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Evaluation of the Floral Diversity and Physicochemical Composition of Honey Procured from some Markets in Anambra State, Nigeria

[1*]IKEGBUNAM, NC; [2]WALTER, OJ; [3]OSAYI, EE

*[1]Department of Botany, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria
[2]Department of Botany, Faculty of Science, University of Lagos, Lagos, Nigeria
[3]Faculty of Science, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Nigeria

*Corresponding Author Email: nc.ikegbunam@unizik.edu.ng
*ORCID: https://orcid.org/0000-0002-8056-7474)
*Tel: +2347065224331

Co-authors email: okwong56@gmail.com; emmanuel.osayi@unn.edu.ng

ABSTRACT: The identification of different plant species in honey is vital as they contribute towards the composition of honey and helps to verify honey authenticity. Therefore, the objective of this study was to evaluate the pollen content and physicochemical composition of honey from different markets (Okpuno Market, Eke Awka, Ifite Market, Eke Nibo and Nkwo Amaenyi) in Anambra State, Nigeria using standard methods. The best recorded values for the tested parameters were; conductivity (0.39±0.03%) in Okpuno sample, pH (2.80± 0.2) in Nkwo Amaenyi Sample, moisture content (10.32±0.03%) in Okpuno sample, ash (0.46±.02%) in Ifite market sample, protein (1.31±0.03%) in Ifite sample, fat (0.16±0.03%) in Eke-Awka sample, polyphenol (8.1±0.25%) in Eke-Awka sample, free acidity (28.93±0.25 meq kg-1) in Nkwo Amaenyi sample, HMF (5.75±0.03 mg/kg) in Nkwo Amaenyi sample, Sucrose (7±1 mg/100g) in Eke-Awka sample, reducing sugar (62.16±0.5%) in Okpuno sample etc. Microscopic examination revealed 37,590 pollen grains, dominated by *Phyllanthus amarus*, *Elaeis guineensis*, Combretaceae/Melastomataceae, *Lannea* sp., and *Parinari excelsa*. The most dominant families of plants present in the samples were Fabaceae, Phyllanthaceae, Arecaceae, Rubiaceae, Euphorbiaceae, Sapindaceae and Anacardiaceae. All the samples were identified as multifloral honey, derived from the nectar of various plant species, with no single species being predominant. The wide variety of pollen types indicates that honeybees travel long distances for nectar and other food sources, indicating authenticity and good quality honey. The sugar assays revealed that all honey samples did not contained the appropriate amount of sugar for acceptable quality honey. However, most measured parameters met international standards, indicating safe human consumption.

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Honey is a natural product produced by honeybees from the nectar of flowers, which they combine with other substances to form honey (Kumar *et al*., 2024). It is one of the oldest food substances and has been used as a major sweetener since ancient times. Honey is made up of pollen grains collected by honeybees, and analyzing pollen in honey can help identify plant species from which it originated (Mehndi *et al*., 2023). Diverse flowers produce honey with varying quantities, qualities, colours, consistencies, and flavours (Sharma *et al*., 2023). Melissopalynology is a scientific discipline that studies pollen grains and spores in honey, helping to determine its geographical and floral origin

*Corresponding Author Email: nc.ikegbunam@unizik.edu.ng
*ORCID: https://orcid.org/0000-0002-8056-7474)
*Tel: +2347065224331

(Zerrouk *et al*., 2021). This identification is crucial as it contributes to the composition of honey and can verify its authenticity (Żak and Wilczyńska, 2023).

The physical characteristics of honey, such as colour, pH, enzyme activity, ash content, and taste, can vary depending on the honeybee species, geographical origin, and the presence of impurities (Tarapoulouzi *et al*., 2023). The colour ranges from pale yellow to dark red to black, and the formation of granules distinguishes honey from other sweeteners (Momtaz *et al*., 2023). The pH of honey indicates the purity or crudeness of the sample, and the chemistry of honey also varies depending on the geographical origin and purity of the sample (Er Demirhan and Demirhan, 2022). Honey typically contains 80-85% carbohydrates, 15-17% water, 0.3% proteins, 0.2% ashes, and small amounts of other substances such as amino acids, phenols, pigments, and vitamins (Alu'dattl *et al*., 2024). The composition of honey varies based on the origin of nectar used by bees, the time of harvest, and the geo-climatic conditions of the honey-producing regions (Kraisoraphong, 2023). Pollen analysis is useful in bee management and beekeeping development, providing reliable information on the floral and geographical origin of honey and the preference of bees among various plant species. Some scientists (Adekanmbi *et al*., 2019; Ikegbunam *et al*., 2023; Daraojimba and Da Luz, 2023; Oyeyemi, 2023; Tesi *et al*., 2024)have carried out physicochemical investigation and pollen analytical studies of some Nigerian honey with the aim of identifying the nectar and pollen sources and as well determine the quality of the honey. However, there is a paucity of information from different market sources hence; the objective of this study is to evaluate the pollen content and physicochemical composition of honey from different markets (Okpuno Market, Eke Awka, Ifite Market, Eke Nibo and Nkwo Amaenyi) in Anambra State.

