8 ± 0.67 | 2.10 ± 0.02 |
| **Anambra** | 36.22 ± 0.01 | 43.87 ± 0.01 | 80.09 ± 1.02 | 1.50 ± 0.01 |
| **Akpanya** | 32.80 ± 0.02 | 40.00 ± 0.02 | 72.8 ± 0.96 | 2.04 ± 0.01 |
| **Anyigba** | 33.67 ± 0.01 | 41.29 ± 0.01 | 74.96 ± 1.02 | 1.90 ± 0.02 |

## DISCUSSION

Pollen grains and nectar are the two principal ingredients required for honey production because they are important sources of nutrient and energy for the honeybees and their broods (Bhattacharya *et al*., 2006). In fact, in addition to nectar, honeybees purposefully forage for pollen grains used as bee bread in order to obtain protein, mineral salts and other biochemical constituents for their survival and fecundity. It has been widely reported that honeybees visit a wide variety of plants for their nectar and pollen grains (Agwu and Akanbi, 1985; Njokuocha and Osayi, 2015) especially during peak of flowering season. This is reflected in the present study in which 104 plant species belonging to 57 families were foraged for nectar and or pollen grains. Similar range in diversity of plant species in honey samples have also been reported in other parts of Nigeria by Agwu and Uwakwe (1992), Njokuocha and Nnamani (2009), Ige and Modupe (2010); Kayode and Oyeyemi (2014) as well as other parts of the world (Moar, 1985; Tsigouri *et al*., 2004; Caccavari and Fagundez, 2010; Ramirez-Arriaga *et al*., 2011). The high number of genera not only demonstrates how active the foraging power of the honeybees, but also the wide range of flora available as sources of nectar and or pollen in the source areas (Eckert, 1942; Njokuocha and Nnamani, 2009). A characterization of the pollen profile showed that nectariferous plants constituted about 72 % of the foraged plant sources most of which are native plants, while the non-nectariferous plants contributed about 16 %. Invariable this shows that the honeys were derived from plant blossoms (Louveaux *et al.,* 1978).

The high values of Shannon-Weiner Diversity and Pielou's Evenness Indices which occurred in the range of 1.987 to 2.676 and 0.580 to 0.705 respectively was an indication of the wide diversity of plant species explored by the bees in the process of the production of bifloral and polyflora honeys as well as the good interaction between the honeybees and vegetation. In particular, the high values of the indices in honey samples from Akpanya, Aguata, Nsukka, Abakaliki and Calabar were indication of the rich nectar and pollen sources in these locations. It also showed the high potential that can be explored for apicultural establishment. The characteristic pollen types identified in the study such as those of *Nauclea latifolia, Lannea* sp., *Alchornea cordifoli*a, Combretaceae /Melastomataceae, *Elaeis guineensis, Syzygium guineense*, Poaceae, *Senna* sp., *Parkia biglobosa*, Moraceae, *Hymenocardia acida, Phyllanthus muellerianus* and *Khaya senegalensis* were comparable to that recorded in Nigeria by Sowunmi (2001), Njokuocha and Ekweozor (2007), Kayode and Oyeyemi (2014) and Njokuocha *et al*. (2019).

Moisture content of honey is one of the most important parameters used to assess the premium quality of honey. This is because the percentage composition of water influences the shelf life and stability to fermentation. It is affected by such factors as level of maturity prior to harvest, season of production, method of harvest and storage conditions among others (Nigussie *et al*., 2012). In the present study the percentage moisture of the honey samples from Calabar, Abakaliki, Nsukka and Aguata were within the limits of internationally acceptable standard (<20 %) and limits set by Codex Alimentarius Commission (2001). However, the honey from Akpanya and Anyigba exceeded the recommended limits and by implication the samples had poor storage potentials and may be subject to easy fermentation by yeast (Molan, 1992). The findings of this study compare favourably to those reported in some regions of Nigeria (Buba *et al*., 2013; Ndife *et al.,* 2014; Njokuocha and Osayi, 2015) and other parts of the world (Can *et al.,* 2015; Boussaid *et al*., 2018).

