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Pollen and Physicochemical Analyses of Honey Samples from Ibaji Local Government Area of Kogi State

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Abstract

Five hives were set up in different locations in Ibaji Local Government Area (LGA) of Kogi State, Nigeria, in 2010 from where honey samples were harvested in June, 2012 and labeled BJ1-BJ5. The honey samples were pressed and filtered with appropriate mesh. They were analyzed for their pollen and physicochemical properties using standard methods. A camera fitted Carl Zeiss microscope was used for pollen analysis. Physicochemical properties analyzed were the pH, Conductivity, Specific Gravity, Sucrose, Protein, and Moisture contents. The data generated were subjected to a one-way ANOVA. There were significant differences in the pH, conductivity, moisture and sucrose contents while the specific gravity and protein contents showed no significant differences. The honey samples complied with the CODEX international standards for all the parameters studied. Pollen analysis showed BJ1 (Sarcocephalus honey), BJ2 (Lannea honey), BJ3 (Lannea honey) and BJ4(Lannea honey) to be monofloral honeys while BJ5 was multifloral. In all, 28 pollen types belonging to 21 families were isolated. 7 were only identified to the family taxon, 6 to the generic level while 15 were identified to the specific level. The quality of honey from the area was of international standard and honey from the area will be suitable for exportation.

Keywords: *Honey, Pollen analysis, physicochemical properties, unifloral, multifloral.*

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Introduction

Honey is produced by bees from nectar of plants, as well as from honeydew. Bees and plants are the sources of some honey components as: carbohydrates, water, traces of organic acids, enzymes, amino acids, pigments; and others like pollen and wax arise during honey maturation. Honey composition depends on great extent on the nectar sources. (Ferna´ndez-Torres *et al.,* 2005)

European Community legislation on honey packaging recommends the use of labels indicating floral and geographical origin, as well as specific quality criteria (CODEX, 2001). The determination of botanical source of honey responds to consumer demands and guarantees the quality of the products, avoiding frauds. Thus an extensive characterization of honey samples becomes a necessary task. Honeys produced in Spain in high quantities, such as eucalyptus, heather, lavender, thymus, citrus, rosemary and honeydew have been extensively studied. Data about volatile and carbohydrate composition of these honey types have been presented by Sanz *et al.* (2007). This is the first known attempt of such bifocal analyses of in situ honey in aserial manner in Nigeria. The very first indigenous attempt was Sowunmi (1976) who analyzed the botanical origin implicating also, the geographical origin of the honey. Other notable contributions include: Agwu and Akanbi (1985); Agwu *et al.* (1989), Agwu and Abaeze (1991); Sowunmi (2001); Ayodele *et al.* (2006); Njokuocha and Ekweozor (2007); Adekanmbi and Ogundipe (2009); Adeonipekun (2010); Ige and Modupe (2010) and Aina and Owonibi (2011). Significant studies on the physicochemical analyses of honey have been done in Cameroon (Joseph *et al.*, 2007), Algeria, (Azzedine *et al.*, 2007), Argentina (Fagundez and Caccavari, 2006). While pollen analyses guaranties against adulteration and determines the geographical origin of honey, the physicochemical attributes imputes identity labels on the samples to prevent wrong labeling and to reveal the nutritional values of the honey. The aim of the study was to characterize honey samples from Ibaji LGA through pollen and physicochemical analyses.

MATERIALS AND METHODS

Study Area

Ibaji is a Local Government Area in Kogi State, Nigeria. It is located to the south of the State, separated from Edo State to the west by Niger River, and bordering Delta State in the South.The north easterly line of equal latitude and longitude passes through the LGA.It has an area of 1,377 km^2 and a population of 128,129 at the 2006 census.

Sample collection

A Kenyan top bar beehive box was placed at each of the five locations within the LGA (Fig. 1-3). They were regularly baited until they were colonized by honey bees (*Apismellifera varadansonii*). Honey samples used for this experiment were harvested in June, 2012. Samples of honey were harvested from the colonized boxes in June, 2012. Harvestings were done in the evenings with the aid of hive tools from the various sites. 10g of each honey sample was acetolysed following Erdtman (1971) and the final residue was made up to 1ml with 50% glycerol and stored in EDTA plastic vials.

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 1000X or 400X (for larger palynomorphs) using Leica DM2500 light microscope. Pollen types were identified by comparison with reference pollen micrographs from Sowunmi (1978; 1995), Agwu and Akanbi (1985), Ybert (1979),Wang and Blackmore (2003) and reference collections, journals and prepared slides of pollen samples at the Department of Archaeology, University of Ibadan, Nigeria. The terminology used was in accordance with Erdtman (1960), Faegri and Iversen (1989).