MATERIALS AND METHODS

Samples collections: In October 2023, five honey samples were collected from markets in Okpuno, Eke Awka, Ifite, Eke Nibo, and Nkwo Amaenyi within Anambra State, Nigeria (Fig. 1). The samples were stored in tightly sealed containers at room temperature to prevent moisture absorption. Each honey sample was produced in Anambra State. This region, located in Southeastern Nigeria, features a diverse vegetation mix, including elements of the Guinea Savannah, which consists of open grasslands and scattered trees, influencing the floral sources.

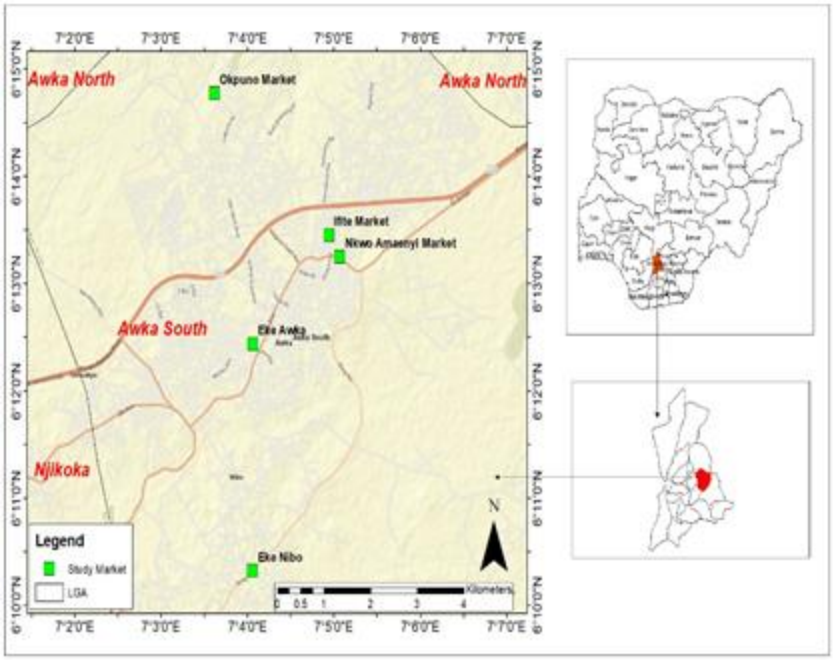

Fig. 1: The vegetation setting of study location
Source: ESRI, 2019

Pollen analysis: Pollen analysis was conducted following the guidelines of the International Commission of Bee Botany (Louveaux *et al*., 1978). Each honey sample, weighing 10 grams, was mixed with 35 ml of warm, acidified water and centrifuged at 5000 rpm for 10 minutes to dissolve colloidal matter and sugars. The supernatant was carefully removed, and 10 ml of

glacial acetic acid was added to eliminate the remaining water before acetolysis. The honey samples were then acetolyzed according to Erdtman's (1969) method. Ten (10 grams) of the resulting polliniferous residue were mounted in glycerine jelly and examined under a compound microscope at X400 magnification. Pollen grains were identified using descriptions and photomicrographs from various references (Y'bert, 1979; Bonnefille and Riollet, 1980; Agwu and Akanbi, 1985; Gosling *et al*., 2013) and compared with reference slides from the Palynology Laboratory, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Pollen types were classified into four frequency categories based on the method recommended by Louveaux *et al*. (1978). Pollen types representing more than 45% of the grains analyzed were classified as 'predominant.' Those making up between (16% - 45%) of the grains were categorized as 'secondary.' Pollen types constituting between (3% - 16%) of the grains were labeled as 'important minor,' while those representing (< 3%) of the grains were grouped together as 'minor' and present pollen (< 1%). Based on the frequency range of pollen and the number of predominant and dominant plant species per 10 grams of honey, three categories were identified (Wingenroth, 2001): monofloral (where a single species constitutes more than 45% of the pollen), bifloral (where two species each make up 22.25% of the pollen), and multifloral (where more than three species each contribute less than 16% of the pollen).The results were expressed as the number of pollen grains per gram of honey (PG g−1) and the Maurizio's classification was used (Maurizio *et al*., 1939), distributing the honey samples in five classes: Class I (less than 2000 PG g−1), Class II (between 2000 and 10,000 PG g−1), Class III (between 10,000 and 50,000 PG g−1), Class IV (between 50,000 and 100,000 PG g−1), and Class V (more than 100,000 PG g−1).

Physicochemical analysis: The pH of the samples was determined with a pH meter according to Arabameri *et al*. (2024), while the moisture content was determined using the drying oven method, by drying a representative 5 g sample in an oven at 105 °C for 3 hours until the weight was constant (A.O.A.C, 2010). The ash content was determined by the incineration of a 4 g sample in a muffle furnace at 600 °C for 6 h until the ash turned whitish-grey colour. The fat content was determined by petroleum ether extraction in a Soxhlet apparatus. A representative 3 g of sample was extracted for 6 hours (A.O.A.C, 2010). Crude protein was estimated by the Kjeldahl method. Total protein was calculated by multiplying the evaluated nitrogen by 6.25 (A.O.A.C, 2010). Using Ten (10) mil of the honey, the colour of the samples was determined by comparing the samples with Munsel colour chart (Giusti *et al*., 2024). Proline was extracted by heating one gram (1 g) of the honey for 20 mins in pure ethanol (10 ml) (Avşar *et al*., 2023). One gram and 2 grams of fructose and glucose respectively were used for the determination of the monosaccarides according to the methods of A.O.A.C (2010). Sucrose contents of the honey samples were analyzed using five (5) mil of the sample according to the methods of (Arabameri *et al*., 2024). The data generated were analyzed using IBM SPSS version 20.

