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## Chemical/Physical Characterization of Nigerian Honey

F.M. Adebisi<sup>1</sup>, I. Akpan<sup>2</sup>, E.I. Obiajunwa<sup>3\*</sup> and H.B. Olaniyi<sup>4</sup>

<sup>1</sup>Department of Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria

<sup>2</sup>Department of Physics, University of Calabar, Calabar, Nigeria

<sup>3</sup>Centre for Energy Research and Development, Obafemi Awolowo University, Ile-Ife, Nigeria

<sup>4</sup>Department of Physics, Obafemi Awolowo University, Ile-Ife, Nigeria

E-mail: eobiajun@oauife.edu.ng

**Abstract:** The TXRF and infrared spectrophotometer techniques were used for the characterization of Nigerian honey. The elemental concentrations and the functional groups present in Nigerian honey samples from five locations in the Southwest and Southeast of Nigeria were determined. Twelve elements - K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Se, Br and Rb - were detected. The honey samples were quite rich in minerals. Potassium was the most abundant trace element in the honey (range 1100 - 2700 ppm) followed by Ca (range 152 - 362 ppm) and Fe range 136 - 407 ppm). There were no significant differences in the elemental contents of honey from the different locations. The infrared spectrophotometer results showed the honey sample to be a mixture of many compounds including carboxylic acids, aldehydes, alkynes, nitrites, alkenes and ethers. Other physical properties - colour, pH, refractive index, moisture and ash contents - were also determined.

**Key words:** Honey, TXRF, infrared spectrophotometer, elemental concentrations, functional groups

### Introduction

Honey, a popular sweetener throughout the world, is made by bees generally from nectars extracted from the nectarines of flowers. From ancient times, honey was used both as a natural sweetener and a healing agent (National Honey Board, 2002). The composition and flavour of honey varies, depending mainly on the source of the nectar(s) from which it originates and to a lesser extent on certain external factors - climatic conditions and beekeeping practices in removing and extracting honey (White, 1975a).

The characterization of honeys aids our understanding of its properties and applications - medicinal properties, anti-bacterial and antioxidant behaviours, and hence its use as a food ingredient in human diet. Whereas there are large volumes of data on the characterization of honeys from North America, Europe, Australia, India and South Africa, there is a paucity of data on Nigerian honeys.

Honey is farmed and used all over Nigeria. Initially local farmers harvested the honey from the wild but today apiculture is a growing industry in many parts of the country. Some studies on the healing effects and antimicrobial activity of Nigerian honey on burns and wounds have been reported (Adesunkanmi and Oyelami, 1994). However, to our knowledge, there is no detailed study on the chemical/physical characterization of the Nigerian honey.

In the paper we present the results of our preliminary studies of Nigerian honey samples from five locations in the Southeast and Southwest regions of Nigeria. This study covers chemical properties - elemental (mineral

content, identification of the functional groups in the honeys; physical properties - colour, pH, refractive index, electrical conductivity, moisture and ash contents of each of the honey samples.

### Materials and Methods

**Samples:** The honey samples were collected from five locations - Ile-Ife (Osun State), Ilara-Mokin (Ondo State), Afikpo (Ebonyi State), Ikot Ukup Itam (Akwa-Ibom State) and Obudu (Cross-River State), in the Southeast and Southwest of Nigeria.

### Chemical analysis

**Elemental analysis:** The elemental analysis of the honey samples was performed using the Total Reflection X-Ray Fluorescence (TXRF) spectrometer in the Department of Physics, Obafemi Awolowo University, Ile-Ife. The TXRF spectrometer consists of a COMPACT 3K5 x-ray generator with x-ray tube (Model IS 221520), with a Mo anode, manufactured by Itai Structures, a Canberra (SL 80175) Si(Li) detector (resolution 175 eV at 5.9 KeV), coupled to a Canberra Inspector-2000 Digital Signal Processor. Canberra Genie-2000 software was used for data acquisition.

0.50g of each honey sample was dissolved with double-distilled water in a 50 ml Volumetric flask and made up to the mark with the double-distilled water. Gallium was added to 50 µl aliquot of the dissolved sample as internal standard and was thoroughly homogenized by shaking. Then 5 µl of the solution was pipetted on a clean quartz sample carrier and dried by evaporation under an Infrared lamp. The carrier with the dried

Table 1: TXRF results for the honey samples

Element	Honey 1 (Ile-Ife)	Honey 2 (Ilara-Makin)	Honey 3 (Afikpo)	Honey 4 (Obudu)	Honey 5 (Ikot-Ukap-Itan)	Mean
K	1100	12500	14700	21600	2700	10520
Ca	250	152	362	265	216	249
Ti	42	30	59	46	80	51.4
Cr	10	17	15	9	5	11.2
Mn	ND	3	3	5	1	3
Fe	140	148	407	136	272	220.6
Ni	10	14	23	15	3.7	13.14
Cu	13	10	35	30	17	21
Zn	106	78	61	41	31	63.4
Se	13	3.5	2.5	2.1	3.8	4.98
Br	0.5	3	4	2	2.5	2.4
Rb	2.2	4	2.4	6	6	4.12