Pollen analysis

Quantitative and qualitative analysis of the pollen contents of samples were carried out. Quantitative analysis followed Terrab *et al.* (2004) but modified in that the samples were acetolysed and covered 22 x 22 mm cover slip surface. The results of the qualitative

analyses are shown in Table 2, expressed as the total number of times a species was encountered in thirty fields of view of the cover slip according to Fagundez and Caccavari (2006) and Maurizio (1979), 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) (Table 3).

Physicochemical properties

Protein analysis was undertaken using Kjeltec 2300; determination of Sucrose followed Anthrone Method; the moisture contents of the honey samples were determined using the oven dry method and pH using pH meter. The 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.

Statistical analysis

The means of the pH, conductivity, specific gravity, protein, sucrose and moisture contents were subjected to a one-way to determine the levels of affinities of these parameters among the samples.

RESULTS AND DISCUSSION

Pollen analysis

Twenty eight pollen types were isolated from the five honey samples. 28 pollen types were recovered in all. 7 were identified only to the family taxon, 8 to the generic while 15 were identified to the specific taxon level. 3 fungal spores were recovered from BJ1 sample.The samples showed less diversity of pollen. This is usual of monofloral honeys (Hermosin *et al.*, 2003, Terrab *et al.*, 2004, Azzedine *et al.*, 2007). The presence of fungal spores, though insignificant, indicated unhygienic processing (Chirife *et al.*, 2006). Pure honey should contain no fungal spores

Physicochemical analysis

There were no significant differences in the specific gravity and protein contents of the 5 samples from Ibaji LGA. There were significant differences in the pH, conductivity, moisture and sucrose contents of the samples.

Table 1: Qualitative analysis of pollen types in honey

Sample	Predominant pollen (>45%)	Secondary pollen (16-45%)	Important minor pollen (3-15%)	Trace pollen (1-3%)	Sporadic pollen (<1%)
BJ1Ihile	*Sarcocephalus nodiflora* 63.016	*Elaeis guineensis* 24.745,	*Danielliaolive ri* 9.395,	*Mangifera indica* 2.515	Combretaceae/Melastomataceae 0.013, *Entada* sp. 0.016, Fungal spore 0.001, Irvingiaceae 0.006, *Lannea* sp. 0.079. Myrtaceae 0.025, *Newbouldialaevis* 0.133, *Nymphaea lotus* 0.010, *Parinari* sp. 0.018, *Parkia biglobosa* 0.006, *Spondia smombin* 0.009, *Tridax procumbens* 0.005, *Vitellaria paradoxa* 0.008
BJ2 Ojigbolo	*Lannea* sp. 77.4		*Sarcocephalu snodiflora* 8.9, *Elaeisguineen sis*5.1	*Newbould ia laevis* 2.1, *Spondia smombin* 1.7, *Alchornea* sp 2.6, *Annona* sp. 1.2, *Entada*sp 1.1	

BJ3 Nwajal	*Elaeisguineensis* 86.95		*Lannea* sp. 12.39,		Anacardiaceae 0.03, Combretaceae/Melastomataceae 0.08, *Entada* sp. 0.04, Euphorbiaceae 0.01, *Mangiferaindica* 0.42, *Morindalucida* 0.04, *Sarcocephalus nodiflora* 0.01, *Spondia smombin* 0.02,
BJ4 Ayeke	*Lannea* sp. 64.1	*Sarcocephalus nodiflora* 19.1	*Alchornea* sp. 5.5, *Entada* sp. 3.8,	*Ormocarpum* sp. 2.4, *Elaeisguineensis* 1.0, Moraceae 1.4, *Spondias mombin* 2.8	
BJ5 Efonu		*Phyllanthusdiscoideus* 27.131, *Lannea* sp. 28.602	*Elaeis guineensis* 12.531, *Vitellaria paradoxa* 13.359, *Sarcocephalus nodiflora* 8.909		*Azadirachta indica* 0.002, *Ceiba pentandra* 0.018, *Daniel liaoliveri* 0.026, *Nesogordoniapaverifera* 0.389, *Parkia biglobosa* 0.181, Poaceae 0.006, *Mangifera indica* 0.006