RESULTSAND DISCUSSION

In the study, 37,590 pollen grains were identified, comprising 53 pollen types and 28 families. These plants were classified based on their production of pollen, nectar, or both (Table 1). Eke Nibo accounted for the largest proportion of the total recovered pollen grains, with 14,846 pollen grains belonging to 25 pollen types and 17 plant families, constituting 39.4% of the total. Eke Awka followed closely with 7,913 pollen grains belonging to 24 pollen types and 15 plant families, comprising 21% of the total. Ifite Market recorded 5,448 pollen grains, belonging to 17 pollen types and 19 plant families, representing 14.4% of the total. Nkwo Amaenyi recorded a total pollen count of 4,830, consisting of 20 pollen types and 18 plant families, accounting for 12.8% of the total pollen grains recovered. Lastly, Okpuno Market recorded 4,553 pollen grains, belonging to 26 pollen types and 20 plant families, representing 12.1% of the total palynomorphs (Table 1). The most dominant families of plants were Fabaceae, Phyllanthaceae, Arecaceae, Rubiaceae, Euphorbiaceae, Sapindaceae and Anacardiaceae which were present in all the samples. The secondary pollen types recorded included *Elaeis guineensis, Lannea* sp., *Phyllanthus amarus, Bombax buonopozense, Hymenocardia acida, Parinari excelsa*, and members of Combretaceae/Melastomataceae (Table 1). The identified species of plants in the sample belong to varying genera of herbs, shrubs, grass, and trees (Table 2). Frequency class analysis revealed that secondary pollen grains (15-45%) accounted for nine pollen types, important minor pollen (3-15%) consisted of 14 pollen types, minor pollen (1-3%) comprised 17 pollen types, and other pollen types

(< 1%) accounted for 20 different types (Table 2). All the samples were identified as multifloral honey, derived from the nectar of various plant species, with no single species being predominant (Table 3).

The absolute pollen grain counts in the honeys studied revealed that 60.5% of the samples from Eke Awka (7,913), Ifite (5,448), Okpuno (4,553), and Nkwo Amaenyi (4,830) were classified as Maurizio's Class II, indicating normal pollen grain representation (Table 3). In contrast, 39.5% of the samples, specifically from Eke Nibo (14,846), were classified as Maurizio's Class III, indicating an over-representation of pollen grains (Table 3).

Table 1: Percentage composition of the various pollen types in the honey samples

S/n	Plant taxa	Family	Okpuno Market	Eke Awka	Nkwo Amaenyi	Ifite Market	Eke Nibo	Nutrient Sources
1.	*Justicia* sp.	Acanthaceae	-	-	-	-	0.03	Nectariferous
2.	Amaranthaceae	Amaranthaceae	0.13	0.03	0.10	-	-	Polliniferous
3.	*Antrocaryon micraster*	Anacardiaceae	10.10	0.37	-	-	-	Polliniferous and Nectariferous
4.	*Lannea acida*	Anacardiaceae	17.35	8.08	0.10	14.86	11.31	Polliniferous and Nectariferous
5.	*Mangifera indica*	Anacardiaceae	1.75	-	-	-	-	Polliniferous and Nectariferous
6.	Apiaceae	Apiaceae	-	-	0.10	-	-	Polliniferous and Nectariferous
7.	*Cocos nucifera*	Arecaceae	0.13	-	-	-	-	Polliniferous and Nectariferous
8.	*Elaeis guineensis*	Arecaceae	8.34	25.78	1.03	2.75	5.25	Polliniferous
9.	*Hyphaene ventricosa*	Arecaceae	-	-	0.31	-	-	Polliniferous and Nectariferous
10.	*Bombax buonopozense*	Bombacaceae	-	-	-	27.53	-	Polliniferous and Nectariferous
11.	*Canarium schweinfurthii*	Burseraceae	-	-	-	-	0.16	Nectariferous
12.	*Commiphora africana*	Burseraceae	-	-	0.58	-	-	Polliniferous and Nectariferous
13.	*Parinari excels*	Chrysobalanaceae	7.90	-	0.62	37.81	-	Nectariferous
14.	Combretaceae/ Melastomataceae	Combretaceae/ Melastomataceae	11.86	10.11	21.53	1.46	27.48	Polliniferous and Nectariferous
15.	*Ipomea* sp.	Convovulaceae	0.22	-	-	-	-	Polliniferous and Nectariferous
16.	*Croton* sp.	Euphorbiaceae	-	0.37	-	-	0.13	Polliniferous and Nectariferous
17.	*Securingea virosa*	Euphorbiaceae	-	-	0.57	-	1.27	Polliniferous and Nectariferous
18.	*Uapaca togoensis*	Euphorbiaceae	-	0.06	-	-	0.04	Polliniferous and Nectariferous
19.	*Alchornea cordifolia*	Euphorbiaceae	1.97	2.90	4.34	1.65	2.22	Polliniferous
20.	*Dalbergia* sp.	Fabaceae	-	-	-	-	0.26	Polliniferous and Nectariferous
21.	*Daniellia oliveri*	Fabaceae	-	-	-	-	0.67	Polliniferous
22.	*Delonix regia*	Fabaceae	-	-	-	0.07	-	Polliniferous and Nectariferous
23.	Fabaceae	Fabaceae	-	-	9.52	1.83	-	Polliniferous and Nectariferous
24.	Papilionioideae	Fabaceae	-	0.08	-	-	-	Polliniferous and Nectariferous
25.	*Pentaclethra macrophylla*	Fabaceae	-	-	-	-	0.10	Polliniferous and Nectariferous
26.	*Acacia* sp.	Fabaceae	-	0.25	-	-	-	Polliniferous and Nectariferous
27.	*Baphia nitida*	Fabaceae	0.08	-	-	-	-	Polliniferous and Nectariferous
28.	*Bauhinia purpurea*	Fabaceae	6.80	-	-	-	0.06	Polliniferous and Nectariferous
29.	*Brachystegia eurycoma*	Fabaceae	-	0.18	-	-	0.03	Polliniferous and Nectariferous
30.	*Cassia* sp.	Fabaceae	1.09	0.25	0.58	0.18	-	Polliniferous and Nectariferous
31.	*Mimosa* sp.	Fabaceae	0.13	-	-	-	-	Polliniferous and Nectariferous
32.	Mimosoideae	Fabaceae	-	-	1.24	-	-	Polliniferous and Nectariferous
33.	*Parkia bicolor*	Fabaceae	0.06	-	-	-	-	Polliniferous and Nectariferous
34.	*Irvingia gabonensis*	Irvingiaceae	0.13	0.31	0.58	-	0.53	Polliniferous

