

# Melissopalynological and Physicochemical Analysis of Honey Samples from Ekiti, Southwestern Nigeria

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## ABSTRACT

**Background and Objective:** The lack of adequate information on parameters that characterize locally produced and marketed honey brands is a problem that needs to be addressed. This is particularly important in providing insight into the quality and commercial value of honey. **Materials and Methods:** To achieve this aim, honey samples were collected from four apiaries in Ekiti State, Southwestern Nigeria. Standard routine palynological procedures (pollen analysis-acetolysis and microscopy) were adopted for the quantification and qualification of pollen types while the AOAC methods were used for the determination of physico-chemical parameters. **Results:** Honey was from unifloral and bifloral sources. Total pollen count (in 10  $\mu\text{L}$ ) ranged from 57 to 1453. Twenty-two palynomorph types (21 pollen and 1 spore) from 15 families were recovered. Frequently encountered pollen types included *Elaeis guineensis*, *Blighia sapida*, *Oldenlandia corymbosa*, *Talinum triangulare*, *Phyllanthus* sp., *Jatropha* sp., *Cardiospermum halicacabum*, Solanaceae. The range for physicochemical parameters was pH, 2.78-3.22, specific gravity, 1.39-1.41, free acidity ( $\text{mEq kg}^{-1}$ ), 18.10-37.95, HMF ( $\text{mg kg}^{-1}$ ), 12.44-32.61, refractive index, 1.42-1.45, electrical conductivity ( $\text{mS cm}^{-1}$ ), 0.49-1.08, total solid (%), 81.44-89.39, total sugar (%), 76.10-82.29 and antioxidant activity ( $\mu\text{g mL}^{-1}$ ), 307.09-974.28. Nutritional parameters were within codex limits and pollen content indicated foraging from the cultivated area and degraded and human-impacted open forests. There were significant differences in the nutritional content of honey samples from the same vegetation belt. **Conclusion:** Despite the level of low pollen content, important biomarkers were recovered and the honey exhibited good quality and purity concerning their physicochemical properties. Although the pollen types did not reflect any major vegetation belt, they provided vital information on the impact of humans on the vegetation of the area where honey samples were collected.

## KEYWORDS

Honey, physicochemical parameters, pollen analysis, nectar, pollen, melissopalynology

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## INTRODUCTION

Honey is majorly a product of nectar and pollen grains from flowering plants. Bees visit plants in search of nectar and pollen, which are returned to the hive for use and storage. The medicinal and nutritional importance of honey depends on its physical and chemical properties. There are reports that some of the



unbranded honey sold in markets have tampered with<sup>1</sup>. Despite the enormous health benefit of honey, consumers still fear purchasing honey from uncertified producers due to honey adulteration. Ayodele *et al.*<sup>2</sup> reported that some of the honey that is sold in the market and groceries in Nigeria are molasses (sugarcane syrup). Adulteration of honey does more dangerous to consumers. There is a need to carry out an analysis of honey to verify its quality, authenticity and botanical and geographical origin. To achieve this objective, pollen, sensory, biological and/or physicochemical analysis is/are carried out. The physicochemical parameters influence the quality, storage, aroma, granulation, flavor and texture of the honey. Many authors have reported the physical and chemical properties of different types of honey. Properties and composition of honey depend on its geographical and/or flora origin, the nectar that the bee utilized, harvesting season, environmental factors, handling of honey during extraction and duration of storage<sup>3,4</sup>.

The melissopalynological analysis provides detailed information on the production process, ecological and geographical origin and quality of honey samples<sup>5</sup> since it is widely used in different industries most especially the health sector. The floral origin and organoleptic properties of honey are determinant factors for its commercial value and quality<sup>6</sup>. In recent times, there has been an increased interest in beekeeping and demand for honey which has led to a proliferation of beekeeping ventures in Ekiti State. Stakeholders in agriculture have actively encouraged farmers in bee farming. Instead of this increase in demand and production of honey, there is a need to allay the fear of consumers about honey adulteration and incorrect labeling. The geographical and prevailing climatic conditions in Ekiti State, Nigeria provide a suitable environment for beekeeping and honey production. Despite these favorable conditions and the increase in honey production in the state, the majority of the honey from this region is not standardized. To achieve this, there need to assess the honey quality through melissopalynological and physico-chemical analyses. Keeping in view the medicinal and nutritional importance of honey, the present study was therefore carried out to evaluate the pollen content and physicochemical properties of honey samples in Ekiti State, Nigeria.

## MATERIALS AND METHODS

**Study area:** Honey bee combs were harvested from four different beehives in Igede (I and II), Igedora and Ado Ekiti localities of Ekiti State, Southwestern Nigeria. The honey contents of the bee combs were carefully extracted using a white clean handkerchief and the honey samples (IG1, ADE, IGE and IG2 representing Igede I, Ado Ekiti, Igedora and Igede II) were kept in a clean plastic bottle in a dark place at room temperature until laboratory analysis.

