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Botanical Affinity and Physico-chemical Parameters of Honey Samples Obtained from Bee Hives in Cross River State Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author EJK designed, supervised and wrote the final manuscript. Author EA managed the experiment, collected data and wrote the first draft, while author JDE performed the statistical analysis and editing. All authors read and approved the final manuscript.

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ABSTRACT

Botanical and physico-chemical parameters were used to assess honey quality obtained from eleven beehives across Cross River State, Nigeria. Possible nectar sources from plant species within 3 km base radius from each hive was enumerated using line transect. The obtained honey samples were subjected to non-acetolytic method of preparation. Of the 69 plant species whose nectar were available for foraging by the bees, only 31 species had their pollen represented in the honey samples. Honey samples 2 and 8 fulfilled the CODEX requirements for unifloral honey. When pH, moisture content, sugar content (fructose and sucrose), free lactic and total acidity content, HydroxyMethylFurfural (HMT), protein and diastase were used to evaluate the quality, only honey sample 2 fulfilled the CODEX requirements for pure honey. This finding was strongly correlated by the result of the cluster analysis and dendrogram.

Keywords: CODEX; Cross River State; plant species; physico-chemical; botanical origin.

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1. INTRODUCTION

1.1 Background to Study

Botanical and biogeographically sources of honey are concerns in food quality and safety. According to the [1], the use of a botanical designation of honey is allowed if it originates predominately from the indicated floral source. Honey may also be designated by the name of a geographical region if it was produced within the area referred to. Several studies on the pollen spectrum of honey had indicated major interplay of nectar and honey dew elements belonging to several plant species (multi floral or poly floral). Regrettably, vast majority of honeys at sale today are simply designated “Honey” or “Pure Honey”. This deceptive-market-strategy designation implies a unifloral honey. Almost nearly all honeys are not pure, thus designating one as pure is misleading and a deliberate ploy to extort money from consumers since unifloral honeys is qualitatively more valuable than polyfloral or multifloral ones. The term unifloral honey is used to describe honey in which the principal part of the nectar or honeydew is derived from a single plant species. Honey composition, flavor, and color vary considerably depending on the botanical source it originates. The physical, chemical, and pollen analytical characteristics of the most important European and African unifloral honeys have been described in various papers [2,3,4]. Contrary to the unifloral honeys, the polyfloral honeys do not exhibit distinct physical or chemical characteristic apart from a huge variability, which makes their authentication particularly difficult. Single and multiple chemicals and components can well indicate the botanical and geographical origins of the honey. Marker chemicals and components include flavonoids, pollen, aroma compounds, oligosaccharides, trace elements, amino acids, and proteins [5]. Similarly, the lack of regional and local floristic data has often times proved a challenge to studies requiring adequate and reliable knowledge of floristic resources. In Nigeria, as well as other countries with near absences of reliable flora bank, effective and prompt pollen characterization and identification has met with limited success.

These challenges had impacted negatively on the huge honey market in Nigeria as little information is available on its quality. More so, the rigorous procedures for honey certification by the National Agency for Food and Drug Administration (NAFDAC) presuppose a holistic characterization of its pollen spectrum. It is in light of these obvious gaps that this study intends to characterize and differentiate honey samples obtained across 11 beehives in four local government areas of Cross river state. The aim of the study is to determine the quality of each of the honey sample. This shall be done by characterization of the floral source (s) of each honey sample and analysis of some of their physico chemical parameters.

2. MATERIALS AND METHODS

Pollen Album /Botanical Affinity: Plant species were identified and enumerated along a 3km long straight line transects lay at the side of each beehive. Plants were identified using standard references by [6 and 7]. Pollen recovered from the plant species was prepared using **Erdtman method**. Field studies for pollen collection and botanical inventory was conducted in two years over four seasons (two wet and two dry seasons).

2.1 Pollen Collection from Beehive

Honey produced by *Apis mellifera* were sampled directly from eleven [11] identified bee hives spread across the Cross River state (Table 1).

All samples after Palynological analysis were kept at -20℃ until physicochemical analyses were performed. All reagents were analytical grade Sigma or Fluke products used without any purification.

2.2 Pollen Analysis

The botanical origin of the honey samples was studied according to the method of [8]. About 10 grams of each pollen honey sample were dissolved in 30 mL of distilled water and centrifuged for at 1000 g for 15 minutes at 2500 rpm. The supernatant was decanted and the sediment was washed twice with 10 mL of distilled water and then centrifuged again at 1000 g for 5 minutes at 2500 rpm. The sediment was spread on a slide, dried at 40℃, and then mounted with stained glycerin gelatin. Pollen grains were identified and counted under Carl Zeiss AG Light Microscope, using x 400 magnification. Pollen grains are counted along 5 parallel equidistant lines uniformly distributed from one edge of the 10 x 10 mm smear to the other (in total at least 500 pollen grains are counted). The identified taxa in the pollen spectrum were expressed in term of relative frequency (RF) and the pollen density was expressed as the absolute number of pollen grain in 10 g of honey (PG/10 g).

Table 1. Beehive co ordinates, town and Local Council Area

Sample no	Town	Local Government Area	Co ordinates
HS1	Okeri	Bekwara	00639 47N 008 54 16E
HS2	Agbokim	Ikom	005 32 53N 008 50 22E
HS3	Akunshie	Obanliku	005 15 27N 008 4652E
HS 4	Iyamitet	Obubra	005 52 28N
HS 5	Bebuabie	Obudu	006 30 15N 009 06 26E
HS 6	Bansara	Ogoja	006 51 28N 008 56 06E
HS 7	Adim	Biase	005 45 18N 008 20 08E
HS 8	Ekuri	Etung	005 48 47N 008 26 34E
HS 9	Ikot Enene	Akpabuyo	004 56 28N 008 27 29E
HS 10	Anantiga	Calabar south	004 50 39N 007 21 29E
HS 11	Issaba	Obubra	005 50 39N 008 22 24E

2.3 Pollen Identification

Pollen was identified using [9] and reference materials in the departments of archeology and Botany of the Universities of Ibadan and Calabar respectively.

2.4 Physicochemical Analyses

Moisture content was determined at 20℃ with an Abbe type refractometer (PDT/001), equipped with a thermometer having a graduation of 0.5℃, and a resolution of 2 x 10-4 units of Refraction Index (RI). Results were expressed as percentages obtained from the Chat table by using the [10] method.

The specific gravity of honey (density) was determined by dividing the weight of specific gravity bottle (50 mL) filled with honey by the weight of the same bottle, filled with water as described [11].

Measurements of pH were performed with a pH meter (Orion 420 A) in a solution containing 10 g of each honey sample in 75 mL of Milli-Q grade water, by the method reported AOAC, 2016. Results were expressed as milliequivalent of NaOH per Kg of honey.

Honey electrical conductivity was measured at 20℃ [12] with a Crison Basic 30 conductimeter. Results were expressed in MicroSiemens per centimeter (µS/cm) [13;14].

Honey color was obtained by Pfund color grader (C221) (HANNA instrument), based on simple optical comparison [15]. The C221 portable microprocessor analyzer measures the percentage light transmittance of honey color compared to the analytical reagent grade glycerol. The transmittance value allows identification of the honey Pfund grade. The instrument operates in the range of 0 to 150 mm Pfund with an accuracy of ±2 mm Pfund. The measurements were expressed in millimeters (mm) Pfund.

Lactone and total acidity were determined by the titrimetric method as follows: Sample was titrated to pH 8.5 using O.O5N NaOH (free acidity). Excess 0.05N NaOH was immediately added and without delay back- titrated with 00.05N HCl to pH 8.3 (Lactone acidity). Total acidity was obtained from the sum of free and Lactone acidities. Results were expressed as meq/kg.