35.	Liliaceae	Liliaceae	-	-	0	0.09	0.06	Polliniferous Nectariferous	and
36.	Loranthaceae	Loranthaceae	0.21	-	0	1.83	0.03	Polliniferous Nectariferous	and
37.	*Corchorus olitorius*	Malvaceae	-	-	0.18	-	-	Polliniferous Nectariferous	and
38.	*Trichilia prieureana*	Meliaceae	0.21	-	-	-	-	Nectariferous	
39.	*Trichillia* sp.	Meliaceae	-	-	-	0.09	-	Nectariferous	
40.	Moraceae	Moraceae	0.13	0.12	0.10	0.55	-	Polliniferous Nectariferous	and
41.	*Psidium guajava*	Myrtaceae	-	0.25	-	-	-	Polliniferous Nectariferous	and
42.	*Syzygium guineense*	Myrtaceae	2.85	5.05	4.34	1.83	5.79	Nectariferous	
43.	*Sesamum indicum*	Pedaliaceae	-	-	-	0.18	0.06	Polliniferous Nectariferous	and
44.	*Antidesma* sp.	Phyllanthaceae	-	0.03	-	0.18	0.10	Polliniferous Nectariferous	and
45.	*Bridelia ferruginea*	Phyllanthaceae	1.76	-	-	-	-	Polliniferous Nectariferous	and
46.	*Hymenocardia acida*	Phyllanthaceae	16.03	7.58	14.28	1.08	14.95	Polliniferous	
47.	*Phyllanthus amarus*	Phyllanthaceae	3.07	25.90	18.21	0.09	-	Polliniferous	
48.	Poaceae	Poaceae	0.21	1.13	0.58	0.73	-	Polliniferous	
49.	*Protea angolensis*	Proteaceae	0.44	1.89	-	-	-	Polliniferous Nectariferous	and
50.	*Ziziphus Mauritiana*	Rhamnaceae	0.13	3.53	-	0.73	0.13	Polliniferous Nectariferous	and
51.	*Crossepferyx febrifuga*	Rubiaceae	-	-	1.24	-	-	Polliniferous Nectariferous	and
52.	*Mussaenda erythiophylla*	Rubiaceae	-	0.37	-	-	-	Polliniferous Nectariferous	and
53.	*Mussaenda* sp.	Rubiaceae	-	-	0.62	-	-	Polliniferous Nectariferous	and
54.	*Sarcocephalus latifolius*	Rubiaceae	0.13	0.25	2.48	2.93	15.42	Polliniferous Nectariferous	and
55.	*Blighia sapida*	Sapindaceae	0.13	-	-	-	-	Polliniferous Nectariferous	and
56.	*Paulinia pinnata*	Sapindaceae	-	-	0.31	-	-	Polliniferous Nectariferous	and
57.	Sapindaceae	Sapindaceae	-	-	-	0.73	0.10	Polliniferous Nectariferous	and
58.	*Solanum melongena*	Solanaceae	-	1.89	-	-	-	Polliniferous Nectariferous	and
59.	*Solanum* sp.	Solanaceae	-	-	4.55	0.91	0.13	Polliniferous Nectariferous	and
60.	*Celtis* sp.	Ulmaceae	0.13	0.18	0.10	-	4.24	Polliniferous	
		Total Pollen counts	**4,553**	**7,913**	**4,830**	**5,448**	**14,846**		