**Pollen analysis:** Palynological analysis of the honey samples was carried out at the Palynology Laboratory of the Department of Archaeology and Anthropology, University of Ibadan, Ibadan, Oyo-State, Nigeria. Ten grams of the honey sample each was weighed into a glass centrifuge tube and dissolved in 20 mL of distilled water (20-25 °C), the solution was centrifuged for 10 min at 1,500 rpm and the supernatant was decanted. The procedure followed a slightly modified method of Louveaux *et al.*<sup>7</sup>. Pollen grains were observed using the Olympus CH30 light microscope (Olympus Life Science (Evident), Shinjuku-Ku, Tokyo, Japan) with DE 1.3M digital camera attachment.

**Pollen grain frequency:** The pollen grain numbers were quantified following the method of Moar<sup>8</sup>. The frequency classes and frequencies of the pollen types of each sample were taken and recorded<sup>7</sup>. As far as possible the pollen types were identified to genus and species levels while in some cases only family identification was possible. The pollen types were identified with the help of standard slides prepared from Nigerian flora in the Palynology Laboratory of the Department of Archeology, University of Ibadan, Oyo State, Nigeria<sup>9</sup>.

**Physicochemical analysis:** Physicochemical parameters such as pH, specific gravity, free acidity, HMF (5-hydroxymethylfurfural), refractive index, electrical conductivity, total solid, antioxidant activity and total sugar were determined according to harmonized methods<sup>10,11</sup>. The physicochemical analyses were carried out at the Laboratory of the Department of Chemistry, University of Lagos, Akoka, Lagos, Nigeria.

**pH:** The Elico LI 120 pH meter (Elico Limited, Telangana, India) was used to measure the pH of the honey samples by diluting 10 g in 75 mL of distilled water using a pH 4 and 9 as standard buffers.

**Acidity:** One gram of each honey sample was dissolved in 75 mL of distilled water and subsequently titrated against 0.05 moles of NaOH using 2 drops of phenolphthalein indicator. The process was continued until a pink colour appeared and persisted for 10 sec. The following calculation was then applied to obtain the percentage acidity:

$$\text{Acidity (\%)} = \frac{0.23 \times \text{NAOH}}{\text{Mass of the honey sample (1g)}}$$

**Electrical conductivity:** Five grams of honey was dissolved in 37.5 mL of distilled water and then the electrical conductivity was determined using the Elico CM 180 (Elico Limited, Telangana, India).

**HMF:** In accordance with Gidamis *et al.*<sup>12</sup>, each honey sample, weighing 5 g, was dissolved in 25 mL of distilled water before being transferred to a 50 mL volumetric flask. After thoroughly mixing the sample with 0.5 mL of Carrez solution I, another 0.5 mL of Carrez solution II was added. Whatman filter paper No. 42 was used to filter the solution. The initial 10 mL of the filtrate was discarded and the remaining 5 mL was divided between two test tubes. One of the test tubes received 5 mL of distilled water, which was added and thoroughly mixed (the sample solution). The reference solution, five milliliters of 0.2% sodium bisulphate, was added to the second test tube and thoroughly mixed. In 10 mm quartz cells during the course of an hour, the absorbency of the sample solution was assessed in comparison to the reference solution. Three duplicates of each determination were made.

The HMF values were calculated using the formula shown below.

$$\text{HMF (mg kg}^{-1}\text{)} = (A_{284} - A_{336}) \times 149.7 \times 5 \times D/W$$

Where:

A<sub>284</sub> = Absorbance at 284 nm

A<sub>336</sub> = Absorbance at 336 nm

149.7 = Constant

D = Dilution factor in case dilution was necessary

W = Weight in g of the honey samples

The results were expressed in mg kg<sup>-1</sup> to the nearest 1 decimal place.

**Refractive index:** Each of the samples was measured with the hand-held Atago 2354 Master-50H (Cole-Parmer Co., Jean-Perrin Quebec, Canada) refractometer at 25°C.

