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# Pollen and Physicochemical Characterization of Honey Samples from Ankpa Local Government Area of Kogi State, Nigeria.

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**Abstract**

Five different honey samples were collected from hives in five locations (Labelled AN 1 - 5) in Ankpa Local Government Area (LGA) of Kogi State, Nigeria, in May, 2012. They were processed by pressing and analyzed for their pollen and physicochemical properties using Standard Methods. For pollen analysis, a camera fitted Carl Zeiss microscope was used. The physicochemical properties screened were the pH, Sucrose content, Protein content, Specific gravity and Moisture content. A total of thirty eight pollen types were recovered from the five honey samples. From AN1, 17, from AN2, 17, from AN3, 10, from AN4 and 25 from AN5 respectively. All the honey samples were heterofloral types. Seventeen pollen types were identified to the specific taxon level, ten to the generic and ten to the family taxon level. A 4-colporate grain remains unidentified. The pollen spectrum revealed species that are typical of Guinea Savanna. Elaeis guineensis was overrepresented in AN1. The physicochemical parameters were subjected to a one way ANOVA. There were significant differences in the pH, conductivity, moisture and sucrose contents of the honey samples while specific gravity and protein contents showed no significant differences. All the physicochemical parameters measured were complied with the CODEX International Standards for honey. It was indicated that honey from the area will be suitable for exportation when processed appropriately.

**Keywords:** Honey samples, pollen analysis, physicochemical properties, beekeeping, heterofloral.

## 1.0 Introduction

The classical approach to verify the botanical origin of honey is to use several complementary analytical methods. Traditionally, the botanical origin of honey is determined by experts evaluating several physical, chemical, pollen analytical as well as sensory characteristics (Bogdanov, Ruoff & Persano Oddo,2004; Persano Oddo & Bogdanov 1995,Persano Oddo, & Bogdanov, 2004).

Presently, Nigerian honey is not sold under any standard control or characterization that is significantly different from the claims of the beekeepers. According to Song & yang (2012), even where the use of a designation of the botanical origin is undertaken, a rate as high as 60 % of incorrect indications of the botanical origin may be made by the beekeepers, showing that conclusions drawn from field observations of foraging bees may not be reliable. Authentication by other analytical methods is therefore absolutely necessary. Honey produced in Nigeria needs to be subjected to these empirical analyses which will standardize the Nigerian honey to enhance its entrance into international market. Nigerian honey cannot enter into international market until the necessary criteria are met.

Pollen analysis has been the major method by which experts evaluate and determine the botanical and geographical origin of honey in Nigeria (Sowunmi, 1976, 2001; Agwu, Obueke & Iwu,1989; Agwu & Abaeze, 1991; Agwu & Akanbi, 1985; Ayodele, Folarin & Oluwalana, (2006); Njokuocha & Ekweozor, 2007; Adekanmbi & Ogundipe, 2009; Adeonipekun, 2010; Ige & Modupe, 2010; Aina & Owonibi, 2011). Very lately however, the awareness to evaluate honey using other analytical methods become a prominent issue, in particular electrical conductivity, protein content, mineral and sugar composition, volatile compounds, etc. Apart from pollen analysis, Agwu and Okeke (1997) carried out chromatographic analysis of honey samples within Nigeria to determine the chemical composition. Some documented studies on the physicochemical properties of honey in Nigeria also include Adenekan *et al*. (2010) and Agbagwa, Otokunefor & Frank-Peterside (2011), among others. The aim of the study was to analyse the pollen and physicochemical properties of honeys from Ankpa Local Government Area of Kogi State, Nigeria. Most of these data are presented for the first time while obtaining the samples from definite locations that enhance the reliability of the results as against samples obtained from retailers or the open market.

## 2.0 Materials and Methods

### 2.1 Study Area

Ankpa is one of the Local Government Areas of Kogi State in Northeast, Nigeria with an area of 1,200 km². The north easterly line of equal latitude and longitude passes through the Local Government Area as shown in Fig. 1-3 below:.