Table 2: Classification of the various pollen types in the honey samples

S.n	Plant taxa	Family	Okpuno Market	Eke Awka	Nkwo Amaenyi	Ifite Market	Eke Nibo	Growth Forms
1.	*Justicia* sp.	Acanthaceae	-	-	-	-	P	Herb
2.	Amaranthaceae	Amaranthaceae	P	P	P	-	-	Herb
3.	*Antrocaryon micraster*	Anacardiaceae	IM	P	-	-	-	Tree
4.	*Lannea acida*	Anacardiaceae	S	IM	P	IM	I M	Shrub
5.	*Mangifera indica*	Anacardiaceae	M	-	-	-	-	Tree
6.	Apiaceae	Apiaceae	-	-	P	-	-	Tree
7.	*Cocos nucifera*	Arecaceae	P	-	-	-	-	Tree
8.	*Elaeis guineensis*	Arecaceae	IM	S	M	M	IM	Tree
9.	*Hyphaene ventricosa*	Arecaceae	-	-	P	-	-	Tree
10.	*Bombax buonopozense*	Bombacaceae	-	-	-	S	-	Tree
11.	*Canarium schweinfurthii*	Burseraceae	-	-	-	-	P	Tree
12.	*Commiphora africana*	Burseraceae	-	-	P	-	-	Tree
13.	*Parinari excels*	Chrysobalanaceae	IM	-	P	S	-	Tree
14.	Combretaceae/ Melastomataceae	Combretaceae/ Melastomataceae	IM	IM	S	M	S	Tree
15.	*Ipomea* sp.	Convovulaceae	P	-	-	-	-	Herb
16.	*Croton* sp.	Euphorbiaceae	-	P	-	-	P	Herb
17.	*Securingea virosa*	Euphorbiaceae	-	-	P	-	M	Shrub
18.	*Uapaca togoensis*	Euphorbiaceae	-	P	-	-	P	Tree
19.	*Alchornea cordifolia*	Euphorbiaceae	M	M	IM	M	M	Shrub
20.	*Dalbergia* sp.	Fabaceae	-	-	-	-	P	Tree
21.	*Daniellia oliveri*	Fabaceae	-	-	-	-	P	Tree
22.	*Delonix regia*	Fabaceae	-	-	-	P	-	Tree
23.	Fabaceae	Fabaceae	-	-	IM	M	-	Tree
24.	Papilionioideae	Fabaceae	-	P	-	-	-	Tree
25.	*Pentaclethra macrophylla*	Fabaceae	-	-	-	-	P	Tree
26.	*Acacia* sp.	Fabaceae	-	P	-	-	-	Tree

27.	*Baphia nitida*	Fabaceae	P	-	-	-	-	Tree
28.	*Bauhinia purpurea*	Fabaceae	IM	-	-	-	P	Tree
29.	*Brachystegia eurycoma*	Fabaceae	-	P	-	-	P	Tree
30.	*Cassia* sp.	Fabaceae	M	P	P	P	-	Tree
31.	*Mimosa* sp.	Fabaceae	P	-	-	-	-	Herb
32.	Mimosoideae	Fabaceae	-	-	M	-	-	Herb
33.	*Parkia bicolor*	Fabaceae	P	-	-	-	-	Tree
34.	*Irvingia gabonensis*	Irvingiaceae	P	P	P	-	P	Tree
35.	Liliaceae	Liliaceae	-	-	-	P	P	Herb
36.	Loranthaceae	Loranthaceae	P	-	-	M	P	Herb
37.	*Corchorus olitorius*	Malvaceae	-	-	P	-	-	Herb
38.	*Trichilia prieureana*	Meliaceae	P	-	-	-	-	Shrub
39.	*Trichillia* sp.	Meliaceae	-	-	-	P	-	Shrub
40.	Moraceae	Moraceae	P	P	P	P	-	Tree
41.	*Psidium guajava*	Myrtaceae	-	P	-	-	-	Tree
42.	*Syzygium guineense*	Myrtaceae	M	IM	IM	M	IM	Tree
43.	*Sesamum indicum*	Pedaliaceae	-	-	-	P	P	Herb
44.	*Antidesma* sp.	Phyllanthaceae	-	P	-	P	P	Shrub
45.	*Bridelia ferruginea*	Phyllanthaceae	M	-	-	-	-	Tree
46.	*Hymenocardia acida*	Phyllanthaceae	S	IM	IM	M	IM	Tree
47.	*Phyllanthus amarus*	Phyllanthaceae	IM	S	S	P	-	Herb
48.	Poaceae	Poaceae	P	M	P	P	-	Grass
49.	*Protea angolensis*	Proteaceae	P	M	-	-	-	Shrub
50.	*Ziziphus Mauritiana*	Rhamnaceae	P	IM	-	P	P	Shrub
51.	*Crossepferyx febrifuga*	Rubiaceae	-	-	M	-	-	Tree
52.	*Mussaenda erythiophylla*	Rubiaceae	-	P	-	-	-	Shrub
53.	*Mussaenda* sp.	Rubiaceae	-	-	P	-	-	Shrub
54.	*Sarcocephalus latifolius*	Rubiaceae	P	P	M	M	S	Tree
55.	*Blighia sapida*	Sapindaceae	P	-	-	-	-	Tree
56.	*Paulinia pinnata*	Sapindaceae	-	-	P	-	-	Shrub
57.	Sapindaceae	Sapindaceae	-	-	-	P	P	Shrub
58.	*Solanum melongena*	Solanaceae	-	M	-	-	-	Herb
59.	*Solanum* sp.	Solanaceae	-	-	IM	P	P	Herb
60.	*Celtis* sp.	Ulmaceae	P	P	P	-	IM	Tree