HydroxyMethylFurfural (HMF) was determined after dilution with distilled water and addition of p-toluidine solution according to Official Methods of Analysis of AOAC International 2016 [12]. Absorbance was determined at 550 nm using a 1 cm quartz cell in a Biochrom Spectrometer. Results were expressed in mg/kg.

The diastase activity was measured with the method of Phadebas [16]. This method was based on the use of an insoluble, dyed starch

substrate. This substrate is hydrolyzed by @-amylase, yielding blue water soluble fragments, determined photo metrically at 620 nm. One unit of diastase activity (or of a-amylase) (Schade), is defined as the amount of enzyme able to convert 0.01 g of starch using 1 g honey in 1 h at 40℃. Results were expressed as invertase number (IN).

3. RESULTS

Botanical Inventory: The floristic resources contained within 3 Km base radius for each of bee hive were determined. The floristic resources censured for each of the bee hive location is shown in Table 2. The result indicated a total of forty eight species around the bee hive where HS1 was collected, forty two where HS2 was collected, fifty seven where HS3 was collected, thirty five where HS4 was collected, thirty three where HS 5 was collected and forty nine where HS6 was collected. This information is as shown in Table 2.

The pollen grains from all the samples were investigated microscopically. Eight honeys samples were considered to be Polyfloral, two were considered to be unifloral, while the remainder was considered to be honeydew honey (Plates 1-11).

Eight different pollen types were observed in HS10, Six in HS1, HS3 and HS8, five in HS6 and HS7, four in HS2, HS4 and HS5, and three in HS9 and HS11. These are as shown in plate 1-11. The pollen type ranges from monoporate, monosulcate, diporate, dicolpate, syncolpate, triporate, tricolpate, tricolporate, zonoporate, syncolpate and pantoporate. The percentages of the most abundant pollen types in each honey sample, as well as the nectar and pollen character of these plants were taken into account. Following the criteria of [17], when the percentage of the most abundant pollen type was over 45%, the honey sample was classified as unifloral. Lower percentages were classified as polyfloral. Honeys with significant indices of fungal spores, mycelia, microscopic green algae and starch grains were considered to be Honey dew elements (Table 3).

As observed in Table 3, the dominant pollen morphoforms were tricolpate and monoporate in HS1, diporate and tricolpate in HS2, tricolporate in HS3, triporate in HS4, monocolpate in HS5 and tricolpate in HS6. Other less dominant pollen morphoforms were pantoporate(6.3%), monosulcate (6.1%), triporate 5.6%) and tricolporate (5.4%) in HS1, dicolpate (12.4%), triporate (9.7%) and honey dew elements (8.0%) in HS2, triporate (21.5%), pantoporate (19.9%), monoporate (15.2%), tricolpate (6.3%), monocolpate (3.1%) and honey dew elements (1.3%) in HS3. In HS 4, the less dominant pollen morphoforms were tricolporate (19.8%), monosulcate (18.4%), monoporate (16.5%) and honey dew elements (6.4%) while tricolporate (27.3%), tricolpate (22.7%) and dicolpate (5.5%) were the less dominant pollen morphoforms in HS5. Dicolpate (21.9%), tricolporate (20.5%), monocolpate (14.1%) and diporate (3.0%) were the least dominant pollen morphoforms in HS6. In HS7, triporate apertural morphoforms accounted for 29.7%, tricolporate (19.8%), dicolpate (18.2%), monocolpate (16.6%), tricolpate (12.7%) and honey dew elements (3.00%). The dominant morphotype in HS8 was monoporate with 5 1.2% of the total pollen count. This was followed by tricolporate (16.4%), triporate (14.2%), dicolpate (12.7%) and pantoporate (5.5%). Percentage pollen count in HS9 showed that monoporate morphoforms was the most dominant with 42.1%, followed by diporate (31.9%), syncolpate (15.6%) and significant members of various honey dew elements (10.4%). Nine various pollen morphoforms were observed in HS10. They range from the predominant monoporate (36.1%) to triporate (18.0%), tricolporate (15.3%), diporate (10.1%), Dicolporate (6.7%), zonoporate (6.1%), tricolpate (3.1%), zonoporate (2.1%), pantoporate (2.0%) and HDE (1.5%). In HS11, monoporate pollen lype yielded a percentage value of 43.1%, dicolpate 27.4%, tricolporate (22.7%) and HDE (4.8%) Based on the strength of the pollen count in the various samples, the pollen class was determined. The classes ranged from 1 in HS3 and 9,2 in HS 7 and 11,3 in HS 5,4 in HS 2,6 and 10 and 5 in HS 1 and 4.

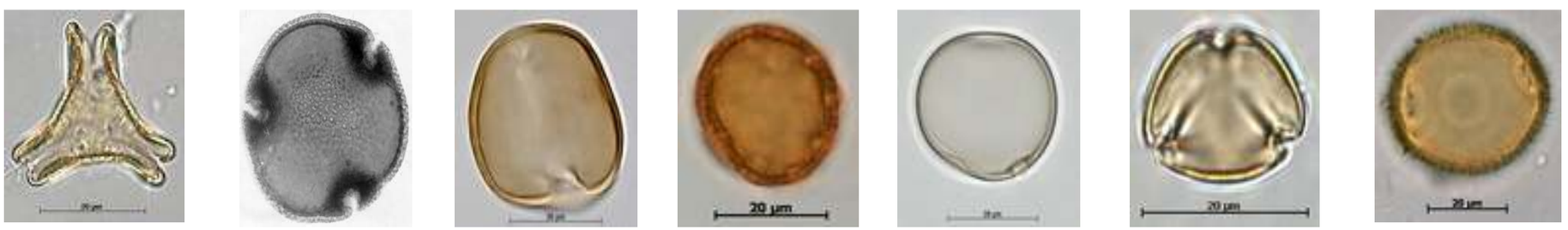
Table 4 shows the result for the physico chemical parameters analyzed for each honey sample. Parameters analyzed and observed include pH, moisture content, specific gravity, color, fructose, glucose, Saccharose, electrical conductivity, diastase, free acidity, lactonic acidity, total acidity, HMF and protein content.

Table 2. Botanical inventory around a 3 KM base radius of 11 beehive locations in Cross River State