The array of the botanical origins of the honey samples revealed low species diversity (Table 1). In BJ1, 16 pollen types were recovered, 8 from BJ2, 10 from BJ3, 8 from BJ4 and 12 from BJ5. *Sarcocephalus nodiflora* was predominant in BJ1, Lannea sp. in BJ2, *Lannea sp.* in BJ3 and Lannea sp. in BJ4. BJ5. BJ5 did not have predominant species. BJ1, BJ2 and BJ4 were thus named *Sarcocephalus, Lannea* and *Lannea* honeys respectively (Hermosin *et al.*, 2003). Though Elaeis guineensis recorded 86.95% frequency and the most abundant pollen type in all the samples, it was not labeled as Elaeis honey because it is anemophilous and nectarless. According to Terrab *et al.* (2004),a minimum of 8% of nectariferous species is considered sufficient to typify a honey as unifloral when considering pollen grains from anemophilous and nectarless plants if nectar is the main source of the studied samples. *Lannea* and *Sarcocephalus* are important sources of honey production in Ibaji LGA.

Table 2: Pollen Spectrum of Honey Samples from Ibaji L.G.A., Kogi East, Nigeria

S/N	PLANT TAXA	BJ1	BJ2	BJ3	BJ4	BJ5	TOTAL
1	ANACARDIACEAE						
	Lannea sp.	178	793	20328	26884	127776	175959
	Mangifera indica	5658		693		33	6384
	Spondia smombin	21	17	37	1153		1228
	Anacardiaceae			43			43
2	ANNONACEAEA						
	Annona sp.		12				12
3	ASTERACEAE						
	Tridax procumbens	11					11
4	BIGNONIACEAE						
	Newbouldia laevis	299	21				320
5	CAESALPINACEAE						
	Entada sp.	37	11	63	1589		1700
6	CHRYSOBALANACEAE						
	Parinari sp.	41					41
7	COMBRETACEAE/MELAST.						
	Combretaceae/*Melastomataceae*	29		131			160
8	EUPHORBIACEAE						
	Alchornea sp		27	2287			2314
	Euphorbiaceae			21			21
	Phyllanthus discoideus					138328	138328

9	IRVINGIACEAE	13					13
10	MALVACEAE						
	Ceiba pentandra					91	91
11	MELIACEAE						
	Azadirachta indica					12	12
12	MIMOSACEAE						
	Daniellia oliveri	21132				131	21263
	Parkia biglobosa	13				924	937
13	MORACEAE				571		571
14	MYRTACEAE	56					56
15	NYMPHAECEAE						
	Nymphaea lotus	22					22
16	PALMAE						
	Elaeis guineensis	55660	52	142688	417	63889	262706
17	*Poaceae*					31	31
18	RUBIACEAE						
	Sarcocephalus nodiflora	141747	91	19	8017	45421	195295
	Morinda lucida			71			71
19	SAPOTACEAE						
	Ormocarpum sp.				1004		1004
	Vitellaria paradoxa	18				68114	68132
20	STERCULIACEAE						
	Nesogordoniapa paverifera					1981	1981
21	*Fungal spore*	3					3
TOTAL		**224935**	**1024**	**166381**	**39635**	**446731**	**878706**

The pollen most gathered by the bees is Elaeis guineensis. *Sarcocephalus nodiflora* was the most abundant nectariferous species. The foraging activities of *Apismellifera* within the Local Government Area showed that they gathered the highest number of pollen grains from the Palmae family with 262706 pollen grains counted, *Rubiaceae* with 195,366, *Anacardiaceae* with 182386 and Euphorbiaceae with 140663 (Table 2). This could indicate bee foraging activities or the ubiquity of the species within the foraging radii of the bees. Seaheng *et al.* (2012) indicated that Elaeis pollen as an important component in bee larvae diet/three fungal spores were encountered in the course of pollen analysis. The array of the species depicted Sudano-Guinean Savannah.

Table 3: Maurizio Classification of Pollen content in honey samples

S/N	Samples	Location	Number of Pollen grains/g	Class
1	BJ1	Ihile	224938	III
2	BJ2	Ojigbolo	1024	I
3	BJ3	Nwajala	164099	III
4	BJ4	Ayeke	41922	II
5	BJ5	Efonu	446731	III

All the samples fell into Maurizio (1979) group I-III. From Table 3, it could be deduced that the honey samples were nutritious, being highly polleniferous (Saeheng *et al.*, 2012).The highest pollen recovery was in BJ5 which is multifloral and the least, BJ2, a monofloral honey.

Table 4: General information on the collected honey samples

Samples	Location	L.G.A	Harvested period	Botanical origin	Mode of extraction
BJ1	Ihile	IBAJI	June, 2012	Unifloral	Pressage
BJ2	Ojigbolo			Unifloral	Pressage
BJ3	Nwajala			Unifloral	Pressage
BJ4	Ayeke			Unifloral	Pressage
BJ5	Efonu			Multifloral	Pressage

Table 4: Honey samples ID and Physicochemical Parameters measured in honey.