Frequency Classes: S = secondary pollen (15–45%), IM = important minor pollen (3–15%), M = minor pollen (1–3%), P = present pollen (< 1%).

Table 3: Quantitative melissopalynological analyses of the different honey samples.

Honey Sample	APC/10g honey	Maurizio's Classes	Botanical sources (Plant Species)	Class honey
NkwoAmaenyi	4,830	II	Combretaceae/Melastomataceae (21.5%), Euphorbiaceae (4.9%), Fabaceae (9.5%), *Hymenoacardia acida* (14.2%), *Phyllanthus amarus*(18.2%).	Multifloral
Ifite Market	5,448	II	*Elaeis guineensis*(2.7%), *Bombax buonopozense*(27.5%), *Parinariexcelsa*(37.8%), *Lannea*sp. (14.8%), *Sarcocephaluslatifelius*(2.7%).	Multifloral
Eke Nibo	14,846	III	Combretaceae/Melastomataceae (27.4%), *Hymenoacardiaacida* (14.9%), *Lannea*sp. (11.3%), *Phyllanthus amarus*(15.4%), *Syzygium guineense*(5.7%).	Multifloral
Okpuno market	4,553	II	*Antrocaryonmicraster*(10.1%), Combretaceae/Melastomataceae (11.8%),*Elaeis guineensis*(8.3%), *Hymenocacardiaacida*(16.0%), *Lannea*sp. (17.3%).	Multifloral
Eke Awka	7,913	II	Combretaceae/Melastomataceae (10.1%), *Elaeis guineensis*(25.7%), *Hymenocacardiaacida*(7.5%), *Lannea*sp. (8.0%), *Phyllanthus amarus*(18.2%).	Multifloral

The conductivity measurements indicated that the highest was observed in Nkwo Amaenyi with a value of 0.87±0.03 mS/cm, while the lowest was recorded in Eke Awka (0.39±0.03 mS/cm). The pH values were found to be highest in Okpuno Market honey with a value of (3.93±0.3), and lowest in honey from Eke Awka (2.9±0.1). Eke-Awka (41.5±0.1 meq/kg) and Nkwo Amaenyi (28.93±0.25 meq/kg) recorded the highest and lowest free acidity respectively (Table 4). The proline content was found to be highest in Nkwo Amaenyi with a value of (3010±3 mg/kg), and lowest in Okpuno Market (19±1.0 mg/kg). Eke-Awka (8.1±0.25%) and Ifite Market (3.9±0.21%) both had the highest and the lowest polyphenol content, respectively. Hydroxylmethylfurfural levels of honey samples sourced from the markets ranged from Okpuno Market (39.75±0.03 mg/kg) to Nkwo Amaenyi (15.76±0.03 mg/kg). The Eke-Awka honey sample had the highest Fructose with a mean of (39.35±0.02 mg/100g), while the lowest was recorded in sample from Okpuno Market (37.86±0.01 mg/100g). In terms of glucose content, sample from Okpuno market had the highest amount at (24.3±0.5 mg/100g), while Eke Awka had the lowest at (13.3 mg/100g) (Table 4).The honey sample from Okpuno market had the highest reducing sugar content at (62.16 ± 0.5 g/100 g), whereas the honey from Eke Akwa had the lowest at (52.65 ± 0.4 g/100 g). For sucrose, Ifite Market had the highest amount at (42.07±1.37 mg/100g), and Eke Awka had the lowest at (7±1 mg/100g). In terms of moisture content, Ifite Market had the highest amount at (14.53±0.03%),

while Okpuno Market had the lowest at (10.32±0.03%). For protein content, Ifite Market had the highest amount at (1.31±0.03%), while Eke Nibo Market had the lowest at (0.06±0.03%). For fat content, Eke Nibo had the highest amount at (0.39±0.04%), while Eke-Awka had the lowest at(0.16±0.03%). Finally, for the ash content, Eke Nibo and Eke-Awka were similar but significantly different from the recorded values from other locations at $P \leq 0.05$ (Table 4).

Table 4: The mean and standard error (mean ± SE) values for the physicochemical content of commercially available honey collected from study areas.