**Fig. 1: Map of Nigeria, showing Kogi State**

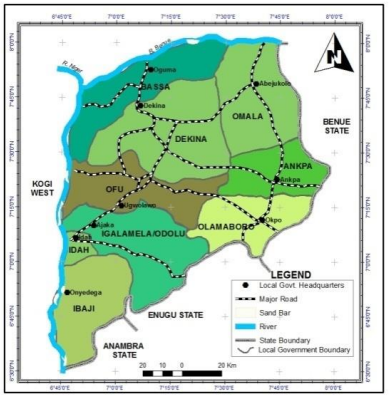


**Fig. 2: Map of Kogi East**

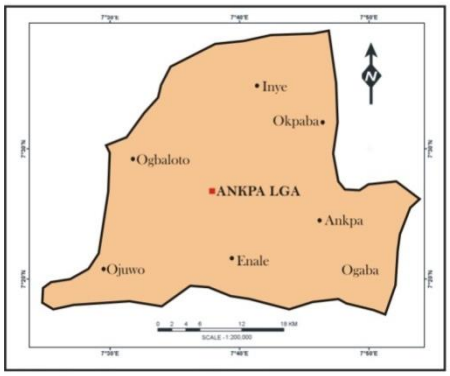


**Fig..3: Map of Kogi East, showing the sites of sample collect**

### 2.2 Sample collection

Five distinct locations were marked out as experimental and sampling sites (coded AN1, AN2, AN3, AN4 and AN5, from Ogbaloto, Enale, Upaba, Ojuwo and Ankpa-Ogoma respectively). Between June and September 2009, a Kenyan top bar beehive box was placed at each of the five locations within the Local Government Area. They were fortnightly baited using honey. The honey was smeared on the slanting sides of the box and few droplets were placed at the corners of the boxes and at the flight entrances. The process was repeated until they were colonized by honey bees (*Apis mellifera* var *adansonii*). Honey samples used for this experiment were harvested in June, 2012.10g of each sample was weighed, diluted with 20ml of

warm ($40^0$ C) dil. $H_2SO_4$ (3ml in 997ml of distilled water), centrifuged at 2500 r.p.m for 10minutes (Louveaux, 1978). The sediment was treated with glacial acetic acid before acetolysis.

### 2.3 Acetolysis

Acetolysis followed the method of Erdtman (1969). Acetolysis mixture was prepared freshand added to samples in centrifuge tubes (Acetolysis mixture consisted of 9 parts acetic anhydride to 1 part conc. Sulphuric acid). They were geated in water bath as from $70^0$C to boiling point for about 10 minutes, centrifuged after cooling and the supernatants poured into the "Acetolysis mixture" bottle. Distilled water was used to wash off the effect of the the acetolysis mixture by adding water to the tubes, mixing , centrifuging and decanting. This was done four times on each sample. After the final washing, the samples were treated with 50% glycerol by adding 50% glycerol , mixing , centrifuging and decanting. The residues in the tubes were then stirred and made up to 1ml with 100% glycerol and transferred to labelled vials before mounting on cleaned and labelled slides.

#### 2.3.1 Mounting and Microscopic Examination

10µl of the acetolysed samples were transferred onto slides and covered with 22 x 22mm cover slips. After three minutes, the slides were inverted for two hours before being sealed with nail vanish. Specimens were studied and photographed at either 1000 X or 400 X (for larger palynomorphs) using Leica DM2500 light microscope. Pollen types were identified by comparison with reference pollen micrographs as documented by Sowunmi, (1973; 1995), Agwu and Akanbi (1985), Ybert, J. P. (1979). The reference collections, journals and prepared slides of pollen samples were done at the Department of Archaeology, University of Ibadan, Nigeria and supported by Wang, (2003). However, the terminologies used were in accordance with Erdtman (1960), Faegeri and Iversen (1989).

#### 2.3.2 Pollen Analysis

Quantitative and qualitative analysis of the pollen contents of the samples were carried out. Quantitative analysis followed was in line with Terrab *et al*., (2001) but modified in that the samples were acetolysed and covered with a 22 x 22mm cover slip. The results of the qualitative analyses are shown in Table 1. Qualitative analysis

was conducted according to Maurizio (1975). as in group I (< 20,000 grains), II (20,000-100,000), III (100,000-500,000), IV (500,000- 1,000,000) and V (> 1,000,000)respectively and shown in Table 2.

### 2.3.3 Physicochemical Properties

Protein analysis was undertaken using Kjeltec 2300; determination of Sucrose was evaluated using Anthrone Method; the moisture contents of the honey samples were determined using the oven dry method and pH was measured using the pH meter. The statistical means of ten replicates of the pH, conductivity, specific gravity, protein, sucrose and moisture contents were subjected to a one- way ANOVA to determine the levels of affinities of these parameters among the samples.

