



Pollen Analysis of Honey Bought from Sokoto Metropolis

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ABSTRACT

Microscopic examination of pollen grains determined the botanical origin and geographical location of honey. This study was aimed at determining the botanical sources of honey using qualitative and quantitative pollen analysis. Ten grams (10g) of honey was diluted with 8ml of distilled water and centrifuged at 2,500 rpm for ten minutes, and the sediments were used to analyse the pollen microscopically. Three thousand six hundred and ninety-two (3692) pollens belonging to fifteen (15) plant species were identified. Among the pollen types, *Azadirachta indica* and *Aspilia africana* were found in all the samples. *Azadirachta indica* was found to be higher ranging from 27.23% to 47.98%, while *Coffea arabica* and *poacea* was rare. Samples A, B and E were classified as unifloral honey with *Azadirachta indica* dominating the pollen assemblage, with a 47.96%, 51.21% and 45.12% relative frequency respectively. Samples C and D are multi-floral honey with a diverse assemblage of frequency classes of pollen and no dominant pollens. The result of pollen analysis showed a wide variety of botanical sources of honey. It is recommended that the pollen analysis of the honey sample produced in the area should be conducted.

Keywords: Pollen analysis, honey, qualitative, quantitative, unifloral and multi-floral honey.

INTRODUCTION

Honey is a natural sweet, viscous substance produced by honeybees (*Apis mellifera*) by collecting nectar and pollen from blossoms or from the secretion or excretion of living parts of the plant, which transform and combine with specific substances of their own, store, and leave in the honeycomb to ripen and mature (Abeshu and Geleta, 2016). Honey has attractive chemical properties for baking and distinctive flavour that attract many people to prefer it over sugar and other sweetness (Estevinho *et al.*, 2012).

Bee honey contains water, sugars and other minor components such as; proteins, free amino acids, flavours, aromas, pigments, vitamins, volatile compounds and carbohydrate (fructose about 38.30%, glucose 31% etc.) (USDA, 2008). The composition of honey varies according to the source of flowers used by the bees, the harvest period and the geo-climatic conditions of the regions concerned (Yédomonhan *et al.*, 2009). However, lighter honey is mild in flavour, while darker honeys are usually more robust in flavour (Kaya *et al.*, 2005). Honey is an alternative to enhance memory, lowering cholesterol and treatment of various ailments which includes; gastric disturbances, ulcer of tongues, healing wounds and burns, and various skin diseases (Al-Wailiet *et al.*, 2011). Although honey has medicinal importance, many people on ingestion of honey suffer from itchy eyes, a runny nose, sneezing and sometimes respiratory problems including asthma, especially if the honey contains heavy pollens (Pierce, 1999). Sometimes honey contains dormant endospores of *Clostridium botulinum*, which can transform into toxins producing bacteria in infant immature intestinal tract leading to illness and even death (Shapiro *et al.*, 1998).

Microscopic analysis of pollen is an essential tool to provide botanical and geographical information of

honey and understand the foraging ecology of bees (Barth, 1990). Pollens allow characterization of vegetation that surrounds the beehives and also the geographical location, from which the nectar was collected (Louveaux *et al.*, 1978). Pollen analysis can be used to determine adulteration in honey; when honey was adulterated with sugar syrup, there is a reduction in pollen content (Alfred, 2004). According to Louveaux *et al.* (1978) pollen in honey can be represented as dominant pollen (> 45% of total grains) (DP), accessory pollen (16 to 45%) (AP), important isolated pollen (3 to 15%) (IIP), and occasional isolated pollen (< 3%) (OIP), and pollen frequency can be used to classify honey into unifloral and multi-floral Honey.

This research aimed to determine the botanical origin of honey through pollen analyses of five (5) commercial honey samples. And the objectives are to identify and determine percentage frequencies of pollen by qualitative and quantitative methods and also to classify the honey.

MATERIALS AND METHODS

Study Area

The study was conducted in Sokoto (capital city) of Sokoto State located in North Western Nigeria, within latitude 13° 00' 59" N and longitude 5° 24' 76" E bordering the Sahara Desert. The state bordered the Niger Republic to the north, shares boundaries with Kebbi State to the west and south, and Zamfara to the south and east. It records annual rainfall between 300mm-800mm and means the temperature of 34.5°C. The dry season temperatures exceed 45° C during the daytime and dominated by the North-East Trade wind (Harmattan) blowing Sahara dust over the land when dust hangs in the air (Adegboyega *et al.*, 2015).