S/N	Species	Family/Sub family	Local name	Flowering time	Okeri	Agbokim	Akunshie	Iyamitet	Bebuabie	Bansara	Adim	Ekuri	Ikot enene	Anantiga	Issaba
1	*Afzelia bipendensis*	Caesalpinoideae			√			√			√				
2	*Spathadea campanulata*	Bignoniaceae		Nov -Dec		√			√			√			
3	*Parkia bicolor*	Mimoisoideae	Efik: Etediuku	Nov-Feb		√			√			√			
4	*Rothmannia hispida*	Rubiaceae	Efik: Obong Boki: Eton	Dec-Mar; July;	√				√			√			
5	*Spondianthus preussii*	Euphorbiaceae	Efik: Ibok-eku	July-Oct	√						√			√	
6	*Cola acuminata*	Sterculiaceae	Efik: Ibong	Dec		√					√			√	
7	*Poga oleosa*	Anisophylleacae	Efik: Inoi Boki: Onyo	Apr-Jun			√			√					
8	*Cocos nucifera*	Arecaceae	Efik: Isip mbaka				√			√		√			√
9	*Lophira alata*	Ochnaceae	Efik: Enwan Boki:kabankik	Nov-Jan		√						√			√
10	*Piptadeniastrum africanum*	Mimoisoideae	Efik: Ubam Boki: Kachi kabiam Ejagam: Ebomme	July-Sept.	√			√				√			√
11	*Enantia chlorantha*	Annonceae	Boki: Kakerim		√				√			√			
12	*Pterocarpus mildbreadii*	Papilinonoideae	Bok:Kakupupu	Nov-Feb	√						√			√	
13	*Brenania brieyi*	Rubiaceaee	Boki Kalang			√		√			√			√	
14	*Guibourtia ehie*	Caesalpinoideae	Boki: Kaluk ofuon	Oct-Nov	√	√		√		√			√		
15	*Diospyros zenkiri*	Ebenaceae	Boki: Kambibri	Feb-Apr		√				√			√		
16*	*Craterispermum cerinanthusm*	Rubiaceaee	Boki:		√					√			√		
17	*Trilepisium madagascariensis*	Moraceae	Boki: Katepnkim	Nov-Feb		√					√			√	
18	*Millettia griffoniana*	Papilionoideae	Boki: Kateposhie	Dec-Apr	√		√			√					
19	*Desbordesia glaucescens*	Irvingiaceae	Boki: Kawo				√			√	√			√	
20	*Hymenostegia afzelii*	Cesalpinoideae	Boki: Kayishuan	Nov-May;Aug	√			√			√			√	
21	*Anonidium mannii*	Annonaceae	Boki: Kechebuchu	Sept-Apr	√			√			√			√	
22	*Amphimas pterocarpoides*	Papilionoideae	Bok: Kechebumpe	Sept-Oct			√			√	√			√	
23*	*Hypodaphnis zenkiri*	Lauraceae	Boki:Kechekawa	Feb-Jul			√			√		√			
24	*Spondias mombin*	Anacardiacea	Boki: Kechibi Ejagam: Ekpi	Mayr-Apr;Jul-Aug	√		√			√		√			
25	*Antiaris toxicaria*	Moraceae	Boki:Kefem Ejagam: Nuwo	Dec-Jan	√			√			√				
26	*Terminalia ivorensis*	Combretacea	Efik: Afia Boki: Kekeku	April-Aug	√			√			√	√			√

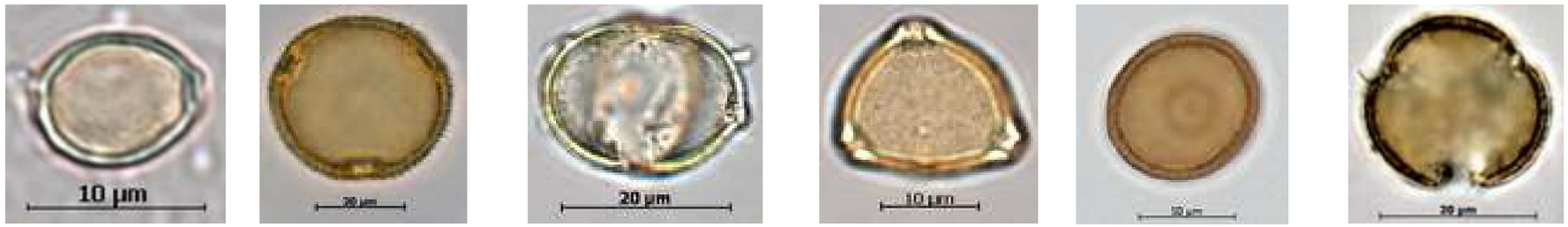
S/N	Species	Family/Sub family	Local name	Flowering time	Okeri	Agbokim	Akunshie	Iyamitet	Bebuabie	Bansara	Adim	Ekuri	Ikot enene	Anantiga	Issaba
27	*Myrianthus arboreus*	Moraceae	Efik: Ndisok Boki: Kekeku	Jan-April		√			√			√			√
28*	*Desplastia dewevrei*	Tiliaceae	Boki: Kelim	Most of the year		√			√	√			√		
29	*Klainedoxa gabunensis*	Irvingiaceae	Boki: kelim kelim	Jan-Apr	√			√		√			√		
3031	*Afzelia Africana*	Caesalpinoidea	Boki: kemk waepe	Feb-Apr	√			√				√			
32	*Cylicodiscus gabunensis*	Mimoisoidea	Efik: anyan Boki: kendum	Feb-Apr & Jun-sept	√			√	√			√			
33	*Bridelia micrantha*	Euphorbiaceae	Boki: Kensange	Atmost season				√	√			√		√	
34	*Bridelia ferruginea*	Euphorbiaceae	Boki: Kensangeabia	Atmost sea son			√			√			√		√
35	*Terminalia superba*	Combretaceae	Efik: Afiaeto	Nov-Jan		√					√		√		√
36	*Erythrophleum suaveolens*	Cesalpinoideae	Efik: Akpa	Jan-Feb				√		√					√
37	*Euphorbia kamernica*	Euphorbiaceae	Efik: Akpambiet	Sept-march	√	√		√	√			√		√	
38	*Macaranga barteri*	Euphorbiaceae	Efik: Akpap	Jun-May			√		√			√	√		√
39	*Entandrophragma cylindricum*	Meliaceae	Efik: Atore	Nov-Apr		√				√				√	√
40	*Entandrophragma utile*	Meliaceae	Boki	March			√			√		√			
41	*Dichaetanthera africana*	Melastomataceae	Boki: Asuoke		√			√			√				√
42	*Xylopia rubescens*	Annonaceae	Efik: Atarabang			√			√		√		√		
43*	*Lepidobotyx staudtii*	Lepidobotryaceae	Boki: Athekakwam				√		√				√		√
44	*Hannoa klaineana*	Irvingiaceae	Boki: Bobet	Jul-Sept &Feb-March	√			√		√				√	
45*	*Ophiobotryxs zenkeri*	Flacourtiaceae	Bok:Bofan			√				√					
46	*Corynanthe pachyceras*	Rubiaceaee	Boki: Bofat	Oct-Dec &May-Jul		√	√	√			√		√		
47	*Irvingia gabonensis*	Irvingiaceae	Efik: Oyo Boki: Bojeb	Nov-March&Jun			√			√		√		√	√
48	*Pterocarpus osun*	Papilionoideae	Efik: Ukpa Boki: Bokoanya	Aug-Nov	√				√			√			
49	*Pterocarpus soyauxii*	Papilionoideae	Efik:Ukpa Boki: Boku	Jun, Sept-Octl		√			√				√		√
50	*Alstonia boonei*	Apocynaceae	Efik: Ukpo	Oct-March		√		√		√			√		
51	*Ceiba pentandra*	Bombaceae	Ibibio: Ukim Boki: Bokum	Dec-Jan			√			√		√		√	√
52	*Musanga cecropoides*	Moraceae	Efik: Uno Boki: Bokuobe	Almost seasons			√				√		√		

S/N	Species	Family/Sub family	Local name	Flowering time	Okeri	Agbokim	Akunshie	Iyamitet	Bebuabie	Bansara	Adim	Ekuri	Ikot enene	Anantiga	Issaba
53	*Distemonanthus bethamianus*	Caesalpinoideae	Boki: Bokwa	Sept-Nov & Feb	√			√				√		√	
54	*Xylopia quintasii*	Annonaceae	Boki:Bolonge						√		√				
55	*Dacrodyes edulis*	Bursaraceae	Efik: Eben	Feb-March	√			√				√			√
56	*Pycnanthus angolensis*		Ibibio: Abakang	Dec-March											
57	*Canarium schweinfurthii*	Bursaraceae	Efik: Eenetridon	Jul-Aug &Nov-Dec		√			√		√				
58	*Bombax buonopozense*	Bombaceae	Boki: Chakun	Dec-Feb	√			√		√			√		
59	*Cola gigantea*	Steruliaceae	Efik:Dikir	Oc-Jan		√		√		√				√	√
60	*Sacoglottis gabonensis*	Humiriaceae	Efik: Ndat	Nov-Jan& July-Aug			√		√		√		√		√
61	*Mammea africana*	Clusiaceae	Efik: eden Boki: Okut	Aug-Dec	√			√		√				√	
62	*Erythrina senegalensis*	Pailionoideae	Efik: Edeng	Sept-Jan		√			√		√				√
63	*Allanblackia floribunda*	Clusiaceae	Efik: Ediang	Sept-Feb											
64	*Treculia africana*	Moraceae	Efik: Ediang	Oct-Feb			√		√		√		√		
65	*Garcinia kola*	Clusisaceae	Efik: efirai	Dec-Jan				√		√				√	
66	*Coula edulis*	Olacaceae	Efik: Ekom	Jan-may		√			√				√		
67	*Porterandia cladantha*	Rubiaceaee	Boki: ekumalin	May-Aug		√			√						
68*	*Gossweilerodendron suaveolens*	Caesal pinoideae	Bok: Emonga	Dec-Feb	√			√				√		√	√