**Specific gravity:** The dry empty pycnometer was weighed at 20°C and distilled water that was kept inside the water bath at 20°C was poured into the ASTM-D8854-00 (ASTM International, West Conshohocken, PA, USA) pycnometer and weighed. The sample kept at the same temperature was used to rinse the pycnometer properly and also weighed at the same temperature:

$$\text{Specific gravity} = \frac{\text{Weight of pycnometer + sample} - \text{Weight of empty pycnometer}}{\text{Weight of pycnometer + water} - \text{Weight of empty pycnometer}}$$

**Total solid:** The empty moisture can be first weighed and about 5 g of the sample was added into the can and re-weighed. This was later transferred into the oven and dried at 105°C until there was no more change in weight change after cooling the can in a desiccator and weighing. As 10 g of sample was weighed into a 250 mL conical flask and then 40 mL of the inversion acid solution was added. This was brought to boiling on a hot plate for exactly 30 sec. Then it was cooled rapidly in cold water and neutralized by adding 1M sodium hydroxide. This was made up to 100 mL in a standard flask. Ten mL of this solution was further diluted to 100 mL before use. Five mL of Fehling solutions A and B were pipette into 250 mL conical flask and 15 mL of distilled water was added. As 25 mL of final sugar solution was run into the conical flask from a burette and boiling chips were added and placed on a hot plate. This was boiled for 2 min and the solution changed to red. Then 3 drops of methylene blue were added which turn the solution back to blue. The remaining sugar solution was quickly added into the boiling solution until there is the final disappearance of the blue colour leaving the red colouration due to cuprous oxide.

$$\text{Total sugar (\%)} = \frac{\text{g sugar}}{\text{Weight of sugar}} \times 100$$

**Statistical analysis:** Two forms of statistical analyses were conducted, inferential and descriptive. Simple percentages and frequency count were used for pollen quantification while the Duncan's Multiple Range test and ANOVA were used for mean separation and to judge the similarity between the physicochemical parameters in the samples at a 0.05 level of significance.

## RESULTS

**Pollen analysis:** The total pollen count ranged from 57 to 1453. A total of 22 palynomorph types (21 pollen and 1 spore type) were recovered from the four honey samples. Most frequency pollen types in 10 µL were *Elaeis guineensis* (n = 1146), Solanaceae (n = 794), *Talinum triangulare* (n = 211) and *Blighia sapida* (n = 44) as shown in Table 1.

Table 1: Frequency count of pollen and spores in honey samples (in 10 µL)

Pollen types/Apiaries	IG1	Percentage	ADE	Percentage	IGE	Percentage	IG2	Percentage	Total
<i>Aspilia africana</i>	0	0.00	11	0.76	0	0.00	0	0.00	11
<i>Blighia sapida</i>	0	0.00	31	2.13	13	22.81	0	0.00	44
Bombacaceae	6	0.91	0	0.00	0	0.00	0	0.00	6
<i>Cardiospermum halicacabum</i>	27	4.11	0	0.00	0	0.00	0	0.00	27
Curcubitaceae	4	0.61	0	0.00	0	0.00	0	0.00	4
<i>Elaeis guineensis</i>	380	57.84	684	47.08	22	38.60	60	26.67	1146
<i>Jatropha</i> sp.	4	0.61	0	0.00	3	5.26	12	5.33	19
<i>Manihot esculenta</i>	8	1.22	0	0.00	0	0.00	0	0.00	8
Monolete spore	0	0.00	0	0.00	0	0.00	2	0.89	2
<i>Oldenlandia corymbosa</i>	0	0.00	15	1.03	4	7.02	0	0.00	19
<i>Parinari</i> sp.	4	0.61	0	0.00	0	0.00	0	0.00	4
<i>Peltophorum pterocarpum</i>	1	0.15	0	0.00	0	0.00	0	0.00	1
<i>Phyllanthus</i> sp.	0	0.00	0	0.00	0	0.00	72	32.00	72
Poaceae	9	1.37	0	0.00	0	0.00	0	0.00	9
Rutaceae	1	0.15	0	0.00	0	0.00	0	0.00	1
<i>Sida acuta</i>	1	0.15	0	0.00	0	0.00	0	0.00	1
Solanaceae	0	0.00	703	48.38	13	22.81	78	34.67	794
<i>Spondias mombin</i>	0	0.00	3	0.21	0	0.00	0	0.00	3
<i>Talinum triangulare</i>	211	32.12	0	0.00	0	0.00	0	0.00	211
<i>Tridax procumbens</i>	0	0.00	6	0.41	0	0.00	0	0.00	6
<i>Triumfetta</i> sp.	1	0.15	0	0.00	0	0.00	0	0.00	1
<i>Zea mays</i>	0	0.00	0	0.00	2	3.51	1	0.44	3
Total	657	100	1453	100	57	100	225	100	2392
Simpson diversity (D)	0.56		0.54		0.75		0.71		



Plate 1: Photomicrographs of pollen recovered from honey samples

1-2: *Elaeis guineensis*, 3-4: *Jatropha* sp., 5: *Parinari* sp., 6: *Phyllanthus* 7-8: *Blighia sapida* sp., 9-11: *Aspilia africana*  
12: *Cardiospermum halicacabum*, 13: *Zea mays* and 14-15: *Talinum triangulare*

Plate 1 and 2 shows some of the pollen types recovered from the honey samples.