Sample Collection

Five (5) honey samples were bought from five different places in Sokoto Metropolis and labelled with letters; A, B, C, D and E. Sample A were brought from Sokoto Kara, sample B from Biological Garden, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto (UDUS), permanent Site, Sample C from Sokoto Old market, Sample D from Gobirawa shop and Sample E from Gwiwa low-cost Sokoto. All the honey samples were prepared using the acetolysis method as adopted (Erdtman, 1960) and then submitted to both a qualitative and quantitative analysis.

Methods

Ten grams (10g) of honey samples were weighed using 0.01g sensitive electrical weighing balance (Sartorius ED224S), the sample was diluted with 8ml of distilled water and stirred with a clean stick. The mixture was then transferred into centrifuge tubes and arranged directly opposite to each other in the centrifuge machine. The set-up was centrifuged at 2,500 rpm for five minutes and the super-natants were discarded. A 10ml of distilled water was then added to the sediments and centrifuge again at the same speed and time (Battesti, 1992). After discarding the super-natants, the sediments were then collected with Pasteur pipette, deposited onto the centre of the clean glass slide and two drops of glycerin-jelly were added on the slide and then covered with 18 x 18 mm coverslip for pollen examination and counting under a microscope.

Microscopic Examination

Microscopic examination was conducted in the Post Graduate Research Laboratory (Zoology Laboratory

II) Zoology Unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto (UDUS) Permanent Site.

Qualitative Analysis

Different pollen types present in the samples were identified under Binocular microscope with X100 magnification by comparison with a reference photomicrographs and pollen atlases (Sowunmi, 2001).

Quantitative Analysis

The slides were divided into 3 portions of the coverslip consisting one side, the median and a 3/4 of the width of the coverslip (Jemmett and Owen, 1990). Consecutive careful counting of pollen grains was done to determined percentages and classes of occurrences such as dominant pollen (> 45% of total grains) (DP), accessory pollen (16 to 45%) (AP), important isolated pollen (3 to 15%) (IIP), and occasional isolated pollen (< 3%) (OIP) (Louveaux *et al.*, 1978).

RESULTS AND DISCUSSION

The study showed that, out of the 5honey samples analysed *Azadirachta indica* and *Aspilia africana* were present in all the samples and the pollen composition of *Azadirachta indica* was found to be higher throughout the samples (Table 1), ranges from 27.23% in sample C to 47.98% in sample A. *Eucalyptus globolus* and *Gliricida sepium* was not found in sample E and A, respectively, while *Acacia albida*, *Coffea arabica*, *Syzygium communis* and *Cocos nucifera* was only found in two samples (which are sample B and D) and *Alchornea cordifolia* 26(2.81%) was only found in Sample D.

Table 1: Number and Percentages of Dry Pollen Grains Analysed in Honey Samples (A, B, C, D and E)

Pollen Grains	Honey Samples					TOTAL
	A No. (%)	B No. (%)	C No. (%)	D No. (%)	E No. (%)	
<i>Acacia albida</i>	15(6.78%)	0%	0%	0%	153(15.07%)	168
<i>Acacia nilotica</i>	19(8.59%)	0%	164(32.60%)	0%	169(16.65%)	352
<i>Alchornea cordifolia</i>	0%	0%	0%	26(2.81%)	0%	26
<i>Anacardium occidentale</i>	0%	0%	26(5.16%)	31(2.81%)	7(0.68%)	64
<i>Aspilia africana</i>	9(4.07%)	9(1.04%)	14(2.78%)	46(4.18%)	36(3.54%)	114
<i>Azadirachta indica</i>	106(47.98%)	441(51.10%)	137(27.23%)	334(30.36%)	458(45.12%)	1476
<i>Borreria verticillata</i>	0%	0%	19(3.77%)	24(2.18%)	4(0.39%)	47
<i>Cocos nucifera</i>	0%	64(7.41%)	0%	160(14.54%)	0%	224
<i>Coffea arabica</i>	0%	0%	0%	16(1.45%)	7(0.68%)	23
<i>Croton urucurana</i>	0%	110(12.74%)	0%	101(9.18%)	96(9.45%)	307
<i>Eucalyptus globolus</i>	37(16.74%)	43(4.98%)	83(16.50%)	198(18.90%)	0%	361
<i>Gliricida sepium</i>	0%	76(8.80%)	38(7.55%)	90(8.18%)	30(2.95%)	234
<i>Mangifera indica</i>	35(15.84%)	0%	22(4.37%)	0%	48(4.72%)	105
<i>Cimnopogon citratus</i>	0%	0%	0%	13(1.18%)	7(0.68%)	20
<i>Syzygium communis</i>	0%	120(13.90%)	0%	51(4.63%)	0%	171
TOTAL	221	863	503	1090	1015	3692