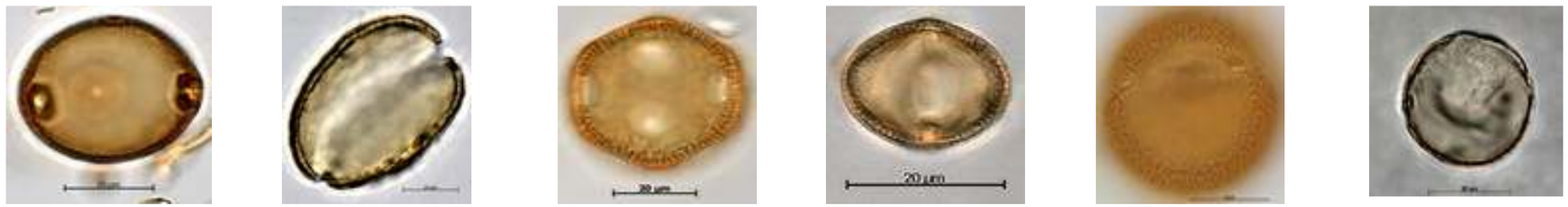
(a) Tricolpate **(b) Tricolpate** **(c) Monosulcate** **(d) Pantoporate** **(e) Monoporate** **(f) Tricolporate** **(g) Triporate**

Plate 1. Pollen morphology of HS1



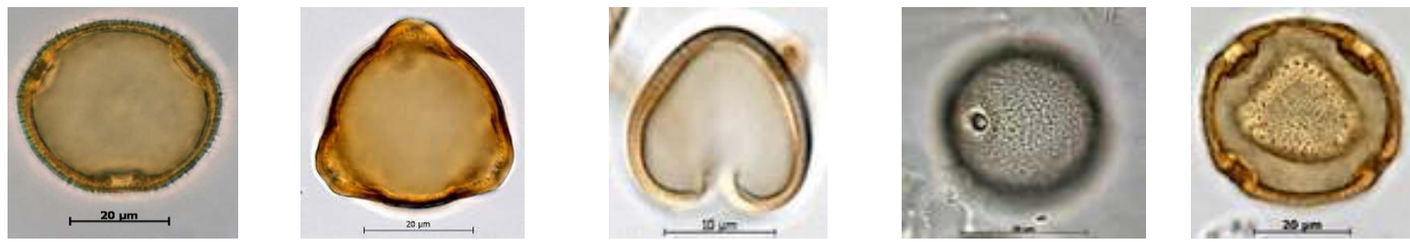
(a) Dicolpate (b)Triporate (c) Tricolpate (d) Triporate (e) Diporate (f) Tricolpate

Plate 2. Pollen morphology of HS2



(a) Triporate (b) Monocolpate (c) Pantoporate (d) Tricolporate (e) Tricolpate (f) Monoporate

Plate 3. Pollen morphology of HS3



(a)Tricolpate (b) Tricolpate (c) Monosulcate (d)Monoporate (e) Triporate

Plate 4. Pollen morphology of HS4

(a) Monocolpate **(b)Tricolporate** **(c) Dicolpate** **(d) Triporate**

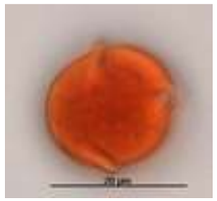
Plate 5. Pollen morphology of HS5

(a) Tricolporate **(b) Monocolpate** **(c) Dicolpate** **(d) Tricolporate** **(e) Diporate**

Plate 6. Pollen morphology of HS 6

(a)Triporate **(b) Tricolporate** **(c) Dicolpate** **(d)Monocolpate** **(e) Tricolpate**

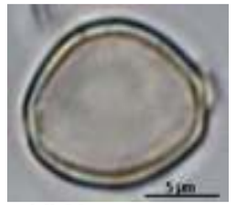
Plate 7. Pollen morphology of HS 7



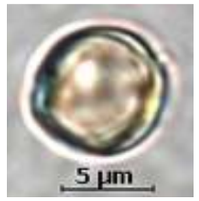
(a) Tricolporate

(b)Triporate

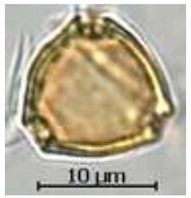
(c) Monoporate



(d) Monoporate

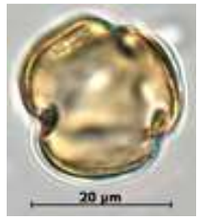


(e) Dicolpate



(f) Triporate

(g) Monoporate



(h) Tricolpate

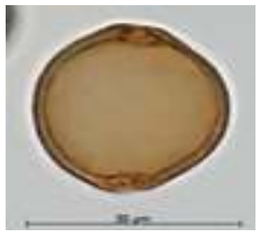
(i) Pantoporate

(j) Monoporate

(k) Tricolporate

Plate 8. Pollen morphology of HS 8

(a) Monoporate



(b) Diporate

(c) Monoporate

(d) syncolpate

(e) Monoporate

Plate 9. Pollen morphology of HS 9

Monoporate

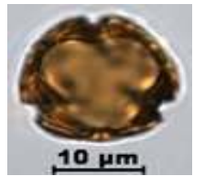
Diporate

Syncolpate

Monoporate

Monoporate

Pantoporate

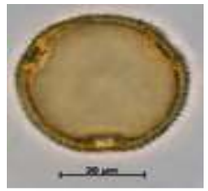


Tricolporate

Tricolpate

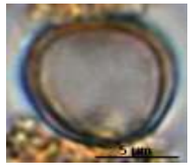
Zonoporate

Pantoporate



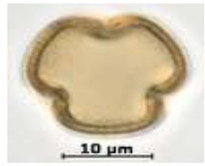
Triporate

Triporate



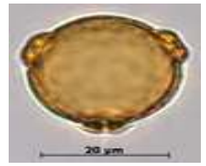
Monoporate

Monoporate



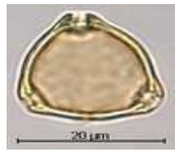
Tricolporate

Zonoporate



Triporate

Monoporate



Triporate

Plate 10. Pollen morphology of HS 10

(a) Monoporate **(b) Monoporate** **(c) Monoporate** **(d) Tricolporate** **(e) Dicolpate**