A total of 15 plant families and one spore were recovered from the honey samples. Figure 1 shows plant families represented in the four samples with a high presence of *Arecaceae* (47%) and *Solanaceae* (33%), the moderate occurrence of *Portulacaceae* (8.8%) and *Euphorbiaceae* (4%) and seemingly insignificant presence of *Poaceae* (0.5%), *Asteraceae* and *Rubiaceae* (0.7-0.8%) and very scarce, almost unnoticed presence of other families like *Malvaceae*, *Bombaceae*, *Fabaceae*, *Verbanaceae*, fern spores, *Chrysoblanaceae*, *Curcubitaceae* and *Rubiaceae* (0.04-0.79%).

**Nature of the foraged site:** Figure 2 revealed three clusters, the top, middle and last (single grain-*Zea mays*) clusters. It indicates the distribution of grains in their habit of occurrence. For instance, *Elaeis guineensis*, *Aspilia*, *Tridax* and *Solanaceae* were all in one cluster indicating the close relationship in distribution which suggests an openness of the vegetation and human interaction where the bees foraged.

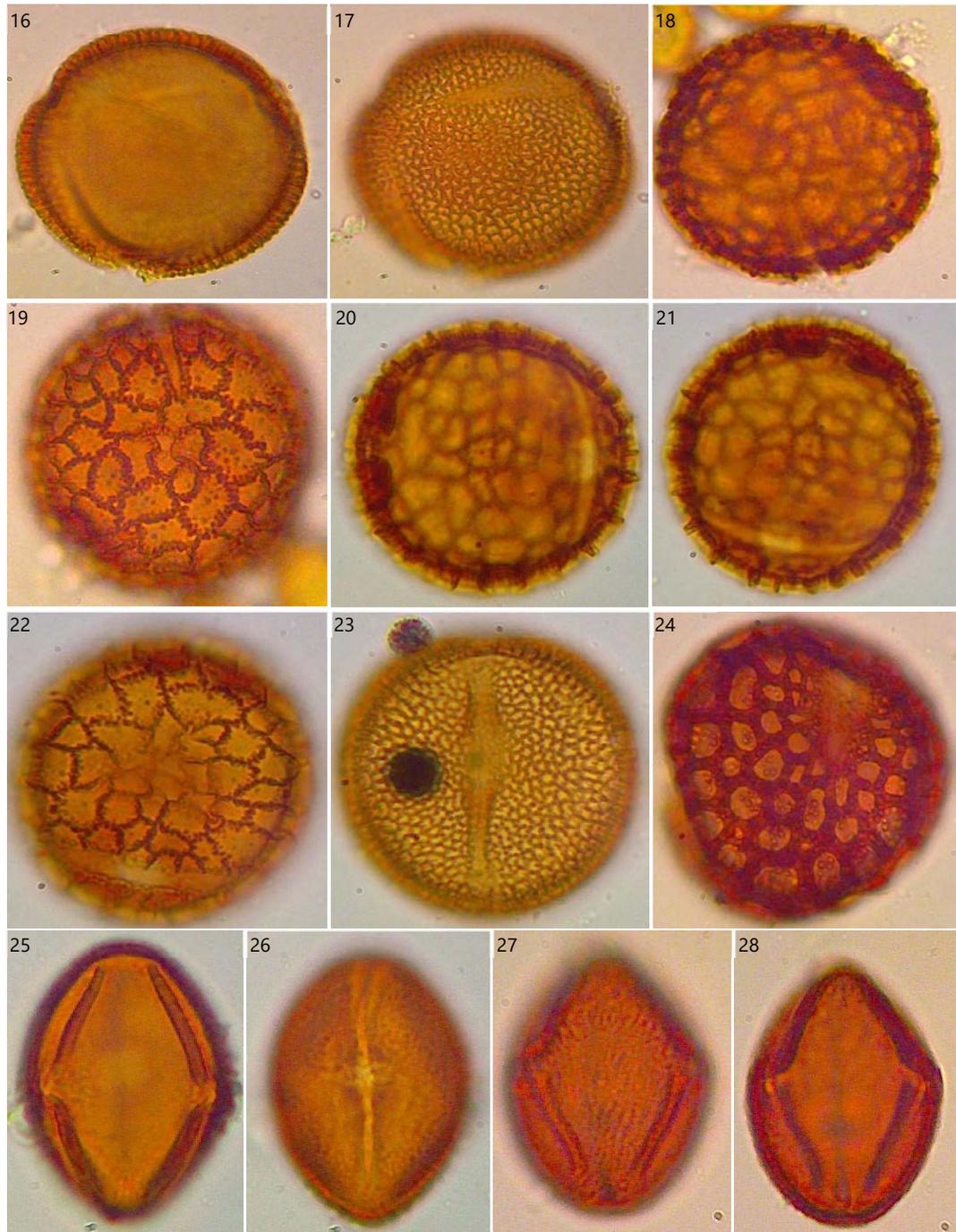


Plate 2: Photomicrograph of pollen recovered from honey samples

16-17: Curcubitaceae, 18-21: Bombacaceae, 23: Curcubitaceae, 24: *Peltophorum pterocarpum* and 25-28: *Spondias mombin*