The percentage crude protein of the honey samples varied considerably across the sampled areas. Protein content of honey is considered low ranging from 0.04 to 0.1 % and may exceed this depending on the primary sources. The source of protein load of honey may be attributed to the quantity and variety of the pollen content (Schafer *et al*., 2006) and enzymes such as glucose oxidase and diastase (Subramanian *et al*., 2007). The presence of protein has also been reported in honey

samples from other parts of Nigeria (Oyeleke *et al*., 2010; Bako *et al*., 2019).

The ash content of honey has a direct relationship with the mineral concentration and is also a parameter for determining the botanical origin of honey. The ash content of honey is a reflection of the level of mineral salts in honey and the darker the honey the more the mineral concentration. In this study, the percentage ash ranged from 0.01 ± 0.01 to 0.29 ± 0.02 % which is within the average percentage for floral honeys (0.6 g). The result of this study was in agreement with earlier studies from Nigeria (Adams *et al*., 2010; Ndife *et al*., 2014; Bako *et al.,* 2019) and other countries (Nigussie *et al*., 2012; Boussaid *et al*., 2018).

Fibre is an important dietary component because of its role in digestive health and steady bowel movement. The percentage fibre in this study ranged from 0.01 ± 0.01 to 0.8 ± 0.01, such low values of fibre have been reported in study on honey by Oyeleke *et al*. (2010).

The results of the HMF analysis was found to range from 3.35 ± 0.01 in honey sample from Calabar to 4.46 ± 0.02 mg/kg in sample from Abakaliki. The overall values showed that HMF in all the honey samples were within the limits of international standard which should not exceed 40 mg/kg (EU Commission, 2002). All the honey samples are therefore considered fresh. An elevated concentration of HMF in honey is an indication of high exposure to heat/temperature or poor storage condition; hence it is a good parameter for determining honey freshness (Codex Alimentarius Commission, 2001).

The free acidity in honey arises as a result of the action of glucose oxidase enzymes which forms gluconic acid and may also be primarily present in honeys. The free acidity as analyzed in the study ranged from 0.04 ± 00 to 0.12 ± 0.01 meg/kg which is less than the maximum limit of 50 meg/kg for blossom honey as recommended by Codex Alimentarius Commission (2001) and EU Commission (2002).

The percentage fat of the honey samples were considerably low and ranged from 0.10 ± 0.01 to 0.40 ± 0.01 %. Similar results have been reported by Buba *et al*. (2013) for honey sample from North-East Nigeria and Ndife *et al*. (2014) for Nigerian honey sourced from different floral locations. It is generally reported that honey contains little or no fat (Singh and Bath, 1997), however recent articles have reported the presence of fatty acids such as linoleic acids, oleic and palmitic acids in honeys, a fact which has been attributed to the presence of pollen grains in honey (Manning, 2015). These polyunsaturated acids are essential for human diet because they are unable to be synthesized in the gastrointestinal tract of humans (Ceksteryte *et al*., 2008).

This is one of the qualities used in differentiating types and quality of honey as well as the moisture content (Chataway, 1932). In this study, the percentage of the Refractive Index of the honey samples ranged from 1.48 ± 0.01 to 1.5 ± 0.01. The values fall within the standard range (1.49 to 1.50) recommended by Codex Alimentarius Commission (2001).

The mean range of pH values of the honey samples which varied from 3.50 ± 0.01 to 4.72 ± 0.01 showed that the honey samples were acidic, but had acidity level within the acceptable limits (3.42 – 6.1) set by Codex Alimentarius Commission (2001). The pH of honey is quite desirable because it influences the shelf life of honey inhibiting the growth of yeast cells that lead to fermentation.