Plate 11. Pollen morphology of HS 11

Table 3. Pollen morphoforms and percentages per honey sample

Sample	Dominant pollen morphology and %	Other recovered pollen morphologies and %	Pollen grain/10 g of sample
HS1	Tricolpate (38.2%) Monoporate (36.4%)	Pantoporate (6.3%) Monosulcate (6.1%) Triporate (5.6%) Tricolporate (5.4%)	6190000-class V
HS2	Diporate (48.3%) Tricolpate (21.6%)	Dicolpate (12.4%) Triporate (9.7%) Honey dew elements (8.0%)	220000-classiv
HS3	Tricolporate (32.7%)	Triporate (21.5%) Pantoporate (19.9%) Monoporate (15.2%) Tricolpate (6.3%) Monocolpate (3.1%) HDE (1.3%)	9500-class I
HS4	Triporate (38.9%)	Tricolporate (19.8%) Monosulcate (18.4%) Monoporate (16.5%) HDE (6.4%)	580000-ClassV
HS5	Monocolpate (44.5%)	Tricolporate (27.3%) Tricolpate (22.7%) Dicolpate (5.5%)	145000-Class III
HS6	Tricolpate (40.5%)	Dicolpate (21.9%) Tricolporate (20.5%) Monocolpate (14.1%) Diporate (3.0%)	275000-Class iv
HS7	Triporate (29.7%	Tricolporate (19.8%) Dicolpate (18.2%) Monocolpate (16.6%) Tricolpate (12.7%) HDE (3.00%)	58000-Class II
HS 8	Monoporate (51.2%)	Tricolporate (16.4%) Triporate (14.2%) Dicolpate (12.7%) Pantoporate (5.5%)	122000-Class III
HS9	Monoporate (42.1%	Diporate (31.9%) Synocolpate (15.6%) HDE (10.4%)	2450-Class I
HS10	Monoporate (36.1%)	Triporate (18.0%) Tricolporate (15.3%) Diporate (10.1%) Dicolporate (6.7%) Zonoporate (8.2%) Tricolpate (3.1%) Pantoporate (2.0%) HDE (1.5%)	298000-Class IV
HS11	Monoporate (43.1%)	Dicolpate (27.4%) Tricolporate (22.7%) HDE (4.8%)	17300-Class II

As could be seen in Table 4, the pH of the samples ranges from 3.74 in HS2 to 4.32 in HS1 with a mean of 4.02 and a standard deviation of 0.20. The moisture content ranges from 12.8% in HS2 to 21.2 in HS1 with a mean of 16.2 and a standard deviation of 3.13. Similarly, the specific gravity of the samples ranged from 1.41 g/100 g in HS1 and HS5 to 1.46 in HS2 with an overall mean of 143 and a standard deviation of 0.021. The color of the honey samples measured in mm/Pfund ranged from 35 in HS2 to 112 in HS11. The fructose content of the samples

varies from 30.6 g/100 g in HS2 to 39 g/100 g in HS5 whereby the mean and standard deviation are 34.9 and 3.42 respectively. In the same vein, the glucose content for the samples varies between 25.4 g/100 g in HS1 to 30.3 g/100 g in HS6 with a mean of 28.01 and a standard deviation of 2.08. The Saccharose contents varied between 0.8 g/100 g in HS4 to 1.8 g/100 g in HS6 yielding a mean average of 1.2 and a standard deviation of 0.36. The electrical conductivity of the samples measured revealed a mean and standard deviation of 0.82 ±0.33 for samples that varied between 0.312 mS/cm in HS2 to 1.204 mS/cm in HS6.The diastase concentration as found to vary between 10.1DN in HS6 to 20.SDN in HS1 with a mean of 14.7 and a standard deviation of 17.39. The free acidity contents varied between 18.7 meq/Kg in 1151 and 23.4 meqKg in 1154 with a mean of 20.8 and standard deviation of 1.93. In the same vein, the lactonic content average and standard deviation was 3.27±1.44 for values that ranged between 1.8 meq/Kg in HS4 to 5.4 meq,/Kg in HS5. The total acidity content for each sample was derived from the free acidity content and that of the lactonic content The result showed a range of 21.4 meq/Kg in HS3 to 26.5 meq/Kg in HS6 with a mean and standard deviation of 24.1±1.80. The HydroxyMethylFurfural content of the samples varied between 2.304 mg/Kg in HS2 to 13.236 mg/Kg in HS1 with a mean and SD of 8.84±4.03. The protein content of the samples varied between 0.5S% in HS4 to 0.91% in HS1 with a mean and SD of 0.72±0.13.

4. DISCUSSION

4.1 Botanical Origin

The analysis for botanical affinities of the different pollen spectrum and HDE shown in plate 1-11 across the eleven honey samples complemented by botanical inventory of 3km base radius around each of the beehive allowed the determination of entirely nectariferous taxa. The pollen spectrum and their botanical affinities are shown in Table 5.

The botanical origin of the various honey samples under investigation showed different variation in the number, type and percentage contribution of plant taxa foraged by the bees. There are thirty-one plant species foraged by the bees. These *species Allanblackia floribunda, Afzelia africana, Anonidium mannii Amphimas pterocarpoides, Antiaris toxicaria, Bridelia micrantha, Brenania brieyi, Cola edulis, Cola acuminata, Cola gigantea, Cocos nucifera, Canarium schweinfurthii, Cylicodiscus gabunensis, Cratet-ispermum cerinanthum, Ceiba pentandra, Corynanthe pachyceras, Desplastia dewevrei, Erythrina senegalensis, Enantia chlorantha, Hypodaphnis zenkeri Lepidobotyx staudtii, Lophira alata, Mammea africana, Macaranga barteri Musanga cecropoides, Piptadeniastrum africanum, Poga oleosa, Porterandia cladantha, Spathodea campanulata, Treculia africana and Xylopia rubescens*.

Several studies on the botanical origins of honey samples have been reported in Nigeria [18], 13, [19,20], [20, and 9], [21 and 22] Some bee foraged genera indentified in studies from Northern Nigeria include *Acacia, Adansonia, Alchornea, Anonidium, Bligha, Ceiba, Spondias, Bridelia, Cussonia, Azadirachta, Borreria, Bombax, Borassus, Cassia, Cleome, Combretum, Delonix, Eucalyptus, Elaeis, Guarea, Gmelina, Faidherbia, Hymenocardia, Hyphaene, Isoberlinia, Lannea, Luffa, Khaya, Parkia, Morus, Parinari, Phoenix, Pterocarpus, Syzygium, Tectona, Triumfetta, Vernonia, Vitellaria, Vitex, Ximenia, Zea mays and Ziziphus. Studies on botanical origins of honey in southern Nigeria include such genera as Cola, Pentaclethra, Citrus, Chrysophyllum, Dacryodus, Lannea microcarpa, Senna spp, Daniellia oliveri, Parkia biglobosa, Hymenocardia acida, Lophira lanceolata, Syzygium guineense, Parinari spp, Elaeis guineensis, Alchornea cordifolia, Khaya senegalensis, Canarium sp, Musanga cecropoides, Elaeis guineensis and Afzelia africana*. Since botanical origins of honey samples is indicative of the floristic resources of the area, about half of the bee foraged genera reported in the south eastern Nigeria was shown in this study in addition to some other genera known to Cross river state and Cameroonian floristic inventories. The number of plant taxa foraged by the bees varied from one beehive to another. It ranged from three in HSs 2, 5, 6 and 11 to 6 in HSs 1 and 9. Albeit about thirteen pollen structures for which botanical affinities could not be confirmed. When the percentage of plant species foraged by the bees were compared to the species diversity available to the bees (using species inventory within 3 km radius of each beehive), the study revealed an average of 2S% (one-fourth) for HS 1 and 3, 14% for HS 2 and 4.19% for HS 4.18% for HS 6, 22% for HS 7 and 8, 10% for HS 9 and 11 and 30% for HS10. The mean percentage of plant foraged by the bees in relation to the species diversity

available for foraging is 18%, translating to about 2 plant species for every 10 species that were available. The choice of foraged plant taxa seems to correlate strongly with sugary and other sensory parameters that were species-specific rather than on seasonality and blossoming times. This assertion was arrived at based on the inventory of the available plant taxa across four seasons (two years) of which all the plants censured blossomed at their different times (see Table 2), yet some were not foraged by the bee. It is instructive to suggest that nectar foraging by honeybees is species - selective, the choice of which is driven predominantly by sensory parameters.