The honey samples are from bifloral (IG1, IG2 and IGE) and unifloral (ADE) sources. The latter is the capital of the sampled state with high degradation of the vegetation, while others are far less impacted by humans hence, honey bees may have foraged from the available flora in the urbanised regions which are usually plant species that are indicative of human impact as shown in Table 2.

**Physico-chemical parameters of the honey:** The range for physicochemical parameters was pH, 2.78-3.22, specific gravity, 1.39-1.41, free acidity ( $\text{mEq kg}^{-1}$ ), 18.10-37.95, HMF ( $\text{mg kg}^{-1}$ ), 12.44-32.61, refractive index, 1.42-1.45, electrical conductivity ( $\text{mS cm}^{-1}$ ), 0.49-1.08, total solid (%), 81.44-89.39, total sugar (%), 76.10-82.29 and antioxidant activity ( $\mu\text{g mL}^{-1}$ ), 307.09-974.28 as presented in Table 3. Each parameter showed a significant difference in their values from the different apiaries.

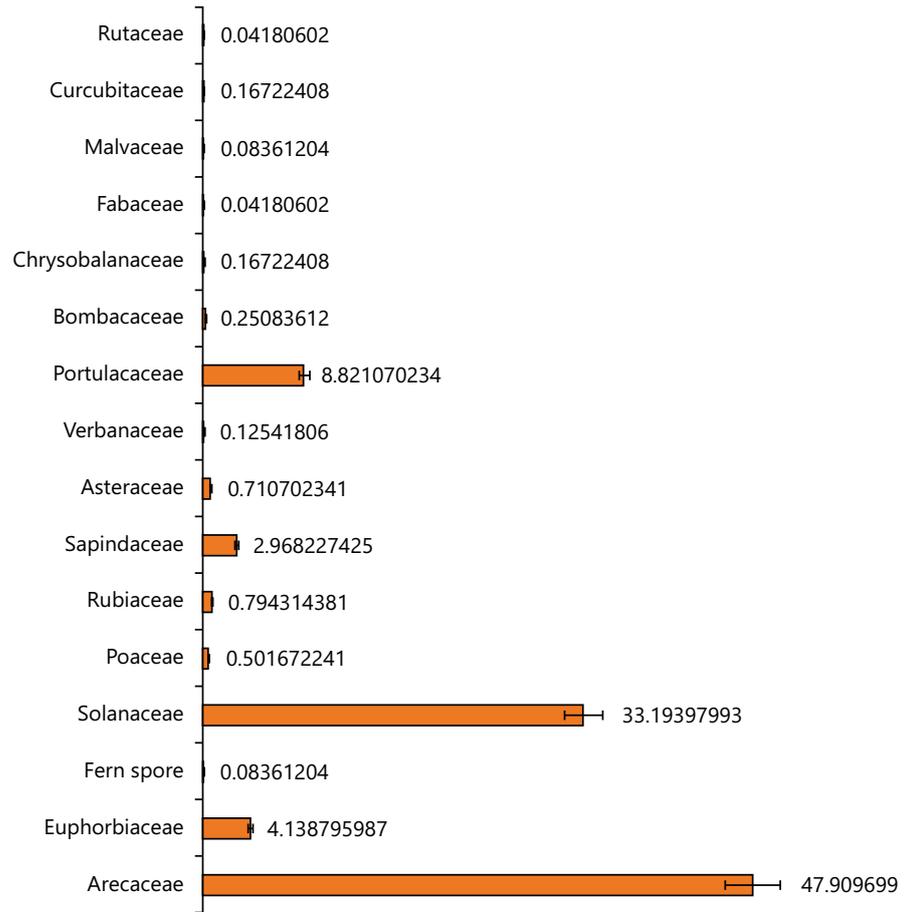


Fig. 1: Chart showing the percentage representation of plant families in the four honey samples

