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Pollen Characterization and Physicochemical Analysis of Six Nigerian Honey Samples; Test for Authenticity

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ABSTRACT

Honey is a popular product consumed for its health benefits. It is an effective antimicrobial an antioxidant agent. Globally, palynological and chemical methods are among the means of authenticating honey quality, geographical origin and floral origin. Six honey samples from six Nigerian towns (Abi, Ikom, Lokpanta, Nsukka, Okigwe and Shaki) were subjected to the aforementioned tests. Eighty-six pollen taxa were recorded in all the samples. The richest sample with seventy-three taxa was from Nsukka, followed successively by Okigwe, Lokpanta, Shaki, Ikom and Abi samples with sixty-eight, sixty-seven, sixty-two, fifty-nine and fifty-seven pollen species respectively. The oil palm Elaeis guineensis pollen dominated the samples in different proportions except Shaki honey dominated by Acacia spp., The commonest plant family was Fabaceae (Caesalpinioideae, Mimosoideae, Papilionideae) with twenty-one taxa followed by Euphorbiaceae, Combretaceae, with four representatives and Rubiaceae with three taxa each. The physico-chemical analysis carried out were total moisture, total ash content, colour assessment, percentage of total solids, relative density, acidity, and Fischer's Test. The samples were found to concur with the international standards for honey.

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Introduction

Honey, a concentrated solution of a complex mixture of sugars dominantly fructose and glucose which is produced by honey bees *Apis mellifera adansonii* has been used by man for thousands of years both as a natural sweetener, source of energy, and a healing agent which suppresses disease causing agents (National Honey Board, 2002; Khalil et al., 2011; Aled et al., 2012; Maddocks et al., 2012; Nwankwo et al., 2014; Ng and Lim, 2015; Adeonipekun et al., 2016; Kaygusuz et al., 2016; Ng et al., 2017; Fatimah et al., 2018, 2019; Al-Kafaween et al., 2020). Furthermore, it contains macro and microelements such as water, carbohydrates, minerals, amino acids, organic acids, proteins, volatile substances, enzymes, phenolic compounds, together with other compounds necessary for normal human growth and development (Jasicka-Misiak et al., 2012; Cimpoiu et al., 2013). The hygroscopic nature of honey which enables dehydrating bacteria by decreasing the moisture of the environment had been reported. Again, the high sugar content and low PH of honey has been documented to hinder the growth of bacteria (Eswaran et al., 2015; Nishio et al., 2016). Nolan et al. (2019) had attributed the antimicrobial potential of honey to its different components such as high sugar contents, low pH, polyphenolic compounds, hydrogen peroxide, 1,2-dicarbonyl compounds, and defensin-1. Good quality honey has been linked to the healing of injured intestinal mucosa as it stimulates the growth of new tissues and works as an anti-inflammatory agent (Kek et al., 2014). In addition, Afrin et al. (2017) had reported the ability of honey at low concentrations to inhibit colon cancer. Apart from these, honey also has the potential to serve as a natural food antioxidant (Saxena et al., 2010; Cimpoiu et al., 2013; Boukraâ, 2015). Nolan et al. (2019) who cited Esteraf-Oskouel and Najafi (2013), who highlighted the uses of honey in their review which included its use by the ancient Egyptians who had used it in embalmment, as a topical agent and for the dressing of wounds. Furthermore, the Greeks had used it also for wound healing, and a remedy for gout, pain, fever. In recent times, there has been high incidences of *Diabetes mellitus* which has promoted the use of natural honey in place of processed sugar and allied products. In Nigeria different honey samples are sold both in the open markets and supermarkets. These are sourced both from the wild and from apiaries. The quality of most of these honey samples need to be ascertained. Siddiqui et al. (2017) had reported that commercial honey is often

adulterated or falsely labeled for economic gains. Presently no established standards exist for certifying the authenticity of these Nigerian honey samples.

Among the major ways of determining the botanical and geographical origin of honey is the assessment of its pollen content (Veitez, 1950; Anklam, 1988; Ghidini et al., 2008; Makhloufi et al., 2010; Jasicka-Misiak et al., 2012). In Nigeria, several authors have worked on different aspects of melissopalynology. The most popular published works are those of Afolabi (1974) and Sowunmi (1976) who set the pace for other researchers. In the last decade and half, honey studies in Nigeria has increase due to the global awareness about *Diabetes mellitus* (Ige and Modupe, 2010; Adeonipekun 2010, 2012; Agbagwa et al., 2011; Aina and Owonibi, 2011; Ayansola, 2012; Agwu et al., 2013; Olugbemi et al., 2013; Kayode and Oyeyemi, 2014; Ndife et al., 2014; Nwankwo et al., 2014; Orijemie 2017; Kayefor et al., 2017; Oyeyemi, 2017).

The use of palynological and physicochemical data in ascertaining how genuine or adulterated a honey sample is having been carried out and is still on in different parts of the world (Saxena et al., 2010; Anklam, 2010; Ramirez-Arriaga et al., 2011; Rateb and Hussein, 2012; Song et al., 2012; Cimpoiu et al., 2013; Jasicka-Misiak et al., 2012; Kek et al., 2014).

This present study was undertaken to enrich the published records of melissopalynological studies in Nigeria, assess the authenticity of honey from the rural areas of Nigeria and compare the results with those already reported from more urban areas like Lagos, Abuja etc and also infer whether their qualities fall within the international standards so as to pave way for export.