Sample ID	pH	µS/cm Conductivity	% Sucrose	% Protein	S.G	% Moisture
BJ1	3.6±1.30	29.6±1.33	4.81±2.00	0.29±0.10	1.26±0.55	19.85±2.00
BJ2	3.4±1.55	36.9±2.00	5.90±1.56	0.17±0.12	1.32±0.25	18.33±1.56
BJ3	3.8±1.75	7.93±1.78	5.31±1.55	0.27±0.15	1.35±0.25	12.32±2.15
BJ4	3.4±1.20	22.2±1.45	4.02±1.65	0.31±0.10	1.03±0.22	22.17±1.75
BJ5	3.4±1.45	488±1.90	5.47±1.87	0.23±0.12	1.3±0.15	18.98±2.25

The pH range was between 3.4% in BJ2, BJ4, BJ5 and 3.8% in BJ3

(Table 4). All the honey samples had pH values that were compliant with the international standards of CODEX (2001), which stipulated blossom honeys' pH range should to be between 3.2 and 4.5.

3.3 Conductivity

Conductivity ranged between 7.93 in BJ3 and 488 μS/cmin BJ5 (Table 4). The codex ceiling is <.8mS/cm which meant that all the honey samples were internationally acceptable for conductivity. BJ3 with the lowest conductivity had the highest pH Ouchemoukh *et al.* (2007) associated high conductivity with high pH.

3.4 Sucrose

The ranges were between 4.02% in BJ4 and 5.90% in BJ2 percent (Table 4). The sucrose contents were low enough for high quality honey. Azzedine *et al.* (2007) obtained as much as 14.78 and Ouchemoukh et al. (2007) 8.3% in Algerian honey samples. Nigerian honeys had low sucrose contents because the bees are not fed with sugar.

Protein

The range of protein contents in the honey samples were between 0.17% and 0.31%. This complied with the CODEX (2001) standard. Azzedine *et al.* (2007) and Ouchemoukh *et al.* (2007) obtained values higher than this.

Specific Gravity

The specific gravity ranged between 1.03 in BJ4 and 1.35 in BJ3. BJ3 with the lowest moisture content also had the highest specific gravity and BJ4 with the highest moisture content had the lowest specific gravity. Moisture content is directly related to specific gravity in that the higher the moisture, the less will be the specific gravity. The value obtained in the study were generally lower than those obtained by Azzedine *et al.* (2007) and Ouchemoukh *et al.* (2007) who worked on Algerian honeys.

Moisture

BJ4 with 22.17% moisture content exceeded the stipulated international standard. Apart from that, all the other samples complied with the stipulated international range.Water content is related to the maturity degree. The water contents obtained ranged from 12.32% and 22.17%. Azzedine *et al.* (2007) has reiterated that the honey having a value ranging between 16- 18% are regarded as best honeys with respect to the conservation and of storage.