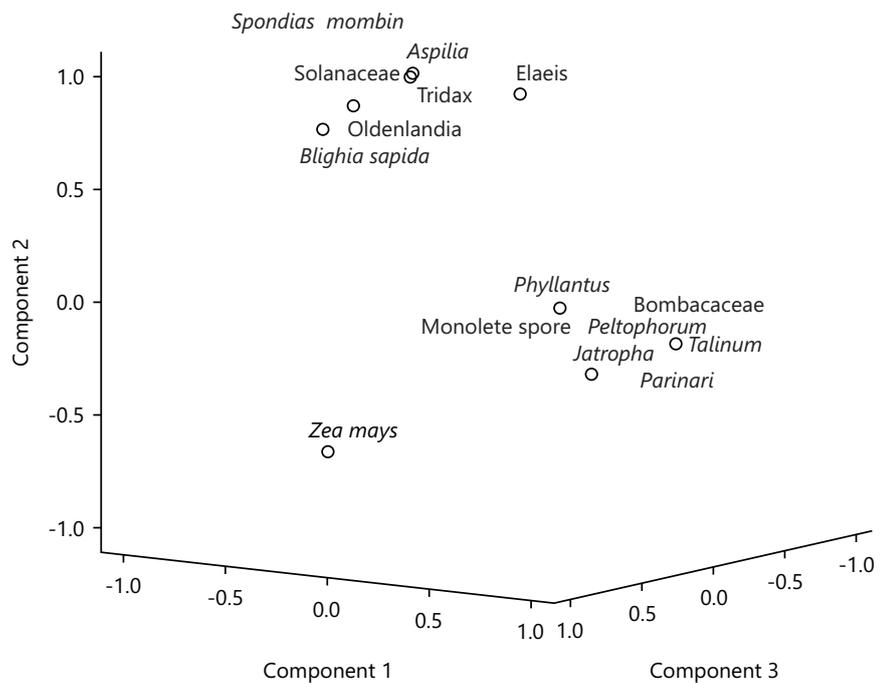


Fig. 2: Principal component analysis for the distribution of bee-foraged plants in the four sample locations

Table 2: Honey classification based on floral sources and category

Samples	Pollen type (%)					Remark on the floral origin	Total pollen count/ category
	Predominant	Secondary	Important minor	Minor	Present		
IG1	<i>E. guineensis</i> (57.84)	<i>Talinum triangulare</i> (32.12)	<i>C. halicacabum</i> (4.11)	<i>Manihot esculenta</i> (1.22) Poaceae (1.37)	<i>Jatropha</i> sp. (0.61) Bombacaceae (0.91) <i>Parinari</i> sp. (0.61) <i>Triumfetta</i> sp. (0.15) Curcubitaceae (0.61) <i>P. pterocarpum</i> (0.15)	Bifloral	657/I
ADE	<i>E. guineensis</i> (47.08) Solanaceae (48.35)	-	-	<i>O. corymbosa</i> (1.05) <i>Blighia sapida</i> (2.13)	<i>Tridax procumbens</i> (0.41) <i>Aspilia africana</i> (0.76)	Unifloral	1453/I
IGE	-	<i>E. guineensis</i> (38.6) Solanaceae (22.8) <i>Blighia sapida</i> (22.8)	<i>Jatropha</i> sp. (5.26) <i>Zea mays</i> (3.51) <i>O. corymbosa</i> (7.02)	-	-	Bifloral	57/I
IG2	-	<i>E. guineensis</i> (26.7) <i>Phyllanthus</i> sp. (32) Solanaceae (34)	<i>Jatropha</i> sp. (5.3)	-	<i>Zea mays</i> (0.44) Monolet spore (0.89)	Bifloral	225/I

Criterion: Predominant (>45%), secondary (16-45%), important minor (3-15.9%), minor (1-2.9%), present (<1%) according to Louveaux *et al.*<sup>7</sup>. Floral origin selected based on most represented (predominant and secondary) plant species and not Family as in the case of Solanaceae. Categories: I (<20,000), II (20,000-100,000), III (100,000-500,000), IV (500,000-1,000,000) and V (> 1,000,000). Sampling locations: IG1: Igede I, ADE: Ado Ekiti, IGE: Igedora and IG2: Igede II