Materials and Methods

Honey Samples and Preparation

Six honey samples were sourced between July 2011 – October, 2011 from the open markets from six towns in six states in Nigeria viz: (Abi, Cross River State; Ikom in Akwa Ibom State; Lokpanta, Abia State; Nsukka, Enugu State, Okigwe, Imo State and Shaki, Oyo State). The honey samples were brought to the Biological laboratory of Redeemer's University and stored prior to preparation. The different honey samples were subjected to palynological and chemical analysis. Standard palynological preparation methods as outlined by Louveaux et al. (1978), with minor modifications after Low et al. (1989) were adopted. The acetolysis were after Erdtman (1969). The prepared slides were analyzed and five hundred pollen grains were counted per sample (de Novais and Absy 2013). The inherent pollen was identified using (Sowunmi 1973,1995; Bonnefille and Riollet, 1980; Willard et al., 2004; Gosling et al., 2013). In addition, fungal materials, charred Graminae cuticles, diatom frustules were all recorded as miscellaneous palynomorphs. These were not included in the total and percentage pollen calculations. Pollen types recorded per sample were classified (Table 1) as predominant pollen types (>45%), secondary pollen types (16-45%), important minor pollen types (3-15%) and minor pollen types (<3%) (Jasicka-Misiak, 2012; Rateb and Hussein, 2012; Schweizer et al., 2014; Sahney, et al., 2018). Photomicrographs (Figure 3 and 4) of the inherent palynomorphs were taken with a United binocular microscope with an inbuilt Motic-2 camera at the palynology laboratory of Earthprobe Nigeria Limited. The chemical analysis followed the International Honey standards (Bogdanov et al., 2009; IHC website) as no standards exist presently for Nigerian honey

Physico-Chemical Analysis

The methods outlined in (Bogdanov and Martin, 2002; Bogdanov et al., 1999) were adopted as no standards exist presently for Nigerian honey. The different parameters investigated were i). Total Moisture (Refractometer Method) ii) Percentage of total solids, iii). Total Ash Content, iv). pH v). Relative Density, vi.) Acidity (% Gluconic Acid), vii). Colour assessment and viii). Fischer's Test.

All physicochemical parameters were done according to the harmonized International Honey Commission (Bogdanov et al.,2009: IHC website). An Abbe refractometer was used in determining the moisture content. Total Moisture (Refractometer Method).

Determination of total solids: the percentage total solid for each honey sample was determined using: Total solids (%) = 100-Moisture content.

Total Ash Content

Determination of total ash content:

An ash dish was initially heated in the electric furnace for 500°C, it was later removed, cooled in the desiccator at room temperature and weighed to 0.001g and the weight (m2) of the empty dish noted. The other procedure outlined by Bogdanov (2009), was followed through for the ashing process until a constant weight was got (m1). Finally, the proportion of ash WA in g/100g of honey was calculated using the formula:

$$WA = ((m1 - m2) \div m0) \times 100$$

Where:

m0 = weight of honey sample taken

m1 = weight of empty dish + ash

m2 = weight of empty dish

The answer is rounded to two decimal places

Relative density: Apparatus: specific gravity bottle, distilled water, water bath, honey sample

A clean and thoroughly washed specific gravity bottle was weighed and filled up with freshly boiled and cooled distilled water which has been maintained at 27°C ± 1°C. The water was removed and the bottle dried again and filled with the honey sample maintained at the sample temperature. The bottle was weighed again and the Relative density calculated thus:

$$\text{Relative Density} = \frac{C-A}{B-A}$$

Where;

C = Mass of the specific gravity bottle with honey in (g)

A = Mass of the empty specific gravity bottle in (g)

B = Mass of the empty specific gravity bottle with water in (g)

Determination of pH; pH was measured using a pH meter, while the titrimetric method was employed in determining the total acidity.

Determination of acidity: The acidity is expressed as the percentage of gluconic acid.

Colour determination: The colour of the different honey samples, were determined, with the aid of a spectrophotometer (Spectronic 20 D). The procedure involved reading the absorbance of the honey against distilled water at a wavelength of 660 nm.

Ficher's Test

Two g of the honey sample was dissolved in 10ml of water and extracted with 30ml ether in a separating funnel and the layer concentrated to 5ml. Later, 2ml of freshly prepared

resorcinol solution was added, the mixture was shaken, and the colour noted. A cherry red colour appearing in a minute indicated the presence of commercially invert sugar. Yellow and other colours were insignificant.

Statistical Analysis

Similarity and dissimilarity level (comparative analysis) between and among the samples from the different locations was determined by constructing a dendrogram (close neighbour analysis) with the physicochemical parameters using SPSS 23.0 (Figure 2).

Results

Melissopalynology

The occurrences of the recovered palynomorphs for each honey sample are highlighted in Table 1a,b below. The richest sample with seventy-three taxa was from Nsukka, followed successively by Okigwe, Lokpanta, Shaki, Ikom and Abi samples with sixty-eight, sixty-seven, sixty-two, fifty-nine and fifty-seven pollen species respectively. The oil palm *Elaeis guineensis* pollen dominated the samples in different proportions except Shaki honey dominated by *Acacia* spp., The commonest plant family was Fabaceae (*Caesalpinioideae, Mimosoideae, Papilionideae*) with twenty-one taxa followed by *Euphorbiaceae, Combretaceae*, with four representatives and *Rubiaceae* with three taxa each. None of the dominant pollen was up to 45% in abundance. Hence all the honey sample s is multifloral. The percentage occurrences of the palynomorps recovered from each honey sample are highlighted below in Table 1a,b.