## 3.0 Results

### 3.1 Pollen Analysis

A total of thirty eight pollen types were recovered from the honey samples in all, 5 from AN1, 17 from AN2, 17 from AN3, 10 from AN4 and 25 from AN5 respectively. All the honey samples were heterofloral types. Seventeen pollen types were identified to the specific taxon level, ten to the generic and ten to the family taxon level. A 4-colporate grain remains unidentified as shown in Table 1. Twelve species occurred in more than 50% of the samples (Fig 4).

**Table 1: Qualitative analysis of pollen types in honey represented in percentages (%)**

| Samples | Predominant pollen (>45%) | Secondary pollen (16-45%) | Important minor pollen (3-15%) | Trace pollen (1-3%) | Sporadic pollen (<1%) |
|---|---|---|---|---|---|
| AN1 Ogbaloto | *Elaeis guineensis* 73.8 | | *Sarcocephalus nodiflora*14.2 | *Hymenocardia acida* 2.3, *Lannea* sp. 1.8 Myrtaceae 2.9 | |
| AN2Enale | | *Lannea* sp. 24.0 Moraceae 21.3 | Combretaceae/Melast.3.7, *Elaeis guineensis* 8.0, *Entada* sp. 3.9, *Ixora/Pavetta* sp. 10.2, Myrtaceae 5.8 *Sarcocephalus nodiflora*5.2, *Spondias mombin* 4.7 | *Daniellia oliveri* 1.3, *Lophira* sp.1.6 *Mangifera indica* 2.2, Meliaceae 2.2, *Parinari* sp.2.1 *Parkia biglobosa* 1.4, Solanaceae 1.8, 4-colporate1.3 | *Hymenocardia acida* 0.6 |
| AN3 Upaba | | *Elaeis guineensis* 22.565Rubia | *Entada* sp. 6.984 *Pavetta/Ixor* | 4-colporate 1.00 | *Alchornea* sp. 0.053, *Blighia sapida* 0.055 |

| Samples | Predominant pollen (>45%) | Secondary pollen (16-45%) | Important minor pollen (3-15%) | Trace pollen (1-3%) | Sporadic pollen (<1%) |
|---|---|---|---|---|---|
| | | ceae32.274 | *a* sp. 22.526 *Phyllanthus pentandrus* 4.030 | | *Daniellia oliveri* 0.020, *Lannea* sp0.011 *Mangifera indica* 8.995 Meliaceae 0.005 Myrtaceae 0.137 *Ormocarpum* type 0.842 *Parinari* sp. 0.038, *Parkia biglobosa* 0.004 *Spondias mombin* 0.114 *Alchornea* sp. 0.04 |
| AN4 Ojuwo | | *Elaeis guineensis* 42.05 *Mangifera indica* 16.76 *Phyllanthus discoideus* 15.02 | *Entada* sp.13.02 *Phyllanthus pentandrus* 5.01 Rubiaceae 4.01 | *Pavetta/Ixora* sp. 3.00 | *Blighia sapida* 0.04, *Ormocarpum* type 0.02 *Parinari* sp. 0.04 |
| AN5 Ankpa Ogoma | | *Cassia* sp. 16.5, *Hymenocardia acida* 16.5 | *Adenia cissampeloides* 7.1, Combretaceae/Melast. | Asteraceae 1.9 *Euphorbia hirta* 1.6, Pipilionacea | *Alchornea cf. cordifolia* 0.2 *Blighia sapida* 0.1, *Bombax buonopozense* |

| Samples | Predominant pollen (>45%) | Secondary pollen (16-45%) | Important minor pollen (3-15%) | Trace pollen (1-3%) | Sporadic pollen (<1%) |
|---|---|---|---|---|---|
| | | | 4.4 *Elaeis guineensis* 9.0 *Lannea* sp. 3.1Myrtaceae 4.5*Sarcocephalus nodiflora*7.5 *Phyllanthus discoideus* 12.7 *Phyllanthus* sp.10.7 | e 1.0, Rutaceae 1.9 | 0.1, *Bridelia ferruginea* 0.2 *Entada* sp. 0.5 *Parinari* sp. 0.1 *Parkia biglobosa* 0.1 *Paullinia pinnata* 0.1 *Piliostigma thonningii*0.1Sapotaceae 0.1 *Vernonia* sp. 0.1 |