Table 3: Physicochemical parameters of honey samples from Ekiti State, Nigeria

Parameter	Honey sample				ANOVA F-value, significance
	IG1	ADE	IG2	IG2	
pH	3.22±0.03 <sup>b</sup>	2.92±0.01 <sup>c</sup>	2.78±0.02 <sup>a</sup>	2.83±0.01 <sup>a</sup>	155.30, 0.000
Specific gravity	1.41±0.02 <sup>c</sup>	1.40±0.01 <sup>a</sup>	1.40±0.05 <sup>bc</sup>	1.39±0.01 <sup>ab</sup>	12.53, 0.017
Acidity (mEq kg <sup>-1</sup> )	18.10±0.01 <sup>a</sup>	22.05±0.05 <sup>b</sup>	26.00±0.02 <sup>c</sup>	37.95±0.50 <sup>d</sup>	5353.03, 0.00
HMF (mg kg <sup>-1</sup> )	12.44±0.02 <sup>a</sup>	26.61±0.19 <sup>b</sup>	24.05±0.10 <sup>c</sup>	32.61±0.01 <sup>d</sup>	7098.74, 0.00
Refractive index	1.45±0.01 <sup>a</sup>	1.42±0.03 <sup>a</sup>	1.43±0.06 <sup>a</sup>	1.42±0.01 <sup>b</sup>	32.08, 0.003
Conductivity (10%) (mS cm <sup>-1</sup> )	1.08±0.01 <sup>c</sup>	0.53±0.01 <sup>b</sup>	0.51±0.10 <sup>ab</sup>	0.49±0.01 <sup>a</sup>	1423.52, 0.00
Total solid (%)	89.39±0.06 <sup>a</sup>	84.29±0.02 <sup>b</sup>	86.33±0.35 <sup>c</sup>	81.44±0.16 <sup>d</sup>	899.05, 0.00
Total sugar (%)	82.29±0.16 <sup>a</sup>	80.18±0.03 <sup>b</sup>	78.25±0.35 <sup>c</sup>	76.10±0.01 <sup>d</sup>	328.72, 0.00
Antioxidant IC50 (µg mL <sup>-1</sup> )	974.28±0.74 <sup>a</sup>	772.25±2.62 <sup>b</sup>	415.77±1.46 <sup>c</sup>	307.09±2.14 <sup>d</sup>	54567.79, 0.00

<sup>a-d</sup>Values in the same row with different superscript letters are significantly different (p<0.05) using Duncan's Multiple Range Test (2 replications were used for each location). \*Sampling locations: IG1: Igede I, ADE: Ado Ekiti, IGE: Igedora and IG2: Igede II

## DISCUSSION

The pH values obtained in this study showed that all the honey samples analyzed are acidic. The pH values of this present study are lower compared to Morocco honeys<sup>13</sup>. The acid nature of honey quality is very important during harvest and storage. Low pH inhibits the growth of microbial organisms<sup>14</sup> thereby improving the honey's stability and shelf life. The specific gravity was higher than other Nigerian honey earlier reported<sup>15</sup>. However, the values fall within the range of established codex standards. Acidity is influenced by the chemical properties of the organic acids and also by the amino acid contents which are influenced by the nectar and salivary enzymes of honey bee<sup>16</sup>. Free acidity determines the freshness or state of deterioration of honey. The free acidity values obtained for the honey samples were similar to previous reports<sup>17,18</sup>. The maximum value of 50 mEq kg<sup>-1</sup> is proposed by the National regulation for free acidity of honey sample<sup>12</sup>. Values higher than the maximum value (50 mEq kg<sup>-1</sup>) may be indicative of fermentation of sugar exceeding the maximum standard value.

The HMF is an indication of honey freshness. It is also considered an indicator of heat processing and or long storage time<sup>19</sup>. The Codex Standard requires that the HMF content of honey after it has undergone processing and or blending shall not be more than 40 mg kg<sup>-1</sup>. The result of HMF in this study agreed with the previous works<sup>20,21</sup>. The low HMF of the honey samples indicates that the honey was kept in good storage condition and had good harvesting practices by beekeepers. Electrical conductivity less than

0.8 mS cm<sup>-1</sup> indicated blossom and more than 0.8 mS cm<sup>-1</sup> indicates honeydew honey. The EC is influenced by the source of honey, acidity, moisture, viscosity as well as ash content. Honey from honeydew has a high conductivity than floral honey. Honey sample A has EC above the prescribed value of <0.80 mS cm<sup>-1</sup> while others are below the recommended value. The results of this present study showed that the refractive index of the honey samples was lower compared with the values reported for other honey samples from southwestern states<sup>22,23</sup>. Refractive index can be used to determine the level of adulteration<sup>24</sup> and the sampled honey did not show any form of adulteration.

The total soluble solids values in this study were high and compared favorably with the report of Nyau *et al.*<sup>25</sup>. Total solid is a parameter used to measure dissolved solids in honey samples. Honey is considered high-grade and highly stable upon storage if its total soluble solids are more than 80% and liable to fermentation if its soluble solids are less than 80%. The results of this finding established that all the honey samples were more than 80% an indication that they are of good grades and will not ferment during storage. The total sugar in the investigated honey samples was high and was similar to the range of 77.6-83.8% for multi-floral honey from Italy<sup>26</sup>. The major composition of honey is sugar. Glucose and fructose are the major contributors to honey composition<sup>19</sup>. Sugar governs honey properties and its content is related to the degree of honey maturity and floral source. It is convenient because grounds that the pollen content does not have any special influence on the physicochemical quality of the honey samples, it is probable that this factor is much determined by the nectar composition.