Keys: A= Sokoto Kara, B= Botanical Garden Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto (UDUS), C= Sokoto Tsohuwar Kasuwa, D = Gobirawa shop and Sample E= Gwiwa low-cost Sokoto.

Pollen composition varied among samples, sample D recorded the highest number of pollen grains of about 1090, followed by sample E, while sample A recorded

the lowest number of pollen grains with 221 pollen grains (Table 1).

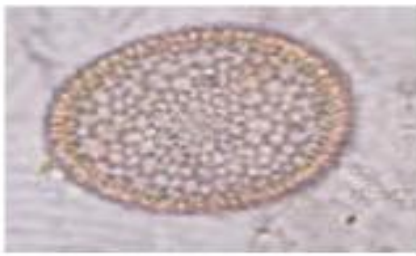


Plate 1. *Croton urucurana* Baill

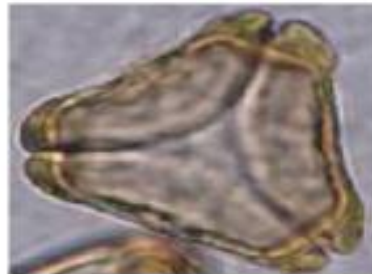


Plate 2. *Cocos nucifera* Linn



Plate 3. *Eucalyptus globulus* Labill

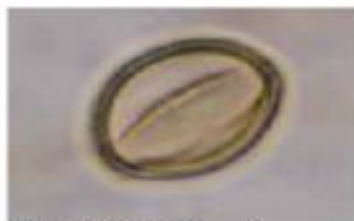


Plate 4. *Gliricida sepium* (Jacq.) Kunth ex Walp



Plate 5. *Cimborogon citratus* Stapf



Plate 6. *Mangifera indica* Linn



Plate 7. *Syzygium communis* Brown ex Gaertn



Plate 8. *Azadirachta indica* Juss.



Plate 9. *Anacardium occidentale* Linn

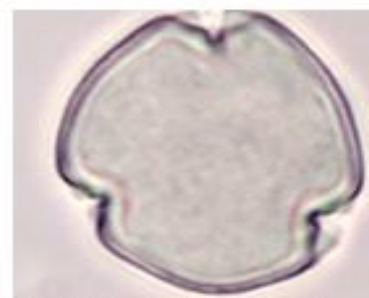


Plate 10. *Coffea Arabica* Linn

Plate 1: Photomicrographs of Pollens Identified in the Honey Samples (A, B, C, D and E) (Source: Singh *et al.* (2017); Shubharani *et al.* (2013) Based on the pollen proportion, Samples A, B and E were classified unifloral honey with *Azadirachta indica* (dominant pollen) constituted the major fraction of the total

pollen assemblage, recorded (47.96%), (51.21%) and (45.12%) respective relative frequency. Sample C and D are multi-floral honey with a diverse assemblage of frequency classes of pollen and no dominant pollens.

Table 2: Frequency Classes of Pollen in Five honey Sample (A, B, C, D and E) and Honey Types