Electrical conductivity in honey is influenced by the presence of organic acids, mineral content, moisture, viscosity as well as the geographical and botanical sources of the honey samples (Subbiah *et al*., 2015). Electrical conductivity can be used to distinguish between blossom honey and honeydew honey because the EC value of honeydew is greater than that of floral honey. In this study the mean value of the Electrical Conductivity ranged from 1.2 ± 0.01 to 17.70 ± 0.01 µS/cm. According to Codex standard the Electrical Conductivity should not be more than 0.08 mS/cm in floral honeys (Codex Alimentarius Commission, 2001). From the standard it means that the honey samples were from floral origin since all of them had values within the international set standard.

The sum of the fructose and glucose in the analyzed samples showed that they had an

average range of 68.8 ± 0.01 to 82.62 ± 0.01, which were within the acceptable international standard of not being less than 60 g/100 g (Codex Alimentarius Commission, 2001) for reducing sugars. Also, the mean percentage values of fructose were found to be more than that of glucose (ratio greater than 1) in entire samples. This is an indication of slower crystallization (Draiaia *et al.,* 2015) and also is in conformity with other studies in Nigeria (Buba *et al*., 2013; Lawal *et al.,* 2017; Nweze *et al*., 2017; Bako *et al*., 2019) and other countries (Nikolova *et al*., 2012; Bogdanov *et al*., 2013; Subbiah *et al*., 2015; El Sohaimy *et al*., 2015; Boussaid *et al*., 2018). The notable variations observed in the mean percentage values of fructose, glucose and ratio of fructose/glucose may be attributed to the differences in floristic composition of the honey sources and their location (Can *et al*., 2015).

The percentage sucrose in honey samples ranged from 1.07 ± 0.01 to 2.10 ± 0.02 % which was an indication that the entire sample met with the recommended standard of not exceeding the acceptable limit of 5 g/100 g (Codex Alimentarius Commission, 2001).

**Conclusion:** The results of the honey analyses from five states of Nigeria showed that the honeys can be classified into three botanical groups; unifloral, bifloral and polyfloral honeys. The number of identified genera and diversity indices of plant sources of nectar and pollen showed that large and diverse meliferous plant species were utilized by the honeybees for the production of the honeys. The biochemical characteristics of the honey samples tested in this study were within the limits of International standard set by Codex Alimentarius and EU Commissions for blossom honeys except water content of honey samples from Akpanya and Anyigba that exceeded the limit.

## ACKNOWLEDGEMENTS

The author wish to acknowledge Chiamaka F. Iwgeonu and Joy N. Ekowo who contributed meaningfully towards the data analyses of this study. My appreciation goes to Devine Laboratory, 12 Ibagwa Road, Nsukka, Enugu State for analyzing the biochemical aspect of this study. I also appreciate the anonymous reviewers for their meaningful contributions.

## REFERENCES

ABU-TARBOUSCH, H. M., AL-KAHTANI, H. A. and EL-SARRAGE, M. S. (1993). Floral-type identification and quality evaluation of some honey types. *Food Chemistry*, 46: 13 – 17.

ADAMS, B. A., OSIKABOR, B., OLOMOLA, A. and ADESOPE, A. A. A. (2010). Analysis of physical and chemical composition of honey samples in selected market in Ibadan metropolis. *Journal of Agriculture and Social Research,* 10(2): 31 – 36.

ADEBIYI, F. M., AKPAN, I., OBIAJUNWA, E. T. and OLANIYI, H. B. (2004). Chemical /physical characterization of Nigerian honey. *Pakistan Journal of Nutrition*, 3(5): 278 – 281.

AGWU, C. O. C. and AKANBI, T. O. (1985). A palynological study of honey from four vegetation zones of Nigeria. *Pollen et Spores*, 25(3-4): 335 – 348.

AGWU, C. O. C. and UWAKWE, G. O. (1992). Melissopalynological study of Abia and Imo States Nigeria honey. *Nigerian Journal of Botany*, 5: 85 – 91.