Parameter	Ifite Market	Eke-Awka	Okpuno Market	NkwoAmaenyi	Eke Nibo
Conductivity (mS/cm)	0.5±0.03^b	0.39±0.03^b	0.39±0.02^a	0.87±0.03^b	0.61±0.03^b
pH	3.1±0.1^a	2.9±0.1^a	3.93±0.3^b	2.8±0.2^a	3.53±0.4^b
Free Acidity (meq/kg)	30.12±0.23^b	41.5±0.1^a	41.27±0.25^b	28.93±0.25^b	29.27±0.15^a
Proline (mg/kg)	1654.67±24.0^b	40.67±2.08^b	19±1.0^a	3010±3^b	1919.33±2.08^b
Polyphenol (%)	3.9±0.21^b	8.1±0.25^b	5.1±0.23^b	7.7±0.2^b	4.53±0.35^b
HMF (mg/kg)	36.51±0.02^a	35.33±0.01^a	39.75±0.03^b	15.76±0.03^b	29.93±0.02^a
Fructose (mg/100g)	39.27±0.03^b	39.35±0.02^a	37.86±0.01^a	38.92±0.03^b	38.9±0.02^a
Glucose (mg/100g)	16.2±0.6^b	13.3±0.4^a	24.3±0.5^b	19.8±0.3^a	21.4±0.53^b
Estimated reducing sugars	55.27±0.6^b	52.65±0.4^a	62.16±0.5^b	58.72±0.3^a	60.3±0.55^b
Sucrose (mg/100g)	42.07±1.37^b	7±1^b	27.3±0.7^a	15.5±0.46^a	19.8±0.3^a
Moisture (%)	14.53±0.03^b	13.2±0.01^a	10.32±0.03^b	12.6±0.03^b	13.12±0.02^a
Protein (%)	1.31±0.03^b	1.1±0.03^b	0.72±0.01^a	0.62±0.03^b	0.06±0.03^b
Fat (%)	0.17±0.03^b	0.16±0.03^b	0.25±0.03^b	0.26±0.02^a	0.39±0.04^b
Ash (%)	0.46±0.02^b	0.48±0.01^a	0.48±0.03^b	0.57±0.03^b	0.58±0.02^a

Data are presented as mean ± standard error. Means with different alphabets on each roll represent significant differences separated using Fishers Least Significant Difference test at $p \leq 0.05$. HMF = Hydroxylmethylfurfural

The examination of the pollen types presents in the honey samples confirmed their botanical origin and provided insight into their geographical source. This finding is consistent with prior research conducted on honey samples from north-central which reflect the vegetation type (Boruah *et al*., 2024).The pollen composition of the samples revealed important information about the local flora, which aligns with the findings of Adler *et al*. (2024).The identification of nectariferous and polliniferous plant species, including *Elaeis guineensis, Syzygium guineense, Ziziphus* sp., *Parkia biglobosa, Irvingia* sp., *Mangifera indica, Dailium guineense, Hymenocardia acida, Phyllanthus amarus,* and *Parinari excelsa*, reflects the lowland rainforest and Guinea Savanna vegetation types. These plant species have been previously reported in Southeastern Nigeria by Ikegbunam *et al*. (2023). The presence of pollen in honey samples can provide valuable insight into determining their authenticity (Mohamadzade Namin *et al*., 2023). The recovery of *Phyllanthus amarus* and *Hymenocardia acida* pollen grains in all the studied honey samples, except for Ifite, aligns with the findings of Essien and Olaniyi (2023) and Ikegbunam *et al*. (2023), who both identified these species in their honey samples from Ondo, Benue, and Kogi States. This abundance could be due to the large landing surface and high number of stamens with long filaments oriented externally (Amarilla *et al*., 2024). The presence of *Parinari excelsa* pollen grains in Ifite honey sample may be attributed to the ability of honey bees to recruit numerous workers for the exploitation of high-yield food sources (Nnamani and Ezikanyi, 2023). This consistency in honeybee foraging behavior is possible due to their ability to memorize and recognize the shape, colour, and odour of flowers visited during previous foraging trips (Desai *et al*., 2024). Although, *Elaeis guineensis* is not a nectar-producing plant, it can be cultivated and protected to increase honey production. Its presence in all studied samples suggests that it is commonly preferred in the tropics, as the juice of the fermenting fruit, which is collected by bees, is ubiquitous in nature. This finding is consistent with Adekanmbi *et al*. (2019), who analyzed pollen and heavy metals in honey samples from southern Nigeria. All the honey samples studied were multifloral, which indicates a unique brand of honey. The analyses of the honey samples showed that they are very nutritious containing various food nutrients varying from proteins, fats, among others. The various components occur in varying quantities which is comparable to the findings of other researchers (Ikegbunam and Okwu, 2021; Ikegbunam and Okwong, 2021). The sugar analysis showed that the monosaccharides (glucose and fructose) were the main sugars in the honey samples. The high values of fructose in the five samples were in agreement with the findings of Ikegbunam and Okwong (2021) and Alaerjani and Mohammed (2024) where the values of

fructose were found to be higher than those of other sugars. The sum of fructose and glucose showed that the values of the monosaccharides in the five samples were less than 60%. They therefore do not meet the acceptable international honey standard which states that the value should not be less than 60% (Codex Alimentarius Commission, 2019). The lower glucose content compared to fructose suggests that the bee colonies were naturally fed, confirming the high quality of the honey types examined. This finding aligns with the results reported by Geană *et al*. (2020). The reducing sugars content of Okpuno Market and Eke Nibo samples was deemed acceptable by Codex Alimentarius, except for Ifite, Eke Akwa and Nkwo-Amaenyi which had a value lower than the standard limit (Codex Alimentarius, 2001). The results showed that fructose and glucose are the main sugars in the honey samples. The codex standard for sucrose is the presence of not more than 5 g of sucrose in 100 g of honey samples (5 g/100g). Therefore, the presence of high amount of sucrose in the studied sample maybe associated with adulteration hence the honey samples did not meet the international standard with respect to sucrose content.