Table 1a: Percentage occurrences of the recovered pollen in the different honey samples. None of the samples fell within the dominant pollen type common in monofloral honeys. Nsukka, Okigwe and Shaki honeys fell within the secondary pollen due to the percentage occurrences of *Elaeis guineensis* with values above 16%

Important minor pollen (IMP) <16%-3%	Minor pollen <3%
Honey Sample: Abi; Dominant pollen (DP) >45%: Nil; Secondary pollen (SP) <45%-16%: Nil	
Elaeis guineensis Jacq. (9.6%), *Parinari kerstingii* Engl. (6.0%), *Rhizophora* spp. (5.2%), *Ceiba pentandra* (Linn.) Gaertn. (4.4%), *Rutaceae* spp. (4.0%), *Combretum* spp. (3.8%), *Poaceae* (3.6%), *Paullinia pinnata* Linn. (3.0%).	*Terminalia* spp. (2.8%), *Bombax buonopozense* P. Beuav. (2.8), *Indigofera* spp. (2.8%), *Alchornea* spp. (2.6%), *Mussaenda* spp. (2.8%), *Nympheae lotus* L. (2.4%), *Solanum* spp. (2.4%), *Capsicum* spp. (2.4%), *Lannea acida* (2.0%), *Pterocarpus santalanoides* L'Her. ex DC. (2.0%), *Brachystegia eurycoma* Harms (2%), *Allophyllus africanus* P. Beauv. (1.6%), *Anogeissus leiocarpus* (DC) Guill & Perr. (1.6%), *Desmodium* sp. (1.6%), *Berlinia grandiflora* (Vahl) Hutch. & Dalz. (1.6%), *Sapotaceae* sp. *(1.6%), Spondianthus preusii* Engl. (1.6%), *Olax* sp. (1.6%), *Sacoglottis gabonensis* (Baill.) Urb. (1.4%), *Pentaclethra macrophylla* Benth. (1.4%), *Milicia excelsa* (Welw.) C.C.Berg. (1.4%), *Hyphaea tiebaica* (Linn.) Mart. (1%), *Proteacidites* sp. (1%), *Albizzia zygia* (DC.) JF Macbride (1%), *Asteraceae* (1%), *Calliacarpa* sp. (1%), *Amanoa* sp. (.8%), *Entanda abyssinica* Steud. ex A.Rich. (.8%), *Irvinga gabonensis* (Aubry-Lecomte ex O'Rorke)Baill. (.8), *Newbouldia laevis* (P.Beauv.) Seem. ex Bureau (.8%), *Ludwigia* sp. (.8%), *Phyllantus reticulatus* Poir. (.8%), *Schinus* sp. (.8%), *Symphonia globulifera* Linn. f. (.8%), *Triplochyton scleroxylon* K. Schum (.6%), *Batis* sp. (.4%), *Pteris* (.4%), *Dalbergia erasi* (.4%), *Polygala* sp. (.4%). *Conocarpus erecta* L. (.4%), *Medinilla mirabilis* (Gilg) Jacq.-Fél. (.4%), *Afzelia africana* Sm. ex Pers. (.2%), *Daniellia oliveri* (Rolfe) Hutch. & Dalz (.2%), *Gardenia imperialis* K. Schum. (.2%), *Myricaria germanica* (L.) Desv. (.2%), *Tetrapleura tetraptera* (Schum. & Thonn.) Taub. (.2%).
Honey Sample: Ikom; Dominant pollen (DP) >45%: Nil; Secondary pollen (SP) <45%-16%: Nil	
Elaeis guineensis Jacq. (10.8%), *Parinari kerstingii* Engl. (5.6%), *Rhizophora* spp. (3.6%), *Rutaceae* spp. (4.8%), *Poaceae* (4.2%), *Ceiba pentandra* (Linn.) Gaertn. (3.6%), *Pterocarpus* spp. (3.4%), *Combretum* spp. (3.2%), *Acacia* spp. (3.2%), *Terminalia* spp. (3.2%).	*Nympheae lotus* L. (2.8%), *Pterocarpus santalanoides* L'Her. ex DC. (2.8%), *Bombax buonopozense* P. Beuav. (2.6%) *Symphonia globulifera Symphonia globulifera* Linn. f. (2.4%), *Mussaenda* spp. (2.0%), *Alchornea* spp. (2.0%), *Indigofera* spp. (2.0%), *Berlinia grandiflora* (Vahl) Hutch. & Dalz. (2%), *Brachystegia eurycoma* Harms (1.6%), *Lannea* acida A. Rich. (1.6%), *Paullinia pinnata* Linn. (1.6%), *Irvinga gabonensis* (Aubry-Lecomte ex O'Rorke) Baill. (1.6%), *Mitragyna* spp. (1.6%), S*olanum* spp. (1.6%), *Sacoglottis gabonensis* (Baill.) Urb. (1.6%), *Ludwigia* spp. (1.4%), *Albizzia zygia* (DC.) JF Macbride (1.2%), *Anogeissus