The geographical locations were marked by the abundance of plant species indicative of human presence. The high occurrence of Arecaceae (*Elaeis guineensis*) showed that the honey samples are not from dense forest regions but from open or secondary forests, Solanaceae predicts some form of cultivation of cash crops and that of Portulacaceae (*Talinum triangulare*) reveals that the areas are inhabited by humans, cultivated and it is likely of a lowland rainforest ecozone (Fig. 1). This could be inferred by the occurrence of pollen of cultivated plants (Solanaceae), highly domesticated ethnobotanicals (e.g., *Elaeis guineensis*) and human-degraded wetter-type, open lowland rainforest species (*Talinum triangulare*). The high representation of pollen of water leaf (*Talinum triangulare*) indicated the openness of the vegetation and the wet climatic conditions, as this plant thrives in such conditions sporadically growing. The openness of the vegetation where the honey bees foraged can also be insinuated from the occurrence of members of the Asteraceae family (*Tridax procumbens* and *Aspilia africana*) and as also expressed in the PCA plot (Fig. 2).

The presence of *Blighia sapida*, a common forest tree species with so many ethnographic uses<sup>27</sup> could have also indicated humans interfered with rainforest. It may have been used as an ornamental plant or for the harvest of fruits. Its pollen was found in ADE and IG2 alongside Solanaceae, *Elaeis guineensis* and *Oldenlandia corymbosa* for which the latter occurs in cultivated lands and the former in open canopy forests, all re-iterating the nature of the geographical origin of the honey samples. The presence of pollen of *Phyllanthus* sp. and of weeds, *Cardiospermum halicacabum* and *Triumfetta* sp. Is known to grow in human-disturbed regions like roadsides and fields and is used for the treatment of illness<sup>28</sup>. The presence of pollen of Curcubitaceae reflects human cultivation of an area further describes the open forest vegetation nature of the region and the presence of human elements interacting with the vegetation either for food (e.g., *Manihot esculenta*) or health. The presence of *Phyllanthus* sp. and *Zea mays* also reflect the openness of the vegetation<sup>29</sup>.

Apart from ADE which was unifloral, all others were bifloral. This clearly showed that the bees were left to forage in a single region and most likely from one of two major plants and pollen or nectar source. The low occurrence of *Zea mays* (0.44-3.51%) in all samples could not have been unconnected with the findings of Crane and Walker<sup>30</sup> that most members of Poaceae are prolific pollen producers who rarely

produce nectar with morphologically unattractive small flowers profusely covered with bracts pollinated by wind and not insects. Honeybees may have foraged on grasses due to the aforementioned reason. It is also worth noting that honeybees periodically feed on grasses or their products when preferred species could not be found<sup>31</sup>.

Despite the high quality of the honey samples rendering them exportable, pollen content was low. This phenomenon reveals the impact of human activities on the foraging patterns of honeybees. It is clear that deforestation activities in the southwest are having an impact on the number of preferred bee plants. More nectar is presumably collected for honey production by bees in this case study. It is arguable that other factors like the honey extraction process, pollen productivity and seasonality may have influenced the quantity of pollen found in the honey, it is important to note that the pollen grains recovered represent some of the now preferred bee plants. The photomicrographs could also be used by other researchers for the identification of pollen from honey samples. This study sampled only four locations and honeys and a single Nigerian State, it is, therefore, important to increase this number of samples and states for better generalization of the findings of this study.

## **CONCLUSION**

The results of the physicochemical parameters and pollen content revealed that the honey samples were produced from nectars or pollen of blossoms of flowering plants. Solanaceae and Arecaceae pollen types were the most abundant. Nectar seems to determine the nutritional quality and anti-oxidative potentials of the honey samples than pollen. The results also provide important information on the geographical source of the honey. The honey is also of good quality and conforms to international regulations hence could be recommended for commercial purposes.

## **SIGNIFICANCE STATEMENT**

Only a few indigenous honeys sold in open markets in Nigeria are branded and well packaged, most times with no specifications about their physical and/or chemical composition. If quality control of Nigerian honey must be achieved, characterization is paramount. This study investigated honey from Ekiti State in Southwestern Nigeria to ascertain its quality (pollen content, physical and chemical attributes) and suitability for public consumption. Results revealed high quality following comparison of physicochemical parameters with set standards by IHC and Codex Alimentarium Commission, although pollen content was grossly low, however, important ecological biomarkers were represented and their photomicrographs along with their distinct morphology can be used for identification and as part of the pollen database of Nigeria. The recovered pollen grains represent important plants for beekeeping in tropical environments and the need for possible conservation is therefore advised.

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