The pH of the honey samples was low enough to slow down or prevent the growth of many species of microorganisms. Honey's acidic nature, with a pH between 3.2 and 4.5, makes it highly effective in inhibiting various bacterial pathogens (Edo *et al*., 2023). From the obtained value, it is evident that the honey samples were acidic in nature which is a general model of assessing honey quality. The moisture content is the most essential quality component of honey because the rate of fermentation, shelf-life span, and processing characteristics are determined by the amount of moisture content. Quality honey should have a low moisture content to avoid fermentation by associated microorganisms and enzymatic factors. Alaerjani and Mohammed (2024) stated that the moisture content of honey can naturally be as low as 13% or as high as 23% depending on the source of honey, climatic conditions, and other factors. Low moisture content was observed in this study which agrees with the values recorded by Ikegbunam and Okwu (2021) and fell within the limit of not more than 22% for quality honey. The protein content of honey is regarded as important nutritional ingredient, though it is low when compared to the sugar content, and its presence in the honey sample indicates that it is nutritionally rich and therefore suitable as dietary supplement (Edo *et al*., 2023). The protein content in all the honey samples is higher than those estimated by international standards (0.26%) on average with a maximum of (0.83%) (Arabameri *et al*., 2024). Hydroxymethylfurfural is defined as the breakdown of fructose that is formed slowly and naturally during the storage of honey and much more quickly when honey is heated. It is highly recognized as an indicator of accurate information on the storage and heat treatment conditions of the honey (Yıldız and Bayraç, 2024). Although, the HMF values are within the range allowed by Codex Alimentarius Commission (2019), they are high. This information serves to improve honey storage conditions. Honey contains organic acids and mineral salts, which chemically are called "ionizable" that is when in solution, they have the property to conduct electric current. Electrical conductivity of honey according to Codex standard for quality honey should not be more than 0.8 mS/cm for nectar honey (Codex Alimentarius Commission, 2019). Conductivity is an indicator of floral origin. From the result, it was observed that samples from Ifite Market, Eke Awka, Okpuno Market and Eke Nibo had values like those reported by Yeboue *et al*. (2021) and conforms to the recommended standard while samples from Nkwo Amaenyi and Eke Nibo had values higher than the acceptable limit of quality honey. This may have resulted from incorrect processes applied by honey producers in extraction, processing, storage, and preservation.

High levels of free acidity in honey may be indicative of fermentation of honey sugars by yeast. The maximum acceptable level of free acidity, as stipulated by the Codex Alimentarius Standard, is 50 mg/kg. Three of the samples under investigation met the recommended limit for quality honey. The ash content of the samples, which reflects the total inorganic minerals present, ranged from 0.46 to 0.58 mg/kg, with honey from Eke Nibo having the highest ash content among all the samples. According to Aneni *et al*. (2023), honey contains very little or no fat, and the small quantity recorded in this study may have come from the melting of wax in the beehive during harvest, as suggested by Dar *et al*. (2024). Polyphenols, which act as antioxidants, are typically found in the form of flavonoids, and protect cells and body chemicals from damage caused by free radicals that contribute to tissue damage (Hassanpour and Doroudi, 2023). All the honey samples analyzed contained polyphenols in varying proportions, in agreement with the findings of Matkovits *et al*. (2023). Proline is a crucial amino acid primarily derived from the

salivary secretions of *A. mellifera* during the conversion of nectar into honey, as reported by Sharma *et al*. (2023). Proline is utilized as an indicator of the ripeness of honey and, in certain cases, as a marker for sugar adulteration. The proline content varied from 19.0 mg/kg to 3411.67 mg/kg, which is higher than the minimum permissible concentration of proline (180 mg/kg) established by the International Honey Commission. The samples collected from Eke-Awka Market and Okpuno Market had values that were lower than the minimum standard. Given that proline content is used as a measure of honey ripeness, these findings suggest that the samples may not have been fully ripened before harvest. Additionally, the lower proline content may indicate that the honey has been adulterated with sugar.

Conclusion: The study confirmed that the honey samples were multifloral, indicating a diverse botanical origin with no single dominant species. The analysis demonstrated a variety of physicochemical properties and nutritional components, including acidic pH levels, moisture content, and varying amounts of protein, fat, and ash. The sugar analysis revealed fructose and glucose as the primary sugars, consistent with international honey standards. However, increased sucrose levels in some samples raise concerns about potential adulteration. Pollen analysis showed that honeybees foraged from a wide range of plants, reinforcing the authenticity of the honey samples. Overall, the study underscores the multifloral nature of the honey and recommends enhanced handling practices to preserve both quality and authenticity.

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Data Availability: Data are available upon request from the first author or corresponding author.

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