leiocarpus* (DC) Guill & Perr. (1.2%), *Cyperaceaepollis* spp. (1.2%), *Spondianthus preusii* Engl. (1.2%), *Anacardiaceae* spp. (1%), *Allophyllus africanus* P. Beauv. (1%), *Pentaclethra macrophyll*a Benth. (1%), Asteraceae (1%), *Coula edulis* Baill. (1%), *Hyphaene tiebaica* (Linn.) Mart. (1%), *Calliacarpa* spp. (1%), *Milicia excelsa* (Welw.) C. C. Berg. (1%), *Triplochyton scleroxylon* K. Schum (1%), *Proteacidites* spp. (1%), *Phyllantus reticulatus* Poir. (.8%), *Vitex doniana* Sweet (.8%), *Cocos nucifera* (L.) (.6%), *Sterculia* spp. (.6%), *Pterocarpus soyauxii* Taub. (.6%), *Desmodium spp*. (.6%), *Uapaca* spp. (.6%), *Capsicum* spp. (.4%), *Tephrosia* spp. (.4%), *Medinilla mirabilis* (Gilg) Jacq.-Fél. (.4%), *Conocarpus erecta* L. (.4%), *Dalbergia erasi* (.4%), *Batis* sp. (.2%), *Cassia senegalensis* (Linn.) (.2%), *Myricaria germanica* (L.) Desv. (.2%), *Newbouldia laevis* (P.Beauv.) Seem. ex Bureau (.2%).
Honey Sample: Lokpanta; Dominant pollen (DP) >45%: Nil; Secondary pollen (SP) <45%-16%: Nil	
Elaeis guineensis Jacq. (11.6%) *Parinari kerstingii* Engl. (7.0%), *Ceiba pentandra* (Linn.) Gaertn. (5.6%), *Rutaceae* spp. (4.0%).	*Indigofera* spp. (2.8%), *Pterocarpus santalanoides* L'Her. ex DC. (2.6%), *Hymenocardia acida* Tul (2.4%), *Mussaenda* spp. (2.4%), *Anacardiaceae* spp. (2.2%), *Poaceae* (2.0%), *Combretum* spp. (2.0%), *Irvinga gabonensis* (Aubry-Lecomte ex O'Rorke)Baill. (2.0%), *Paullinia pinnata* Linn. (2.0%), *Lannea acida* A. Rich. (2.0%), *Pentaclethra macrophylla* Benth. (1.8%), *Asteraceae(1.6%),Berlinia grandiflora* (Vahl) Hutch. & Dalz. (1.6%), *Bombax buonopozense* P. Beuav. (1.6%), *Brachystegia eurycoma* Harms (1.6%), *Pterocarpus soyauxii* Taub. (1.6%), *Solanum* spp. (1.6%), *Spondianthus preusii* Engl. (1.4%), *Alchornea* spp. (1.4%), *Annonaceae* spp. (1.4%), *Cleome spp.* (1.4%), *Hyphaene tiebaica* (Linn.) Mart. (1.4%), *Terminalia* spp. (1.4%), *Tetrapleura tetraptera* (Schum. & Thonn.) Taub. (1.4%), *Crudia klainei* Pierre (1.2%), *Phyllantus reticulatus* Poir. (1.2%), *Triplochyton scleroxylon* K. Schum (1.2%), *Allophyllus africanus* P. Beauv. (1.2%), *Sapotaceae* spp. (1.2%), *Afraegle paniculata* (Schumach.) Engl. (1%), *Carapa procera* DC. (1%), *Anogeissus leiocarpus* (DC) Guill & Perr. (1%), *Nymphea lotus* L. (1%), *Olax* spp. (1%), *Tephrosia* spp. *(1%), Cyperaceaepollis* spp. (1%), *Khaya senegalensis* (Desv.) A. Juss. (1%), *Mitragyna* spp. (.8%), *Phyllantus reticulatus* Poir. (.8%), *Amanoa* spp. (.6%), *Capsicum* spp. (.6%), *Coclospermum planchonii* Hook f. (.6%), *Desmodium spp.* (.6%), *Dissotis* spp. (.6%), *Entadrophragma angolense (*Welw.) C. DC. (.6%), *Ximenia americana* Linn. (.6%), *Milicia excelsa* (Welw.) C.C.Berg.a (.6%), *Proteacidites* spp. (.6%), *Afzelia africana* Sm. ex Pers. (.4%), *Albizzia zygia* (DC.) JF Macbride (.4%), *Polygala* spp. (.4%), *Sacoglottis gabonensis* (Baill.) Urb. (.4%), *Syzygium guineense* (Willd.) DC. (.4%), *Sterculia* spp. (.4%), *Medinilla mirabilis* (Gilg) Jacq.-Fél. (.4%), *Daniellia oliveri* (Rolfe) Hutch. & Dalz (.4%), *Crotolaria retusa* L. (.4%), *Myrtaceae* spp. (.4%), *Calliacarpa* spp. (.4%), *Conocarpus erecta* L. (.2%), *Myricaria germanica* (L.) Desv. (. 2%), *Vitex doniana* Sweet (.2%), *Vernonia* spp. (.2%).