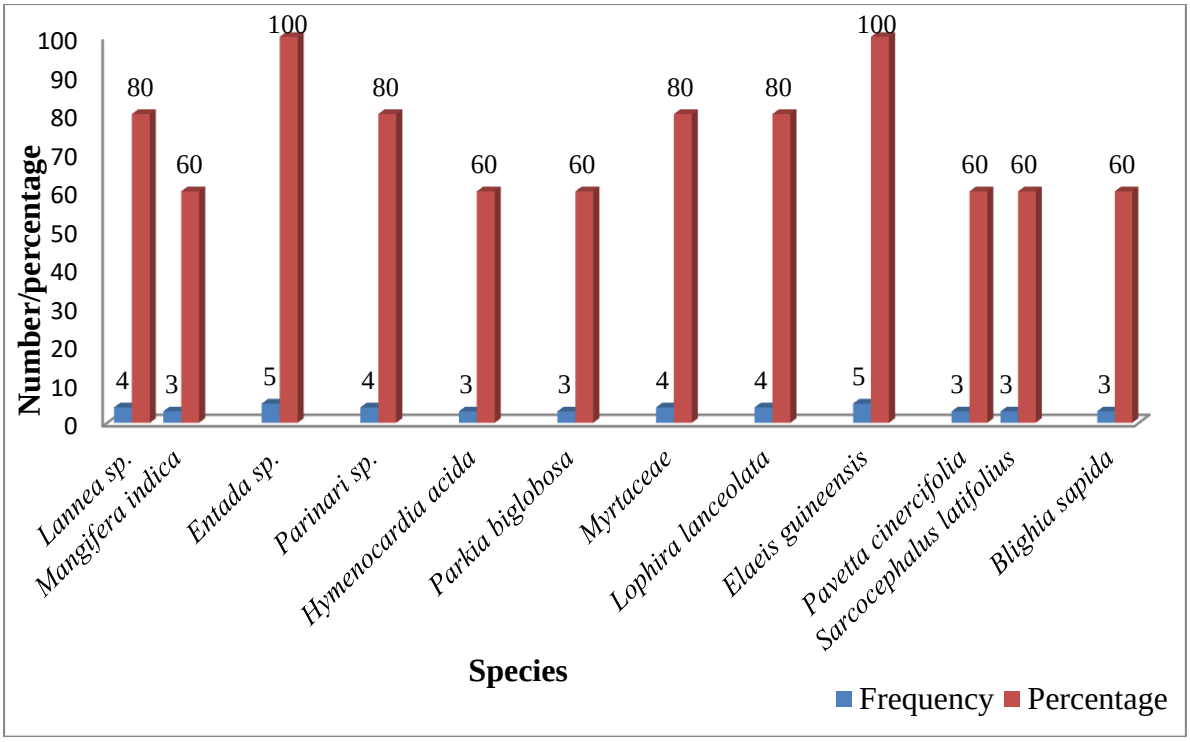


**Fig. 4: shows the taxa with frequency of 50% and above.**

### 3.2 Physicochemical Analysis

The pH, conductivity, sucrose, protein, specific gravity and moisture of the honey samples were measured and analyzed. They all fell within the CODEX (2001) International Standard (Table 2). The pH ranged between 3.6 and 4.1. The CODEX (2001) range is 3.2 - 4.5. AN2 with the highest conductivity had the lowest pH, protein content, moisture and specific gravity. The physicochemical parameters showed significant differences in the pH, conductivity, moisture and sucrose contents of the honey samples while specific gravity and protein contents showed no significant differences (Fig. 4). All the physicochemical parameters measured complied with the CODEX international standards for honey.