Concentrations of elements are given in  $\mu\text{g/g}$

Table 2a: Assignment of possible Functional Groups in Ile-Ife Honey samples

Wave Number ( $\text{cm}^{-1}$ )	Possible Assignment
3400 (broad)	OH, Carboxylic acids
2950	CHO(str), Aldehydes
2250	C / C and C / N(str), Alkynes and Nitrites
1642.5	C = C(str), Alkenes
1411	C - C(bending)
1059.4	C - O(str), Ethers

Table 2b: Assignment of possible Functional Groups in Ilara-Mokin Honey samples

Wave Number ( $\text{cm}^{-1}$ )	Possible Assignment
3400 (broad)	OH, Carboxylic acids
2950	CHO(str), Aldehydes
2250	C / C and C / N(str), Alkynes and Nitrites
1642.4	C = C(str), Alkenes
1411	C - C(bending)
1070.7	C - O(str), Ethers

Table 2c: Assignment of possible Functional Groups in Afikpo Honey samples

Wave Number ( $\text{cm}^{-1}$ )	Possible Assignment
3400 (broad)	OH, Carboxylic acids
2950	CHO(str), Aldehydes
2250	C / C and C / N(str), Alkynes and Nitrites
1642.4	C = C(str), Alkenes
1411	C - C(bending)
1060.2	C - O(str), Ethers

sample on it was then presented to the TXRF spectrometer.

Each sample was irradiated for 1000 seconds at fixed tube operating conditions of 40 kV and 20 mA. The spectral data analysis was done with the AXIL fitting

Table 2d: Assignment of possible Functional Groups in Obudu Honey samples

Wave Number ( $\text{cm}^{-1}$ )	Possible Assignment
3369 (broad)	OH, Carboxylic acids
2943.4	CHO(str), Aldehydes
1642.4	C = C(str), Alkenes
1411.3	C - C(bending)
1061.8	C - O(str), Ethers

Table 2e: Assignment of possible Functional Groups in Ikot-Ukap-Itan Honey samples

Wave Number ( $\text{cm}^{-1}$ )	Possible Assignment
3400 (broad)	OH, Carboxylic acids
2937.4	CHO(str), Aldehydes
2200	C / C and C / N(str), Alkynes and Nitrites
1641.5	C = C(str), Alkenes
1411.3	C - C(bending)
1060.7	C - O(str), Ethers

program contained in the QXAS software package supplied by the International Atomic Energy Agency (I.A.E.A) (IAEA, 1993).

**Identification of functional groups:** For the identification of the functional groups present in the honey samples, each sample was run raw in the Scanning Infrared Spectrophotometer (Buck Model M500) in the Department of Chemistry, Obafemi Awolowo University, Ile-Ife. The Spectrophotometer was first calibrated using polystyrene.

**Physical analysis:** Six physical parameters - colour, pH, refractive index, electrical conductivity, moisture and ash contents were determined for each sample. The colour of the samples was determined using the Lovibond comparator.

The pH was measured using a digital pH meter Model

Table 3a: Physical characterization of the honey samples

Location	Colour (Pt. Co)	pH	Refractive Index	Conductivity ( $\mu\text{S cm}^{-1}$ )	Moisture Content (%)	Ash Content (%)
Ile-Ife	52.00	6.02	1.489	9.419	16.380	0.095
Ilara-Mokin	255.00	4.31	1.460	172.900	30.820	0.219
Afikpo	127.00	4.42	1.479	39.965	21.300	0.357
Obudu	60.00	4.40	1.488	18.624	17.720	0.319
Ikot-Ukap-Itam	132.60	4.60	1.488	22.176	18.540	0.518
Range	(52.00-255.00)	(4.31-6.02)	(1.460-1.488)	(9.419-172.900)	(16.380-30.820)	(0.095- 0.518)
Mean	125.42	4.75	1.481	52.617	20.952	0.302

Table 3b: Comparison of the measured and calculated moisture content of the honey samples

Location	Moisture Content (%) (measured)	Moisture Content (%) (calculated)
Ile-Ife	16.380	18.19
Ilara-Mokin	30.820	30.00
Afikpo	21.300	22.20
Obudu	17.720	18.59
Ikot-Ukap-Itam	18.540	18.59
Range	16.380-30.820	18.19-30.00
Mean	20.952	21.51

HI 8519 (Hannan Instrument), while the refractive index measurements were done with an Abbe refractometer. The refractometer's sample compartment and window were first cleaned with acetone. The measurements were made at room temperature (28°C). The electrical conductivity measurement was done at 25°C using pH/Conductivity meter Model 20 (Denver Instrument). The instrument was calibrated using 0.01M KCl (potassium chloride solution).