Table 1b: Percentage occurrences of the recovered pollen in the different honey samples. None of the samples fell within the dominant pollen type common in monofloral honeys. Nsukka, Okigwe and Shaki honeys fell within the secondary pollen due to the percentage occurrences of *Elaeis guineensis* with values above 16%

Important minor pollen (IMP) <16%-3%	Minor pollen <3%
Honey Sample: Nsukka; Dominant pollen (DP) >45%: Nil; Secondary pollen (SP) <45%-16%: *Elaeis guineensis* Jacq. (16%)	
Lannea acida A. Rich. (8.4%), *Parinari kerstingii* Engl. (5.0%), *Rutaceae* spp. (4.8%), *Combretum* spp. (3.6%).	*Poaceae* (2.8%), *Acacia* spp. (2.6%), *Terminalia* spp. (2.4%), *Anacardiaceae* spp. (2.4%), *Indigofera* spp. (2.4%), *Tephrosia* spp. (2.0%), *Ceiba pentandra* (Linn.) Gaertn. (2.0%), *Paullinia pinnata* Linn. (2.0%), *Sapotaceae* spp. *(1.*6%), *Entadrophragma angolense* (Welw.) C. DC. (1.6%), *Crudia klainei* Pierre (1.6%), *Bombax buonopozense* P. Beuav. (1.6%), *Syzygium guineense* (Willd.) DC. (1.6%), *Ludwigia* spp. (1.2%), *Pentaclethra macrophylla* Benth. (1.2%), *Albizzia zygia* (DC.) JF Macbride (1.0%), *Vernonia* spp. (1.0%), *Annonaceae* spp. (1.0%), *Berlinia grandiflora* (Vahl) Hutch. & Dalz. (1.0%), *Cassia senegalensis* (Linn.) (1.0%), *Cyperaceaepollis spp.* (1.0%), *Brachystegia eurycoma* Harms (1.0%), *Irvinga gabonensis* (Aubry-Lecomte ex O'Rorke)Baill. (1.0%), *Gardenia imperialis* K. Schum. (1.0%), *Hymenocardia acida* Tul (1.0%), *Myrtaceae* spp. (1.0%), *Khaya senegalensis*(1.0%), *Asteraceae* (.8%),*Mussaenda* spp. (.8%), *Phyllantus reticulates* Poir. (.8%), (.8%), *Polygala* spp. (.8%), *Crotolaria retusa* L. (.8%), *Cocos nucifera* (L.) (.6%), *Allophyllus africanus* P. Beauv. (.6%), *Olax* spp. (1.0%) *Prosopis africana* (Guill. & Perr.) Taub. (.6%), *Proteacidites* spp. (.6%), *Sacoglottis gabonensis* (Baill.) Urb. (.6%), *Solanum* spp. (.6%), *Triplochyton scleroxylon* K. Schum (.6%), *Ximenia americana* Linn. (.6%), *Spondianthus preusii* Engl. (.6%), *Dissotis spp.* (.4%), *Afzelia africana* Sm. ex Pers. (.4%), *Anogeissus leiocarpus* (DC) Guill & Perr. (.4%), *Calliacarpa spp.* (.4%), *Capsicum spp.* (.4%),*Coula edulis* Baill.(.4%), *Delonix regia* (Boj. ex Hook) Raf. (.4%), *Entanda abyssinica* Steud. ex A.Rich. (.4%), *Hyphaene tiebaica* (Linn.) Mart. (.4%), *Medinilla mirabilis* (Gilg) Jacq.-Fél. (.4%), *Milicia excelsa (*Welw.) C.C.Berg. (.4%), *Myricaria germanica* (L.) Desv (4%), *Nymphea lotus* L. (.4%), *Pterocarpus santalanoides*(.4%), *Pterocarpus soyauxii* Taub. (.4%), *Vitex doniana* Sweet (.4%), *Amanoa* spp. (.2%), *Coclospermum planchonii* Hook f. (.2%), *Carapa procera* DC. (.2%), *Daniellia oliveri* (Rolfe) Hutch. & Dalz (.2%), *Conocarpus erecta* L (.2%), *Sterculia* spp. (.2%), *Symphonia globulifera* Linn. f. (.2%), *Tetrapleura tetraptera* (Schum. & Thonn.) Taub. (.2%).
Honey Sample: Okigwe; Dominant pollen (DP) >45%: Nil; Secondary pollen (SP) <45%-16%: *Elaeis guineensis Jacq.*(23.6%)	
Parinari kerstingii Engl. (6.4%), *Ceiba pentandra*(Linn.) Gaertn. (5.2%).	*Bombax buonopozense* P. Beuav. (2.6%), *Nympheae lotus* L. (2.4%), *Paullinia pinnata* Linn. (2.4%), *Brachystegia eurycoma* Harms (2.0%), *Combretum* spp. (2.0%), *Indigofera* spp. (2.0%), *Lannea acida* A. Rich. (2.0%), *Rutaceae* spp. (2.0%), *Pterocarpus santalanoides* L'Her. ex DC. (2.0%), *Amanoa* sp. (1.6%), *Berlinia grandiflora* (Vahl) Hutch. & Dalz. (1.6%), *Cleome spp.