Honey Sample	Type of Honey	DP (>45%)	AP (16-45%)	IIP (3-15%)	OIP (<3%)
A	Unifloral	<i>Azadirachta indica</i> (47.96%)	<i>Eucalyptus globolus</i> (16.74%), <i>Mangifera indica</i> (15.84%)	<i>Aspilia africana</i> (4.07%), <i>Acacia albida</i> (6.78%), <i>Acacia nilotica</i> (8.59%)	
B	Unifloral	<i>Azadirachta indica</i> (51.21%)		<i>Croton urucurana</i> (12.74%), <i>Cocos nucifera</i> (7.41%), <i>Eucalyptus globolus</i> (4.98%), <i>Gliricida sepium</i> (8.84%), <i>Syzygium communis</i> (13.90%)	<i>Aspilia africana</i> (1.04%)
C	Multifloral		<i>Azadirachta indica</i> (27.23%), <i>Acacia nilotica</i> (32.60%), <i>Eucalyptus globolus</i> (16.50%)	<i>Mangifera indica</i> (4.37%), <i>Borreria verticillata</i> (3.77%), <i>Anacardium occidentale</i> (5.16%), <i>Gliricida sepium</i> (7.55%)	<i>Aspilia africana</i> (2.78%)
D	Multifloral		<i>Azadirachta indica</i> (30.36%), <i>Eucalyptus globolus</i> (18.90%)	<i>Croton urucurana</i> (9.18%), <i>Cocos nucifera</i> (14.54%), <i>Syzygium communis</i> (4.63%), <i>Aspilia africana</i> , (4.18%), <i>Gliricida sepium</i> (8.18%)	<i>Borreria verticillata</i> (2.36%), <i>Anacardium occidentale</i> (2.18%), <i>Alchornea cordifolia</i> (2.81%), <i>Poaceae</i> (1.18%), <i>Coffea arabica</i> (1.45%)
E	Unifloral	<i>Azadirachta indica</i> (45.12%)	<i>Acacia nilotica</i> (16.65%), <i>Cacia albida</i> (15.07%)	<i>Mangifera indica</i> (4.72%), <i>Croton urucurana</i> (8.72%), <i>Aspilia africana</i> (3.54%)	<i>Borreria verticillata</i> (0.36%), <i>Anacardium occidentale</i> (0.63%), <i>Poaceae</i> (0.63%), <i>Gliricida sepium</i> (2.72%), <i>Coffea arabica</i> (0.68%)

Keys: DP= Dominant Pollen, AP= Accessory Pollen, IIP= Important Isolated Pollen and OIP= Occasional Isolated Pollen.

DISCUSSION

The result showed, the honey samples used contained a variety of pollen grains, and occur in different shape, size and pollen composition. In this study fifteen (15) different plant species were identified, and the honey originates from several plant sources but *Azadirachta indica* and *Aspilia africana* are the highest occurring pollens among the five samples used. The dominant pollens identified indicated high abundance in the area where the honey produced and serve as the chief source of nectar and pollen for foraging bees. The number of pollens present in the honey indicated the richness and health of the vegetation (Agwu and Uwakwe, 1992). According to Kaya *et al.* (2005), predominant and secondary pollen grains supply the nectar sources, which play a crucial role in the production of honey, while, the taste, aroma and colour of honey change according to the flower nectar. The pollen richness of the honeys is influenced by the floral morphology, phenology and floristic composition (Lobreau-Callen and Damblon, 1994). The number of pollen in the honey sample indicates the richness of the vegetation of the area where the honey produced (Agwu and Njokuocha, 2004)

The analysis revealed that pollen diversity was greater among the Important Isolated Pollen (IIP) range from 3-15%, followed by Occasional Isolated Pollen (OIP) pollen with <3% and accessory pollen range from 16-

45% and dominant pollen > 45% has the least pollen diversity. This result is in agreement with the pollen analysis of Olusola and Oluwatoyin (2000), who reported that variability in pollen grains is always small among the dominant pollen group (>45%), while greater among the OIP <3%, IIP 3-15% and AP 16-45%.

CONCLUSION

The result showed that three thousand six hundred and ninety-two pollens were identified in the five honey sample and revealed a variety of botanical sources. Fifteen (15) different plant species were identified; *Azadirachta indica* and *Aspilia africana* were present in all the five samples. The results showed that the pollen types of *Azadirachta indica* were the most abundant among the samples, *Acacia albida*, *Coffea arabica*, *Syzygium communis* and *Cocos nucifera*, *Alchornea cordifolia* was also present. However, *Coffea arabica* and *Poaceae* was found rarely in the samples. Sample A, B and E were classified unifloral due to the presence of dominant pollen of *Azadirachta indica* 47.96%, 51.21% and 45.12% relative frequency respectively. Sample C and D are multifloral honey with a diverse assemblage of frequency classes of pollen and no dominant pollens. It is recommended that the pollen analysis of the honey sample produced in the area should be conducted.

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