Two of the honey samples under investigation; HS 2 and HS 8 qualify as unifloral honeys since they met the minimum pollen percentage count requirement of 45% and above. The remainder of the 9 samples was therefore not derived predominantly from a single plant nectar source, hence multifloral. Several melissopalynology studies in Nigeria and elsewhere had indicated a higher ratio of multifloral honey samples to unifloral ones [20,23,24,25,26 and 17] except in some limited studies which reported a higher percentage of unifloral honeys among the studied samples. These few were typified by [19]. In this study the unifloral honey of HS 2 and HS 8 was obtained predominantly from *Erythrina senegalensis* while the species affinity that yielded the predominant nectar/non nectar source in HS 8 could not be confirmed. When the percentage pollen counts were subsequently analyzed further using predominant pollen (>45%) secondary pollen (16-45%), minor (3-15%) and important minor pollen (< 3%), *Cylicodiscus gabunensis, Desplastia gabonensis, Afzelia africana, Craterispermum cerinanthum, Ceiba pentandra, Treculia africana, Anonidium mannii, Bridelia micrantha, Mammea africana, Corynanthe Pachyceras, Cocos* nucifera*, Canarium schweinfurthii,, Cola acuminata, Erythrina senegalensis, Porterandia Claudantha, Poga oleosi, Piptadeniastrum africana, Macaranga barteri Desplastia dewevrei, Antiaris toxicaria, Xylopia rubescens, Allanblackia floribunda, Lepidobotyx staudtii and* Spathodea campanulata *contributed on the average between 16% and 45% to the pollen spectrum of the 11 honey samples under investigation. On the other hand, species that contributed to minor pollen classification are Amphimas pterocarpoides, Mammea africana, Hypodaphnis zenkeri Cola gigantea, Lophira alata, Brenania brieyi, Musanga Cecropoides, Cola edulus and Entandrophragma cylindricurn. Enanthia chlorantha* was the only species with less than 3% pollen count and hence was classified important minor pollen. In the same vein, the foraging preference of the honey bees for *Cylicodiscus gabunensis, Amphimas pterocarpoides, Mammea africana, Erythrina senegalensis, Afzelia africana, Lepidobotyx staudtii and Poga oleosa* were observed as their pollen were observed in at least two of the honey samples as against one for the other contributing pollen from other inventoried species. The most plausible suggestion for this preference could be the nectar and sensory characteristics of these species since blossoming sequence for all species within 3 km radius of each beehive was monitored for two years before each hive was harvested for its honey content

4.2 Physico Chemical Parameters

Fourteen physico chemical parameters were determined for each of the 11 honey samples under investigations. The parameters are pH, moisture, specific gravity, color, glucose, fructose, Saccharose, electrical conductivity, free, lactic and total acidity, diastase activity, HydroxyMethylFurfural (HMF) and protein.

4.3 pH

Acidity of honey is a quality parameter used to indicate honeys susceptibility to fermentation and microbial growth. [1 and 27] stipulated a pH of 3.5-4.5. Values below the 3.50 threshold is prone to microbial degradation while values of between 4.5- 5.5 indicates a honey sample from plant and bees secretion (Honey dew elements) other than nectar source. The pH of most honey samples are acidic [28]. This is evident in this study where a range of 3.74-4.32 was observed. Similar values were observed in studies by [29] (3.10-4.20) for samples obtained in Umuahia and a mean of 3.90 for imported honey samples. [30] reported low pH values for polyfloral honeys than those of *Acacia* dominated (unifloral) honey samples while [22] observed a slightly higher p11 of about 5.21 honey samples obtained in Ogun state, south west Nigeria. When the values obtained in this study (3.74-4.32) was tested at .05 confidence limit the result of the p value ($p<0.001$) was statistically insignificant Similarly, these values were all within the regulatory limits and they were all of a blossoming honey type as suggested by [24].

Table 4. Results for physico chemical parameters for eleven samples of Cross River State Honey

Sample	Ph	Moisture (%)	Specific gravity (g/100 g)	Colour (mm/Pfund)	Fructose (g/100 g)	Glucose (g/100 g)	Saccharose (g/100 g)	Electrical conduct(µs/cm^{-1})	Diastase (DN)	Free acidity (meg/kg)	Lactonic acidity (meq/kg)	Total acidity (meg/kg)	HMF(mg/kg)	Protein (%)
HS1	4.32	21.2	1.41	54	32.2	25.4	1.2	0.546	20.5	18.8	4.2	22.9	13.32	0.91
HS2	3.74	12.8	1046	35	30.6	25.6	0.9	0.312	15.1	21.3	2.5	23.8	2.304	0.68
HS3	4.04	16.9	1.45	39	38.5	29.8	1.1	0.874	11.3	19.5	1.9	21.4	6.239	0.72
HS4	3.96	15.4	1.42	67	35.7	28.7	0.8	0.912	12.6	23.4	1.8	25.2	9.132	0.55
HS5	3.89	17.7	1.41	44	39.0	28.3	1.4	1.082	18.7	19.4	5.4	24.8	12.026	0.64
HS6	4.15	13.2	1.43	98	33.4	30.3	1.8	1.204	10.1	22.7	3.8	26.5	10.005	0.84
HS7	3.79	13.0	1.42	41	31.9	26.0	1.1	0.657	14.6	19.4	2.3	21.7	9.543	0.64
HS 8	4.13	16.8	1.44	53	33.2	25.8	1.3	0.437	13.9	18.9	4.1	23.0	5.602	0.71
HS9	2.86	15.1	1.41	43	34.7	27.6	0.9	0.823	18.6	22.4	3.6	26.0	11.765	0.59
HS10	4.22	13.2	1.41	62	38.4	29.4	1.7	0.90	17..3	18.8	4.1	22.9	12.002	0.87
HS11	4.09	19.7	1.45	112	35.7	26.9	1.5	1.12	10.2	20.3	3.4	23.7	4.321	0.82
Mean	4.02	15.9	1.43	51.5	34.8	27.6	1.25	0.81	15.3	20.4	3.37	23.8	8.75	0.72
SD	0.18	2.85	1.43	51.58	34.85	27.62	1.25	0.81	15.3	20.4	3.37	23.8	8.75	0.72
Range	3.74-4.32	12.8-21.2	1.41-1.46	35-112	30.6-39	25.4-30.3	0.8-1.8	1.312-1.204	10.1-20.5	18.7-23.4	1.8-54	21.4-26.5	2.304-13.326	0.55-0.91
Code X/EAS limits	3.5-4.5	≤20%	1.37-1.5	≤85pfund	31.2-42.4%	≤5%	≥0.8µs/cm^{-1}	≤8DN	≤50/meq/kg				≤8 mg/100 g	90%

Table 5. Pollen morphoforms and botanical affinity of the eleven honey samples obtained from beehives in Cross river State, Nigeria