* (1.6%), *Crudia klainei* Pierre (1.6%), *Olax* spp. (1.6%), *Pterocarpus soyauxii* Taub. (.4%), (1.6%), *Solanum* spp. (1.6%), *Albizzia zygia* (DC.) JF Macbride (1.4%), *Alchornea cordifolia* (Schum. & Thonn.) Mull. Arg (1.4%), *Annonaceae* spp. (1.4%), *Tetrapleura tetraptera* (Schum. & Thonn.) Taub. (1.4%), *Vernonia* spp. (1.4%), *Carapa procera* DC (1.2%), *Irvinga gabonensis* (Aubry-Lecomte ex O'Rorke)Baill. (1.2%), *Mitragyna* spp. (1.2%), *Phyllantus reticulatus* Poir. (1.2%), *Triplochyton scleroxylon* K. Schum (1.2%), *Allophyllus africanus* P. Beauv. (1%), Asteraceae (1%), *Pentaclethra macrophylla* Benth. (1%), *Polygala* spp. (1%), *Afraegle paniculata* (Schumach.) Engl. (.8%), *Ludwigia* spp. (.8%), *Spondianthus preusii* Engl. (.8%), *Terminalia* spp. (.8%), *Anogeissus leiocarpus* (DC) Guill & Perr. (.8%), *Poaceae* (.8%), *Sapotaceae* spp. (.8%), *Cyperaceaepollis* spp. (.8%), *Antiarias africana* Engl. (.6%), *Dissotis* spp. (.6%), *Desmodium* spp. (.6%), *Hymenocardia acida* Tul. (6%), *Khaya senegalensis* (.6%), *Calliacarpa spp*. (.4%), *Cocos nucifera* (L.) (.4%), *Coula edulis* Baill. (.4%), *Coclospermum planchonii* Hook f. (.4%), *Crotolaria retusa* L. (.4%), *Daniellia oliveri* (Rolfe) Hutch. & Dalz (.4%), *Hyphaene tiebaica* (Linn.) Mart. (.4%), *Medinilla mirabilis* (Gilg) Jacq.-Fél. (4%), *Crudia klainei* Pierre, (.4%),*Milicia excels* (Welw.) C.C.Berg. (.4%), *Mussaenda* spp. (.4%), *Vitex doniana* Sweet (.4%), *Symphonia globulifera* Linn. f. (.4%), *Schinus* spp. (.2%), *Cassia senegalensis* (Linn.) (.2%), *Conocarpus erecta* (.2%), *Myricaria germanica* (L.) Desv. (.2%), *Proteacidites* spp. (.2%), *Sacoglottis gabonensis* (Baill.) Urb. (.2%), *Ximenia americana* Linn. (.2%).
Honey Sample: Shaki; Dominant pollen (DP) >45%: Nil; Secondary pollen (SP) <45%-16%: *Acacia* spp. (22.8%)	
Tephrosia spp. (11.2%), *Parinari kerstingii* Engl. (7.6%); *Ceiba pentandra* (Linn.) Gaertn. (4.0%), *Combretaceae* (4.0%), *Poaceae* (3.2%), *Isoberlinia doka* Craib & Stapf (3.0%), *Bombax buonopozense* P. Beuav..(3.0%).	*Brachystegia eurycoma* Harms (2.2%), *Rutaceae* spp. (2.0%), *Terminalia* spp. (2.0%), *Lannea acida* A. Rich. (2.0%), *Heliotropium* spp. (2.0%), *Cassia senegalensis* (Linn.) (2.0%), *Paullinia pinnata* Linn. (2.0%), *Khaya senegalensis* (1.6%), *Crudia klainei* Pierre (1 %), *Entadrophragma angolense* (Welw.) C. DC. (1%), *Gardenia imperialis* K. Schum. (1%), *Hymenocardia acida* Tul (1%), *Indigofera spp.* (1%), *Proteacidites* spp. (1%), *Pterocarpus santalanoides*(1%), *Sterculia* spp.(1%),*Syzygium guineense* (Willd.) DC. (1%), *Vernonia* spp. (1%), *Capsicum* spp. (.8%), *Triplochyton scleroxylon Triplochyton scleroxylon* K. Schum (.8%), *Afzelia africana* Sm. ex Pers. (.6%), *Albizzia zygia* (DC.) JF Macbride (.6%), *Berlinia grandiflora* (Vahl) Hutch. & Dalz. (.6%), *Medinilla mirabilis* (Gilg) Jacq.-Fél. (.6%), *Conocarpus erecta* L (.6%), *Crotolaria retusa* L. (.6%), *Cleome spp.* (.6%), *Myrtaceae* spp. (.6%), *Cyperaceaepollis* spp. (.6%), *Prosopis africana* (Guill. & Perr.) Taub. (.6%), *Solanum* spp. (.6%), *Afraegle paniculata* (Schumach.) Engl. (.4%), *Anacardiaceae* spp. (.4%), *Annonaceae* spp(.4%),*Antiarias africana* Engl. (.4%), *Asteraceae*(.4%),*Coula edulis* Baill. (.4%), *Daniellia oliveri* (Rolfe) Hutch. & Dalz (.4%), *Desmodium spp.* (.4%), *Dissotis spp.* (.4%),*Schinus* spp. (.4%),*Elaeis guineensis Jacq.*(.4%), *Tetrapleura tetraptera* (Schum. & Thonn.) Taub. (.4%), *Vitex doniana* Sweet (.4%), *Allophyllus africanus* P. Beauv. (.2%), *Anogeissus leiocarpus* (DC) Guill & Perr. (.2%),*Calliacarpa* spp.(.2%), *Cocos nucifera* (L.) (.2%), *Dalbergia erasi*(.2%), *Entanda abyssinica* Steud. ex A.Rich. (.2%), *Ludwigia* spp. (.2%), *Mussaenda* spp.(.2%), *Myricaria germanica* (L.) Desv..(2%), *Phyllantus reticulatus* Poir. (.2%).