AINA, D. O. (2016). Pollen and physicochemical characterization of honey samples from Ankpa Local Government Area of Kogi State, Nigeria. *FUTO Journal Series (FUTOJNLS),* 2(2): 160 – 172.

ANYANSOLA, A. A. and BANJO, A. D. (2011). Physico-chemical evaluation of authenticity of honey marketed in southwestern Nigeria. *Journal of Basic and Applied Scientific Research,* 1(12): 3339 – 3344.

AOAC (2005). *Official Methods Analysis of the Association of Official Analytical Chemists.* 18th Edition, Association of Official Analytical Chemists (AOAC), Maryland, USA.

APLF (1974). *Pollen et Spore d'Afrique Tropicale*. Trauaux et Documents de Geographic Tropicale, CEGET, Association des

Planetariums de Langue Francaise (APLF), Talence 16.

AZONWADE, F. E., PARAISO, A., DOSSA, C. P. A., DOUGNON, V. T., N'TCHA, C., MOUSSE, W. and BABA-MOUSSA, L. (2018). Physicochemical characteristics and microbiological quality of honey produced in Benin. *Journal of Food Quality*, 2018: 1 – 13.

BAKO, T., MAMAI, E. A. and BATURE, B. J. (2019). Determination of quality parameters of honey from Taraba State, Nigeria. *Chemical and Biomolecular Engineering,* 4(1): 1 – 9. 352

BHATTACHARYA, K., MAJUMDAR, M. R. and BHATTACHARYA, S. G. (2006). *A Textbook of Palynology (Basic and Applied)*. New Central Book Agency (P) Limited, London.

BOGDANOV, S., JURENDIC, T., SIEBER, R. and GALLMANN, P. (2013). Honey for nutrition and health: A review. *Journal of the American College Nutrition*, 27(6): 677 – 689.

BONNEFILLE, R. and RIOLETT, G. (1980). *Pollen de Savanes d'Afrique Orientale.* Centre National de la Recherche Scientifique, Paris, France.

BOUSSAID, A., CHOUAIBI, M., REZIG, L. HELLAL, R., DONSI, F., FERRARI, G. and HAMDI, S. (2018). Physicochemical and bioactive properties of six honey samples from various floral origins from Tunisia. *Arabian Journal of Chemistry,* 11: 265 – 274.

BUBA, F., GIDADO, A. and SHUGABA, A. (2013). Analysis of Biochemical composition of honey sample from North-East Nigeria. *Biochemistry and Analytical Biochemistry* 2(3): 139. doi:10.4172/2161-1009.1000139.

CACCAVARI, M. and FAGUNDEZ, G. (2010). Pollen spectra of honey from the Middle Delta of the Parana River (Argentina) and their environmental relationship. *Spanish Journal of Agricultural Research*, 8(1): 42 – 52.

CAN, Z., YILDIZ, O., SAHIN, H., TURUMTAY, E. A., SILICI, S. and KOLAYLI, S. (2015). An investigation of Turkish honeys: Their physico-chemical properties, antioxidant capacities and phenolic profiles. *Food Chemistry*, 180: 133 – 141.

CEKSTERYTE, V., RACYS, J., KASKONIENE, V. and VENSKUTONIS, P. R. (2008). Fatty acid composition in beebread. *Biologija*, 54(4): 253 – 257.

CHATAWAY, H. D. (1932). The determination of moisture in honey. *Canadian Journal of Research*, 6(5): 532 – 547.

CODEX ALIMENTARIUS COMMISSION (2001). *Revised Codex Standard for Honey*. Codex STANS 12-1981, Rev.1 (1987). Rev.2 (2001); FAO/WHO.

DAFNI, H., LENSKY, Y. and FAHN, A. (1988). Flower and nectar characteristics of nine species of *Labiatae* and their influence on honey visits. *Journal of Apicultural Research*, 27(2): 103 – 114.