Honey sample	Pollen morphofonns	Botanical affinity
HS1	Tricolpate (38.2%)	*Cylicodiscus gabonensis*
	Monoporate (36.4%)	*Desplastia gabonensis*
	Pantoporate (6.3%)	*Aphimas etrocarpoides*
	Monosulcate (6.1%)	*Mammea africana*
	Triporate (5.6%)	*Hypodaphnis zenkiri*
	Tricolporate (5.4%)	*Cola gigantea,*
HS2	Diporate (48.3%)	*Erythrina senegalensis*
	Tricolpate (21.6%)	*Afzelia africana*
	Dicolpate (12.4%)	*Unidentified*
	Triporate [9.7%)	*Lophira alata*
	Honey elements [8%)	*Secretions*
HS3	Tricolporate (32.7%)	*Craterispermum cerinanthum*
	Triporate (21.5%)	*Ceiba pentandra*
	Pantoporate (19.9%)	*Treculia africana*
	Monoporate (15.2%)	*Lepidobotyx staudtii*
	Tricolpate (6.3%)	Unidentified
	Monocolpate (3.1%)	Unidentified
	HDE (1.3%)	Fungal spores
HS4	Triporate (38.9%)	*Anonidium mannii*
	Tricolporate (19.8%)	*Bridelia micrantha*
	Monosulcate (18.4%)	*Mammea africana*
	Monoporate (16.5%)	*Corynanthe pachyceras*
	HDE (6.4%)	*Fungal spores*
HS5	Monocolpate (44.5%)	*Cocos nucifera*
	Tricolporate (27.3%)	*Canarium schweinfurthii*
	Tricolpate (22.7%)	*Cylicodiscus gabunensis*
	Dicolpate (5.5%)	*Brenania brieyi*
HS6	Tricolpate (40.5%)	*Unidentified*
	Dicolpate (21.9%)	*Unidentified*
	Tricolporate (20.5%)	*Cola acuminata*
	Monocolpate (14.1%)	*Musanga cecropoides*
	Diporate (3.0%)	*Erythrina senegalensis*
HS 7	Triporate (29.7%)	*Porterandia cladantha*
	Tricolporate (19.8%)	*Poga oleosa*
	Dicolpate (18.2%)	*Piptadeniastrum africanum*
	Monocolpate (16.6%)	*Unidentified*
	Tricolpate (12.7%)	*Afzelia africana*
	HDE (3.00%)	*Dinofiagellate cysts*
HS 8	Monoporate (51.2%)	*Unidentified*
	Tricolporate (16.4%)	*Macaranga barteri*
	Triporate (14.2%)	*Coula edulis*
	Dicolpate (12.7%)	*Unidentified*
	Pantuporate (5.5%)	*Amphimas pterocarpoides*
HS 9	Monoporate (42.1%)	*Desplastia dewevrei*
	Diporate (31.9)%	*Antiaris toxicaria*
	Syncolpate (15.6%)	*Xylopia rubescens*
	HDE (10.4%)	
HS10	Triporate (18.0%)	*Allanbiackia floribunda*
	Tricolporate (15.3%)	*Poga oleosa*
	Diporate (10.1%)	*Unidentified*
	Dicolporate (6.7%)	*Unidentified*
	Zonoporate (6.1%)	*Entandraphragma cylindricum*
	Tricolpate (3.1%)	*Unidentified*
	Zonoporate (2.1%)	*Enanthia chiorantha*
	Pantoporate (2.0)	*Unidentified*
	HDE (1.5%)	*Fungal cysts*
HS11	Monoporate (43.1%)	*Lepidobotyx staudtii*
	Dicolpate (27.4%)	*Unidentified*
	Tricolporate (22.7%)	*Spathadea campanulata*
	HDE (4.8%)	Plant secretions

4.4 Moisture

Moisture content defined as the amount of water present in a honey sample is one of the most important quality assurance criteria as it affects storage life, prevailing temperature, processing and harvesting methods and degree of maturation [28 and 29]. Low moisture content below the regulatory limit of 20% [1] forms an important component of the honey characteristics that inhibits microbial attack. [31] reported the deteriorating activities of moulds and yeast in honey samples with moisture content above the permissible limit of 20%. The values of moisture content of honey samples recorded across Asia, Africa and Nigeria were compared with those obtained in this study. For instance, [32] reported a moisture content of 184% or *Acacia* honeys while [33,34,35] reported values ranging from 14% - 23.36%. Values of the range 15.01-18.16% were reported by [28] for honey samples obtained in Ethiopia. In studies of honey samples obtained in Nigeria, [36,29] observe values higher than 20%, while [13,37,38,39] reported values lower than 20%. The values obtained in this study (12.8-21.2%) with the exception of HS1 (21.8%) were within the regulatory of not above 20% and could be classified either moisture content grade A (<18.6%) or grade B (>18.6 - >20%) USDA 2012.

4.5 Specific Gravity (S.G.)

Specific gravity also known as density is a measure of the sample weight to that of equal volume of water. The values for S.G. obtained in this study (1.41-1.46) is slightly higher than those reported for Obudu, Nsukka, Umuahia and Indian honey samples [40,36] but were within the maximum EAS [24] limits of 1.5. These obtained values are indicators of low honey viscosity. It is equally a quality assurance index of measuring the extent of dilution (adulteration) with water, processing/harvesting protocols and shelf life. There is a linear relationship between low S.G. values and low quality honey and vice versa. The minimum regulatory [1] limit for honey specific gravity is 1.37.

4.6 Colour

The pigmentation showed by honey samples is defined as its color. Honey color varies naturally in a wide range of tonalities, ranging from light yellow to amber, dark amber and black in extreme cases; sometimes green or red hues may also occur [41]. Colour serves as a criterion for the determination of the botanical origin of raw honey samples. However, its appearance changes with storage, human alterations and age. Color classes ar. expressed in millimeters (mm) Pfund grades, compared to an analytical grade Glycerol Standard Reference. Table 6 shows the United State Department of Agriculture [41].

Table 6. Classification of honey color using the Pfund scale

USDA color standards designation	Color range Pfund scale (mm)
Water White	8 or less
Extra White	5 to 17
White	17 to 34
Extra Light Amber	34 to 50
Light Amber	50 to85
Amber	85 to 114
Dark Amber	More than 114

The color and aroma of honey produced has a linear correlation with the type of flower foraged by the honey bee. The samples under investigations with color values ranging from 35 to 112 Pfund indicated that none of the honey samples under examination were either water white, extra white or white pigmentations. On the contrary, HSs 2, 3, 5, 7 and 9 had Pfund values corresponding to extra light amber, while HSs 1, 4, 8 and 10 had values categorized as Light amber as against HSs and 11 with Pfund values of 98 and 112 (Amber). The maximum [24] regulatory color for honey samples is the Light amber (85 pfund). For this study, HSs 6 and 11 could be suggested to be of lower quality in terms of color variation as it would have been exposed to overheating during hive harvesting. This possibility was also corroborated by the low diastase activity for these two honey samples (10.1 Schade unit and 10.3 respectively).

4.7 Sugar

The fructose/glucose ratio and the glucose/water ratio are honey assurance parameters used in predicting crystallization potentials of honey samples. Sixty percent (60%) of the constituents of every honey sample is composed of one sugar or the other [1]. The amount of which present in any giving sample is influenced by storage procedures and heating and therefore an indicator of how fresh or stale the sample is. The concentration of glucose and fructose observed in this study (30.6 -39%) and (25.4-30.3%) are

generally within the [1] regulatory limits (31.2-42.4%) and (23.2% - 32%) for fructose and glucose respectively. The only exception is HS 2 with fructose value of 30.6%. However, as observed by [42, and 22], the fructose content contained in honey is generally higher than that contained in glucose. [1] stipulated a minimum fructose and glucose percentage of not less than 60% (hr each honey sample. In this study, honey samples 1, 2, and 7 and had a combined fructose and glucose of less than the 60% indicating a low quality honey samples. In addition to fructose and glucose, sucrose is the most important sugar constituent which according to [43] constitutes about 95 % of honey dry weight The amount of sucrose contained in honey is directly related to the nectar of the plant taxa foraged by the bees [28]. In this study, the sucrose content varies between 0.8-1.8g/100g. This range is in agreement with several studies reviewed for this parameter [18 and 44] and well within the CODEX limit of not greater than 5%.