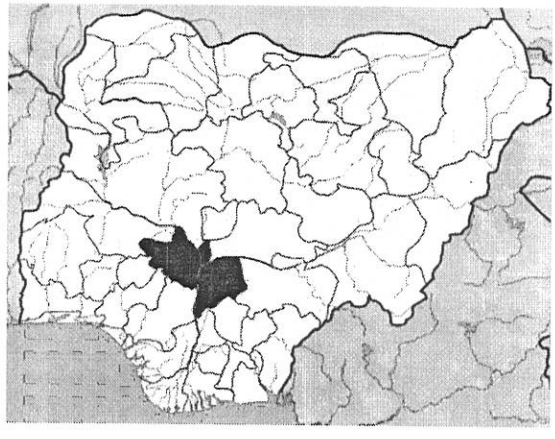


Fig. 1: Map of Nigeria showing Kogi State
(Coordinates: 7°30?N 6°42?E

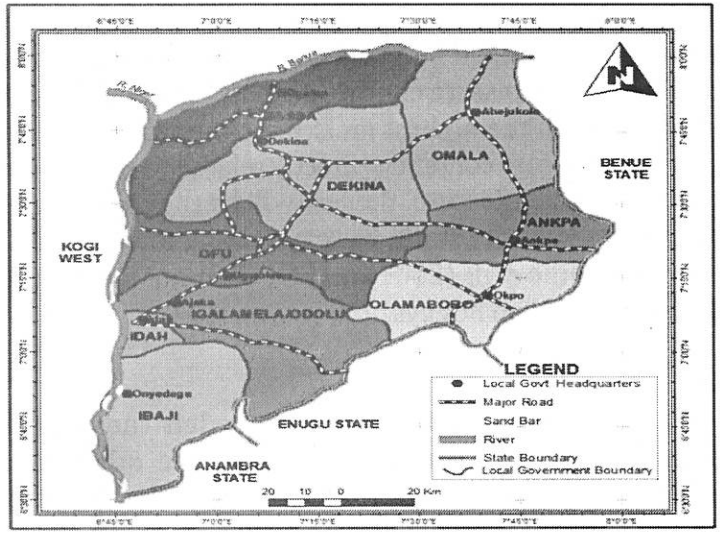
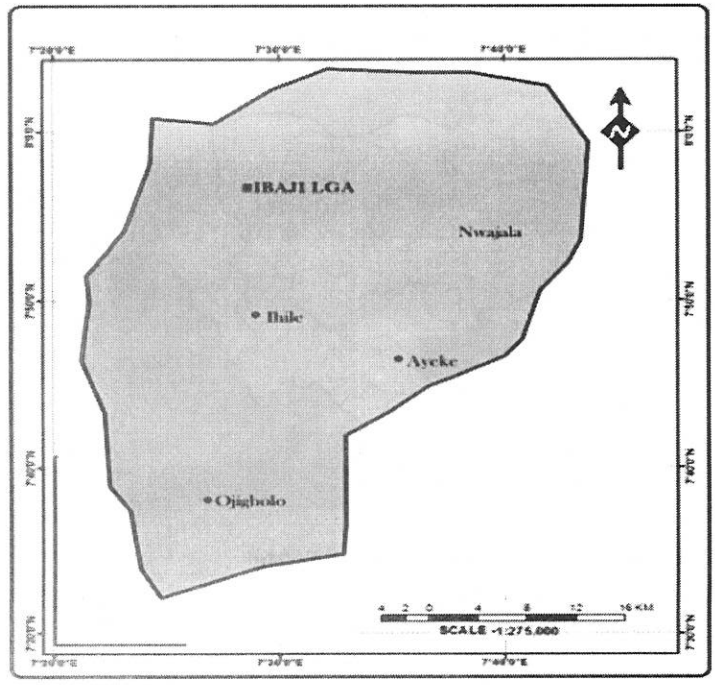


Fig. 2: Map of Kogi State



IBAJI LOCAL GOVERNMENT AREA

Fig. 3: Map of Kogi East, showing the sites of samples collection.

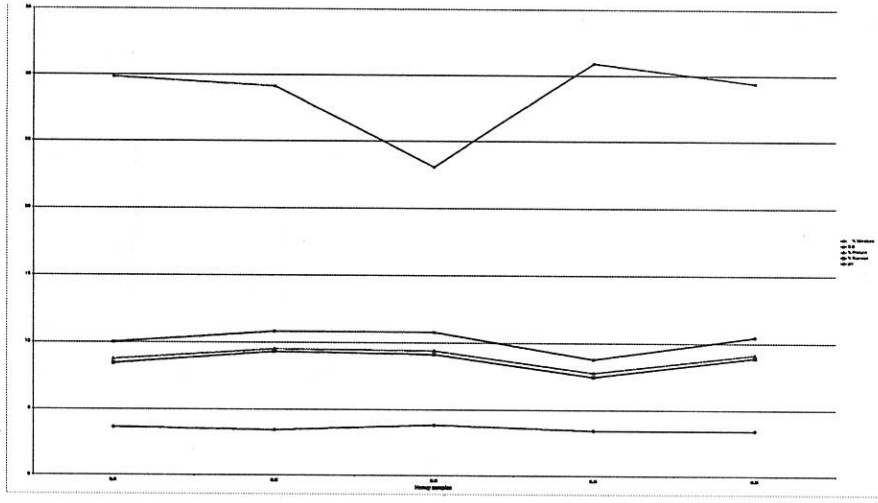


Fig. 4: Physicochemical Parameters measured in Honey Samples

CONCLUSION

The honey samples obtained from Ibaji LGA and analysed revealed their pollen contents which enabled the determination of the botanical and geographical origins. The physicochemical properties analysed also revealed that Ibaji LGA has a great potential in the production of high quality honey that meets international standards. *Lannea sp.* and *Sarcocephalus nodiflora* are species that needs to be preserved and/or propagated to enhance honey production. The characterization of honey from various areas should be encouraged and the parameters screened could be expanded to make analyses more comprehensive.

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