DRAIAIA, R., CHEFROUR, A., DAINESE, N., BORIN, A., MANZINELLO, C., GALLINA, A. and MUTINELLI, F. (2015). Physico-chemical parameters and antibiotics residuals in Algerian honey. *African Journal of Biotec*hnology, 14(14): 1242 – 1251.

EBIGWAI, J. K., EGBE, A. and JUSTIN, D. E. (2017). Botanical affinity and physicochemical parameters of honey samples obtained from bee hives in Cross River State Nigeria. *Journal of Scientific Research and Reports*, 14(6): 1 – 23.

ECKERT, J. E. (1942). The pollen required by a colony of honeybees. *Journal of Economic Entomology*, 35(1): 309 – 311.

EL SOHAIMY, S. A., MASRY, S. H. D. and SHEHATA, M. G. (2015). Physicochemical characteristics of honey from different origins. *Annals of Agricultural Science,* 60(2): 279 – 287.

EU COMMISSION (2002). Council Directive 2001/110/EC of 20 December, 2001 relating to honey. *Official Journal of the European Commission*, L10: 47 – 52.

HASSAN, H. M. M. (2011). Chemical composition and nutritional value of palm pollen

grain. *Global Journal of Biotechnology and Biochemistry*, 6(1): 01 – 07.

IGE, O. E. and MODUPE, T. O. (2010). Pollen characterization of honey samples from North Central Nigeria. *Journal of Biological Sciences,* 10(1): 43 – 47.

KAYODE, J. and OYEYEMI, S. D. (2014). Pollen analysis of *Apis mellifera* honey collected from Nigeria. *American Journal of Agriculture and Forestry*, 2(5): 226 – 231.

LAWAL, O. O., OBOH, E. O., BASSEY, S. C. and OBETEN, O. O. (2017). Composition of sugars in honey produced in the South-South and South-West regions of Nigeria. *International Journal of Sciences*, 6(8): 179 – 184.

LOUVEAUX, J., MAURIZIO, A. and VORWOHL, G. (1978). Methods of melissopalynology. *Bee World*, 59(4): 139 – 157.

LULLAH-DEH, J. A., KHAN, M. E. and ENEJI, I. S. (2018). Physicochemical characteristics of honey sample from Mambilla Plateau, Nigeria. *Journal of Biomaterials*, 2(1): 7 – 11.

MANNING, R. (2015). Fatty acids in pollen: a review of their importance for honey bees. *Bee World*, 82(2): 60 – 75.

MOAR, N. T. (1985). Pollen analysis of New Zealand. *Journal of Agricultural Research,* 28: 39 – 70.

MOLAN, P. C. (1992). The antibacterial activity of honey 1. The nature of antibacterial activity. *Bee World*, 73: 5 – 28.

MOORE, P. D. and WEBB, J. A. (1978). *An Illustrated Guide to Pollen Analysis*. 1st Edition, Hodder and Stoughton, London.

NDIFE, J., ABIOYE, L. and DANDAGO, M. (2014). Quality assessment of Nigerian honey sourced from different floral locations. *Nigerian Food Journal*, 32(2): 48 – 55.

NIGUSSIE, K., SUBRAMANIAN, P. A. and MEBRAHTU, G. (2012). Physicochemical analysis of Tigray honey: An attempt to determine major quality markers of honey. *Bulletin of Chemical Society of Ethiopia*, 26(1): 127 – 133.

NIKOLOVA, K., PANCHEV, I., SAINOV, S., GENTSCHEVA, G. and IVANOVA, E. (2012). Selected physical properties of lime bee honey in order to discriminate between pure honey and honey adulterated with glucose. *International Journal of Food Properties*, 15(6): 1358 – 1368.

NJOKUOCHA, R. C. and EKWEOZOR, C. C. (2007). Pollen contents of commercial honeys of Opi, Nsukka, Enugu State, Nigeria. *Plant Product Research Journal*, 11: 5 – 11.