4.8 Electrical Conductivity (EC)

The amount of all ionizable and non ionizable substances found in a honey sample is measured using the electrical conductivity. Electrical Conductivity (EC) values depend on the mineral content of the honey. Examples of some ionizable and non ionizable substances in honey include K, Ca, Cu, Fe, Zn, Cr and Pb, complex sugars and polyps contents. The EC in addition to the ash content is often used as an index to measure the degree of pollution in the environment where the honey sample was produced [45]. The range of values obtained in this study (0.8-1.8 is/cm-^{1}) is within the CODEX limit of not less than 0.8 ps/cm-^{1} and indicates a non-polluted environment. The values for this study were in agreement with that obtained by [46] but differ significantly from that of [31].

4.9 Free, Lactic and Total Acidity

The acidity of honey is due to the presence of organic acids, particularly the gluconic acid and other minor acids (formic acid, acetic, lactic, maleic, malic, oxalic, pyroglutamic and succinic acids), in equilibrium with their lactones or esters and inorganic ions such as phosphate and chloride [47]. Free acidity is an important criterion for honey taste. The free acidity values for samples in this study ranged from 18.7 — 23.4 meq/kg. All the samples were within this limit On the other hand, lactonic acidity, the acidity reserve of the honey samples when it becomes progressively alkaline [48] is governed principally by the lactic acid bacteria found in the guts of honey bees. Lactonic acidity in honey helps in the deactivation of harmful bacteria, yeast and moulds that are found in the nectar, flowers and pollen of the foraged plant taxa [49]. In this study the lactonic acidity values ranged from 1.8 - 5.4 meq/Kg. The total acidity, which is a summation of the free acidity and lactonic aridity values ranged from 21.4 -26.5 meq/Kg. These values were within the CODEX regulatory limit of not more than 50 milliequivalent acid per 1000g. The results obtained for the acidity are slightly low when compared to those observed in Italian honeys (mean of total acidity 443.3 meq/kg) and honeys from La Rioja (mean of total acidity 39.5 meq/kg) but within range observed for Nigeria sample [20].

4.10 Hydromethylfufural (HMF)

HydroxyMethylFurfural, an organic acid derived from dehydration of glucose, fructose, Saccharose and other trace amount of hexoses in honey samples is one of the indicators of honey quality. The amount present in any honey sample increases with increase storage and heating regime. Review of literatures showed the availability of trace amounts of HMF in fresh honey. The mean HMF values for Ethiopian honeys was 32.4 mg/kg [50] while [22] reported a range of 1.20 mg/kg for Nigerian samples. The values obtained in this study (0.23-1.33 mg/100 g) were within the [24] regulatory limit of not more than 8 mg/kg.

4.11 Protein

The amount of protein contained in the honey samples under investigation varied between 0.55-0.91 percent. This range was higher than those reported by [51 and 22], but were within ranges by [50]. However, the value of 0.91% obtained for HS1 have values exceeding the CODEX standard of 0.90%. This is explained by the strong content of pollen in the sample.

4.12 Diastase

Diastase activity as an index of enzymatic (amylase) conversion of starch into maltose is a quality assurance criterion for freshness of honey samples. The diastase activity in these samples ranged between 10.1-20.0 DN. This range was above the minimum CODEX standard of 8DN but lower the maximum limit. The range is in agreement with previous studies of Nigerian sample.

4.13 Classification of the Samples Using Cluster Analysis

Classification analysis of the eleven honey samples were conducted using hierarchical cluster analysis. This was done in order to characterize honey samples that met the various discussed regulatory benchmarks into a common group while also noting the extent of the conformity (quality assurance) to the standards of the various samples. The variables of physico chemical parameters and botanical origins were used to determine the degree of similarity among the various honey samples using simple binary notation of one (1) for compliance to regulatory limits and zero (0) for non-compliance to regulatory benchmarks. Samples of unifloral types were assigned a value of one (1) as against those of polyfloral types with an assigned value of zero (0). Table 7 shows the conversion of these qualitative data into a quantitative one using the binary notion domain.

Three deductions are possible from Table 7. First, no one of the honey samples met all the regulatory benchmarks and second, no one of the honey sample failed all the regulatory benchmarks. Three cluster groups were evident in the study. Cluster group 1 (Honey samples 4, 7 and 8), with samples failing only one of the regulatory benchmarks. The second cluster group consists of samples failing at least three of the regulatory benchmarks. This group comprises of samples 3,5,6,9 and 10. The third of the cluster group consist of samples failing three and above of the benchmark criterion. This group is made up of honey samples 1 and 11. Fig. 1 is a dendrogram illustrating these findings.

Table 7. Binary notation of the botanical and physico chemical parameters of 11 honey samples in Cross River state

	1	2	3	4	5	6	7	8	9	10	11
Botanical	0	1	0	0	0	0	0	1	0	0	0
pH	1	1	1	1	1	1	1	1	1	1	1
Moisture	0	1	1	1	1	1	1	1	1	1	1
Specific gravity	1	1	1	1	1	1	1	1	1	1	1
Colour	1	1	1	1	1	0	1	1	1	1	0
Fructose	1	0	1	1	1	1	1	1	1	1	1
Glucose	1	1	1	1	1	1	1	1	1	1	1
Saccharose	1	1	1	1	1	1	1	1	1	1	1
Electrical conductivity	0	0	1	1	1	1	1	1	1	1	1
Diastase	0	0	1	1	0	1	1	1	0	0	0
Free acidity	1	1	1	1	1	1	1	1	1	1	1
Lactose acidity	1	1	1	1	1	1	1	1	1	1	1
Total acidity	1	1	1	1	1	1	1	1	1	1	1
HMF	1	0	0	1	1	1	1	0	1	1	0
Protein	0	1	1	1	1	1	1	1	1	1	1

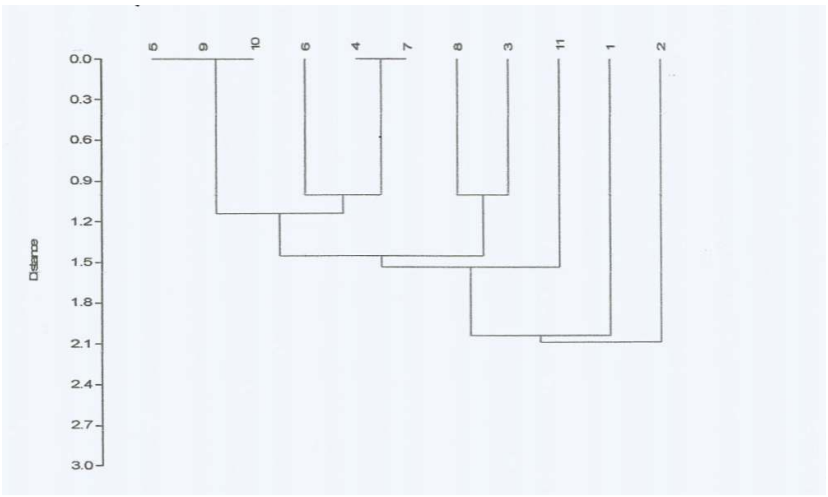


Fig. 1. A hierarchical Dendrogram showing cluster analysis among eleven honey samples from beehives in Cross River state Nigeria

5. CONCLUSION

It is evident from this study that an integrated approach of botanical and sensory parameters is essential in characterization of honey samples. Using botanical source alone, samples 2 and 8 would have been adjudged the best since they are unifloral but with an integrated approach of physico chemical parameters, honey sample 2 failed more benchmark criteria, leaving honey sample 8 as the best in terms of quality. Honey samples 4 and 7, despite being polyfloral, is considered of high quality also. It is also instructive to note that using geographical origin as a brand name to suggest honey quality or otherwise should be applied with caution. This is so because, honey samples termed "Obudu honey" is usually adjudged the best in Cross River state but this study do not agree with this universal assertion as honey sample 8, obtained from Etung was the best according to this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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