**Table 2: Honey samples ID and Physicochemical Parameters measured in honey.**

| Sample ID | pH | µS/cm Conductivity | % Sucrose | % Protein | S.G | % Moisture |
|---|---|---|---|---|---|---|
| AN1 | 3.8±0.12 | 20.8±0.40 | 4.61±1.00 | 0.32±0.50 | 1.41±0;20 | 19.35±1.20 |
| AN2 | 3.6±0.02 | 506±1.55 | 4.03±0.30 | 0.18±0.55 | 1.25±0.33 | 17.69±1.22 |
| AN3 | 3.9±0.02 | 74±0.02 | 5.86±0.60 | 0.38±0.25 | 1.37±0.27 | 18.11±0.65 |
| AN4 | 4.1±0.10 | 39.5±0.45 | 4.95±0.22 | 0.36±0.20 | 1.31±0.15 | 18.65±0.90 |
| AN5 | 3.6±0.02 | 78.8±1.00 | 3.86±0.10 | 0.27±0.10 | 1.44±0.40 | 17.85±1.00 |

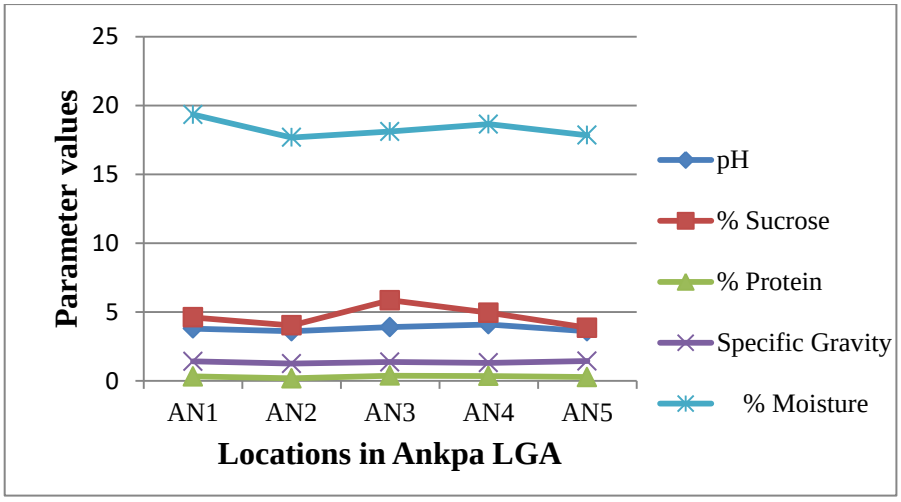


**Fig 5: Physicochemical Parameters measured in Honey Samples**

## 4.0 Discussion and Conclusion

The pollen spectrum is indicative of the botanical sources of honey though not exhaustively the focus of this paper because the presence of pollen in honey is processed by pressing and may not be from nectariferous species. Thus, the percentage predominance of *Elaeis guineensis* (oil palm) was 73.8 in AN 1, the honey is not labeled as monofloral. It is considered as overrepresentation since the plant is not nectariferous. The female inflorescence of *Elaeis guineensis*is heavily scented though it is not clear if the bees are also rewarded by some access to the palm sap. The predominance of a species will only be of value in the determination of the honey status by the quantity of nectar it contributed in the honey production process and is usually inferred from the pollen quantity. This however, will equally depend on the rate of the species' nectar and pollen production. This fact is corroborated by Ingram (2011).

Different locations showed variant pollen diversities. AN 1 had the least pollen diversity with only 5 pollen types as a result of anthropogenic activities. However, the four constituent species were prolific nectar producers. Ingram (2011) indicated certain species as good nectar sources. AN 5 with 25 pollen types was a less disturbed location. Generally it was observed that anthropogenic activities had direct effects on the available number of pollen types in the honey samples. The 12 species occurring in more than 50% of the samples indicated their importance as foraging species. This could guide our attitude towards these species to preserve, protect and propagate them.

The sample AN 2 with the least pH, protein content, specific gravity and moisture content had exceptionally higher conductivity than all other samples. The trend, noticed in AN 5 with the second highest conductivity, is seen in pH, protein and moisture contents as depicted in Figure 5 It could be but it is not clear if those factors affected conductivity or *vice versa*. The homogeneity of the specific gravity and moisture content was as a result of being from a single factor whereby it is expected that the higher the moisture content, the less the specific gravity. It is not clear why this was not identified in AN 1 and AN 2 samples. Generally , the results have shown that when processed in an appropriate manner, such as , centrifugation, honey from Ankpa Local Government Area will meet the International standards and so suitable for exportation. It may be necessary to include more physicochemical properties for greater precision on the delineation of honey samples from Ankpa Local Government Area therefore pose a new direction of research.

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