Physicochemical Analysis

Table 2. Results of the different physic-chemical tests on the different honey samples

Parameters	Abi	Ikom	Lokpanta	Nsukka	Okigwe	Shaki
Relative Density	1.40	1.39	1.39	1.38	1.37	1.38
Total Ash	0.59	2.40	1.78	2.00	1.82	0.20
P_H	3.49	6.14	6.18	6.71	6.24	3.49
Acidity	0.18	0.07	0.17	0.07	0.17	0.24
Colour	A	LA	LA	A	LA	A
Moisture content	18.8	18.9	19.0	18.9	18.8	19.0
Total solids (%)	81.20	81.10	81.00	81.10	81.20	81.00
Fischer's Test	CRC	N	CRC	N	CRC	CRC

A: Amber; LA: Light amber; CRC: Cherry red colour; N: Negative

Figure 1. Map of Nigeria showing the location of the Sources of the Honey Samples

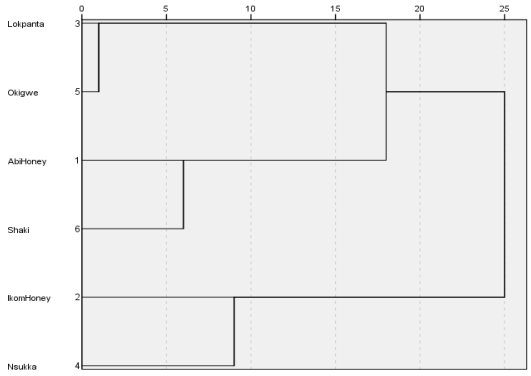

Figure 2. Comparative Dendrogram of the chemical parameters

Discussion

The presence of abundant pollen taxa (Tables 1) attests to the good quality of the analysed honey samples (Selvaraju et al., 2019; Rodopoulou et al., 2018; Shubharani et al., 2012). The pollen content of the current Nsukka honey closely resembled those of Njokuocha and Ekweozor (2007) in being multifloral. Again, most of the pollen recorded for Nsukka, Lokpanta and Okigwe all in south eastern Nigeria especially *Elaeis guineensis, Parinari kerstingii, Hymenocardia acida /Combretum* spp., *Alchornea cordifolia, Daniellia oliveri, Melastomataceae: Dissotis* sp., closely resembled those they recovered. Majority of these same pollen were later reported by Njokuocha (2019) from his study of seven honey samples from seven towns in three local governments areas of Anambra state south eastern Nigeria. A critical analysis of the recovered pollen clearly reflected the dominant vegetation and nectar sources of the honey bees. For the Okigwe and Lokpanta sample from south eastern Nigeria Samples from Okigwe and Lokpanta which are closely located, showed over 95% similarity (Figure 2), possibly due to similarity in flora which is dominantly rainforest with elements of derived savanna due to over cultivation and high population density, the pollen assemblage contained *Ceiba pentandra, Pentaclethra macrophylla, Pterocarpus santalanoides, P. soyauxii* (common vegetables in the south east) *Irvinga gabonensis, Berlinia grandiflora* and *Alchornea cordifolia*, with common fungal spores, Charred Graminae Cuticle and rare *Poaceae*. The comparative analysis of the samples from the different locations revealed an interesting trend (Figure 2). Abi and Shaki honeys showed 75% similarity, while Ikom and Nsukka samples showed 62% similarity. However, honeys from Ikom and Nsukka had some inherent qualities that differed from the other four samples. Furthermore, savanna pollen characterized the Shaki honey which appeared slightly similar to the results of Ige and Modupe (2010) from Abuja. *Acacia* spp. pollen dominated the assemblage possibly from the *Acacia* trees which are common around the Shaki-Ogbomosho area. Other pointers to the savanna vegetation were *Cassia senegalensis, Khaya senegalensis, Combretum* spp., *Parinari kerstingii, Tephrosia* spp., *Terminalia* spp., *Isoberlinia doka, Bombax buonopozense, Sterculia* sp., *Hymenocardia acida, Gardenia imperialis, Heliotropium* spp., among others.

The moderate records of fungal elements and Charred Graminae cuticles indicated savanna fires and preponderance of fungal elements in the air. The common recovery of *Ceiba* pollen further attest to its being a common source of nectar for honeybees in Nigeria just as (Ramirez-Arriaga et al. 2011) had reported from Mexico.

Generally, the common records of *Elaeis guineensis* and other forest species in these samples contrasts the reports of Adekanmbi and Ogundipe (2009) and Adeonipekun (2012) who reported the preponderance of *Asteraceae* and other pollen in the Lagos and Ibadan samples they studied. These differences could have arisen from the fact that these samples from the rural areas reflected the more closed forest canopies compared to Lagos and Ibadan where the main vegetation cover had been cleared for construction and other developmental purposes. The results of the present study further revealed the common occurrence of *Elaeis guineensis* pollen in Nigerian honey samples just as (Afolabi,1974; Njokuocha and Ekweozor, 2007; Ige and Modupe, 2010) had all reported. Moreover Njokuocha (2019) had reported a 43.45% *Elaeis guineensis* for the Nsukka honey samples. This is close to 45%, the acceptable quantity for branding unifloral honey samples (Jasicka-Misiak et al. 2012). Should the percentage of *Elaeis guineensis* exceed 45%, then such honey sample will be branded as oil palm honey. Selvaraju et al. (2019) had reported the preponderance of pollen of oil palm *Elaeis guineensis* and coconut *Cocos nucifera* in honey samples from the west coast of Malaysia.

The results of the melissopalynological assessment coupled with the results of the Physico-chemical analysis (and Table 2). These values for the relative density conformed to international standards. Total ash: The ash content of the honey samples were measured by incinerating 3g of each honey overnight at 550°C in a furnace (Carboline, Sheffield, U.K.) until a constant weight is reached (Stefan 2009). The pH values of the six samples which ranged from 3.49 to 6.71 (Table 2), revealed that they were all acidic which concurs with the assertion of Saxena et al. (2010) that honey is normally acidic no matter where it came from. However, the Abi and Shaki samples with pH of 3.49 were more acidic than those with values above 6.0 for the Ikom**,** Lokpanta, Okigwe, and Nsukka with the highest value of 6.71. According to Khalil et al., (2012) the Abi and Shaki samples were fresh compared to the rest as pH values between 3.4 and 6.1 indicated freshness of honey. However, higher acidic values suggest possible fermentation of sugars into organic acids. They pointed out that pHinfluences honey texture, stability and shelf life.