NJOKUOCHA, R. C. and NNAMANI, N. A. (2009). Palynological analysis of seven samples of honey from the rainforest-savannah vegetation of east-middle belts of Nigeria. *Nigerian Journal of Botany,* 22(1): 189 – 202.

NJOKUOCHA, R. C. and OSAYI, E. E. (2015). Physicochemical assessment and pollen analysis of honey from Nsukka, Enugu State, Nigeria. *Nigerian Journal of Botany*, 28(1): 95 – 107.

NJOKUOCHA, R. C., DIM, K. I., ONYEJEKWE, O. K. and NWOKORIE, V. U. (2019). Determination of the concentration of some mineral elements and pollen spectra of *Apis mellifera* L. honeys from different locations in Nigeria. *Animal Research International*, 16(1): 3186 – 3197.

NWEZE, J. A., OKAFOR, J. I., NWEZE, E. I. and NWEZE, J. E. (2017). Evaluation of physicochemical and antioxidant properties of two stingless bee honeys: a comparison with *Apis mellifera* honey from Nsukka, Nigeria. *BMC Research Notes*, 10: 566. doi: 10.1186/s13104-017-2884-2

ODDO, L. P., PIAZZA, M. G., SABATINI, A. G. and ACCORTI, M. C. (1995). Characterization of unifloral honeys. *Apidologie*, 26: 453 – 465.

OYELEKE, S. B., DAUDA, B. E. N., JIMOH, T. and MUSA, S. O. (2010). Nutritional analysis antibacterial effects of honey on bacterial wound pathogens. *Journal of Applied Scientific Research*, 6(11): 1516 – 1565.

RAMIREZ-ARRIAGA, E., NAVARRO-CALVO, L. A. and DIAZ-CARBAJAL, E. (2011). Botanical

characterization of Mexican honeys from a subtropical region (Oaxaca) based on pollen analysis. *Grana*, 50: 40 – 54.

SCHAFER, M. O., DIETEMANN, V., PIRK, C. W. W., NEUMANN, P., CREWE, R. M., HEPBURN, H. R., TAUTZ, J. and CRAILSHEIM, K. (2006). Individual versus social pathway to honeybee worker reproduction (*Apis mellifera*): pollen or jelly as protein source for oogenesis. *Journal of Comparative Physiology A,* 192: 761 – 768.

SINGH, N. and BATH, P. K. (1997). Quality evaluation of different types of Indian honey. *Food Chemistry*, 58(1-2): 129 – 133.

SONG, X-Y., YAO, Y-F. and YANG, W-D. (2012). Pollen analysis of natural honeys from the central region of Shanxi, North China. *PLoS One*, 7(11): e49545. doi: 10.1371/journal.pone.0049545

SOWUNMI, M. A. (2001). Microscopic analysis of honey. *The Nigerian Field*, 66: 125 – 133.

SUBBIAH, B., STEMBRIDGE, A. L. and MORISON, K. R. (2015). *Measurement and calculation of the electrical conductivity of model honey solutions*. APCCHE 2015 Congress incorporating Chemeca 2015, 27 Sept – 01 Oct 2015, Melbourne, Victoria.

SUBRAMANIAN, R., HEBBAR, H. U. and RASTOGI, N. K. (2007). Processing of honey: a review. *International Journal of Food Properties,* 10(1): 127 – 143.

TSIGOURI, A., PASSALOGLOU-KATRALI, M. and SABATAKOU, O. (2004). Palynological characteristics of different unifloral honeys from Greece. *Grana*, 43(2): 122 – 128.

Y'BERT, J. P. (1979). *Atlas de Pollen de Cote D'Ivoire*. Official de la Redurche Scientifique et Technique Outremer, Paris.

YADATA, D. (2014). Detection of the electrical conductivity and acidity of honey from different areas of Tepi. *Food Science and Technology*, 2(5): 59 – 63.