For the determination of the moisture content, 2.50g of each sample was put in a flat dish and dried in the oven at 105°C for three hours, covered, cooled in a desiccator and weighed. The sample was re-dried for one hour in the oven, cooled and reweighed. The process was repeated at one hour drying intervals until a constant weight was obtained. Also for the ash content determination, 2.50g of each sample was put in a crucible and dried in an oven at 105°C for three hours to prevent loss by foaming. After cooling it was ashed in a Muffle model furnace at 600°C overnight. It was cooled and weighed to a constant weight.

### Results and Discussion

The results of the TXRF elemental analysis of the honey samples are given in Table 1. Twelve elements (K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Se, Br and Rb) were detected and their concentrations determined. Potassium was the most abundant trace element (range 1100 - 2700 ppm), followed by Ca (range 152 - 362 ppm) and Fe range 136 - 407 ppm). The results show that these Nigerian honey samples are quite rich in minerals.

There were no significant differences in the elemental content of honeys from the different locations.

The identification of functional groups present in the honey samples was done using the Infrared (IR) spectrophotometer. The assignments of the possible functional groups for the honey samples are given in Table 2 (a-e). The Infrared spectra of all the samples show similar peaks except C/C and C/N (str) of Alkynes and Nitrites respectively that are not present in the Afikpo and Obudu samples. The IR results show that the honey samples are a mixture of many compounds including Carboxylic acids, Aldehydes, Alkynes, Nitrites, Alkenes and Ethers. This finding is in accord with many other studies on honey that show that honeys are mixtures of carbohydrates, acids, lipids, proteins, minerals and vitamins (White *et al.*,1962, White *et al.*,1967, Gryuner and Arinkina, 1970, National Honey Board, 1999)

The results of the other measured physical parameters - colour, pH, refractive index, electrical conductivity, moisture and ash contents respectively, are given in Table 3a. The colours of all honey samples collected were in the ember to dark ember shade, which on the Lovibond comparator scale range from 52.00 to 255.00. The dark honeys are known to contain more minerals than the lighter ones (White, 1975b). The pH of the Nigerian honey samples range from 4.31 to 6.02, with a mean of 4.75. These values are comparable to the pH values of honeys from U.S. (range 3.4-6.1) (White, 1975a,b).

The mean refractive index of the honey samples was 1.481 with a range of 1.460 - 1.489. This value is similar to those reported by other researchers (White, 1975b). The moisture content of the honey samples ranged from 16.38 to 30.82%, which is also comparable to values obtained for U.S. honeys. The refractive index of honey is said to be a rapid, accurate and simple measure of its moisture content (White, 1975b). Wedmore in 1955 gave the following relationship between the moisture content and refractive index of the honey.

$$\text{Moisture content} = \frac{1.7390 - \log(n_{20} - 1)}{0.002243}$$

where  $n_{20}$  is the refractive index measure at 20°C. If the refractive index is measured above 20°C, 0.00023 per °C

above 20°C is added before using the above relationship. In Table 3b, the measured moisture content is compared to the calculated value using the Wedmore's relationship. The measured and calculated values compare favourably well. The mean ash content of the honey sample was 0.302% with a range of 0.095 to 0.518%. The Ile-Ife honey sample had particularly low ash content - 0.095%. The mean ash content of U. S. honey is mean 0.169% with a range 0.020 - 1.028%.

**Conclusion:** The TXRF and infrared spectrophotometer techniques were used to measure elemental concentrations and the function groups respectively, present in Nigerian honey samples from five locations in the Southwest and Southeast of Nigeria. Twelve elements - K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Se, Br and Rb - were detected. The honey samples were quite rich in minerals. Potassium was the most abundant trace element in the honey (range 1100 - 2700 ppm). There were no significant differences in the elemental contents of honey from the different locations.

The infrared spectrophotometer results showed the honey sample to be a mixture of many compounds including carboxylic acids, aldehydes, alkynes, nitrites, alkenes and ethers. This result is in accord with many other studies that showed honey to be a mixture of carbohydrates, acids, lipids, proteins, minerals and vitamins.

Other physical properties - colour, pH, refractive index, moisture and ash contents - were also determined. The colours of the honey samples were in the amber to dark shade or 52.00-255.00 range of the Lovibond comparator scale. The pH, refractive index, moisture and ash content values were comparable to equivalent values obtained for U.S. honey.

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