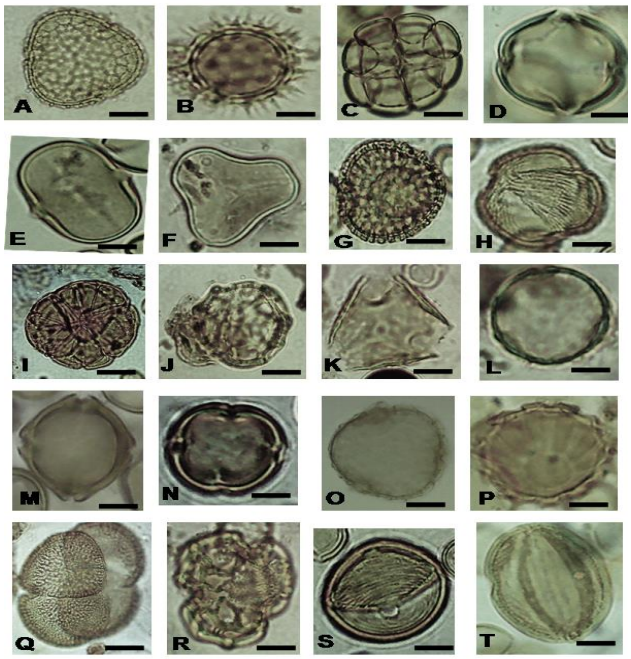

Figure 3. Photomicrographs of some selected palynomorphs recovered from the Nigerian honey samples
Names of the palynomorphs
A. Bombacaceae: *Ceiba pentandra* (Linn.) Gaertn. B. Asteraceae C. Fabaceae: *Albizzia* zygia (DC.) JF Macbride D. Sterculiaceae: *Afraegle paniculata* (Schumach.) Engl. E. Fabaceae: *Tephrosia* spp. F. Arecaceae: *Elaeis guineensis* Jacq. G. Sapotaceae: *Delonix regia* (Boj. ex Hook) Raf. H. Fabaceae: *Berlinia grandiflora* (Vahl) Hutch. & Dalz. I. Boraginaceae: *Heliotropium* spp. J. Fabaceae: *Afzelia africana* Sm. ex Pers. K. Chrysobalanaceae*: Parinari kerstingii* Engl. L. Rutaceae: *Citrus* sp. M. Guttiferae: S*ymphonia globulifera* Linn. f. N. Meliaceae: *Khaya senegalensis* (Desv.) A. Juss. O. Bombacaceae: *Bombax buonopozense* P. Beuav. P. Polygalaceae: *Polygala* spp. Q. *Annonaceae* spp. R. Fabaceae: *Brachystegia eurycoma* Harms S. Anacardiaceae: *Lannea acida* A. Rich. T. Fabaceae - *Isoberlinia doka* Craib & Stapf Scale bars: 10µm

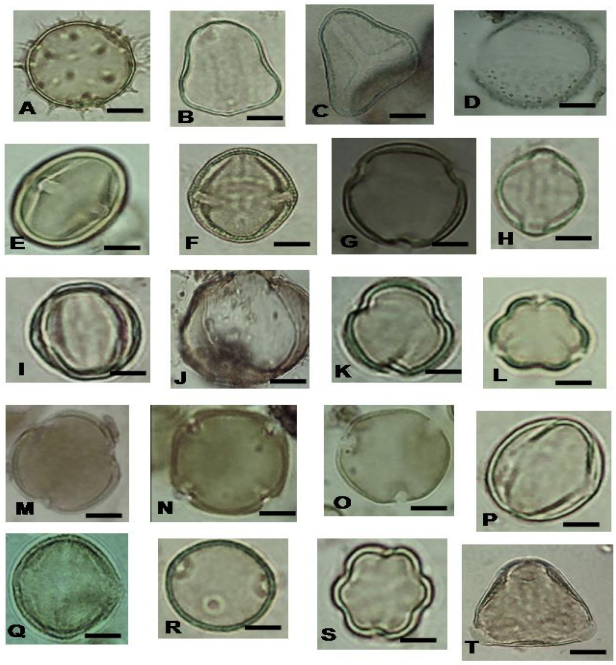

Figure 4. Photomicrographs of some selected palynomorphs recovered from the Nigerian honey samples
Names of the palynomorphs
A. Asteraceae B. Cyperaceae C. Arecaceae: *Elaeis guineensis* Jacq. D. Arecaceae: *Hyphaene tiebaica* (Linn.) Mart. E. Rubiaceae: *Mussaenda* spp. F. Euphorbiaceae sp. G. Rutaceae: *Citrus* sp. H. Rhizophoraceae: *Rhizophora* sp. I. Olalaceae: *Ximenia americana* Linn. J. *Nympheaceae: Nymphea lotus* L. K. *Vitex* doniana Sweet L. Melastomataceae: *Dissotis* sp. M. *Cleome* sp. N. Meliaceae: *Khaya senegalensis* (Desv.) A. Juss. O. Cochlospermaceae: *Coclospermum planchonii* Hook f. P. Combretaceae: *Combretum* spp. Q. Fabaceae-Papilionoideae: *Heliotropium cf. cliffordiana* R. Celtis sp. S. *Terminalia* sp. T. Chrysobalanaceae*: Parinari kerstingii* Engl. Scale bars: 10µm

Acidity

The colours which ranged from Amber to light amber especially for the samples from Abi, Nsukka, and Shaki denotes good quality as lighter colours are caused by over mixing with water or other materials (White, 1975, Crane,1980).

The moisture contents which ranged from 18.8% to 19.0% agreed with the reports of Saxena *et al*. (2010), from India in which the moisture content of six out of the seven samples they studied ranged from 17.2% to 21.6%. Khalil et al., (2012) had also documented moisture contents which ranged between 11.59-14.13% for four honey samples from Algeria. These values they pointed out were below the maximum prescribed limit for moisture content according to Codex standard for honey (Saxena *et al*. 2010). Khalil et al., (2012) had reported (≤ 20%) as the limit of the International quality regulations (Codex Alimentarius, 2001). They further asserted the importance of water content for the shelf life of honey in storage. High levels of water encourages fermentation due to osmotolerant yeasts.

The result of the total solids which ranged between 81% for Lokpanta and Shaki to 81.20% for Okigwe and Abi fell within the Codex Alimentarius (2001) and European Union Standard Reports (2001). This implies that that the honeys have not undergo further processing as all the organic and inorganic contents were still intact (Kayode and Oyeyemi, 2014). The total solids were highest in the Abi and Okigwe samples with values of 81.20%, followed successively by 81.10% for the Ikom and Nsukka samples while the lowest values of 81.00 were obtained for the Lokpanta and Shaki samples. These results fell within the acceptable range indicating that the samples were not subjected to further processing (Khalil et al. (2012).

Conclusion

The honey samples were all multifloral as no single species had values above 45%. The pollen contents point to the geographical origin of the honey as they reflected different vegetation zones of Nigeria. Those from Southeastern Nigeria were dominated by rainforest species (*E. guineensis, Bombax, Ceiba*, etc while those from the derived savanna and savanna regions were dominated by savanna species (*Acacia* spp., *Combretum* spp., *Terminalia* spp., *Khaya senegalensis*, and *Tephrosia* spp.). The Nsukka samples yielded an admixture of rainforest and some savanna species which is characteristic of a derived savanna due to over cultivation in the area possibly brought about by high population density. Chemical analysis revealed that the honey samples were of moderately good quality when compared to international standard and their acidic $_{P}$H values reveals that they are unadulterated and have potentials to stay long as suggested by Lawal et al. (2009).

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