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Research Article

Physicochemical and Infrared Spectroscopic Profiles of Honey Samples from Nine Nigerian States

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Abstract

Although pure honey samples had been characterized in many parts of the World, there is a dearth of information on the physicochemical indices of quality honey in Nigeria. The present study attempted fingerprinting/characterization of honey samples in Nigeria using their physicochemical parameters. Physicochemical parameters of 36 honey samples (including two locally sourced quality honey designated standard) collected from 9 Nigerian states were analyzed for their functional groups and mineral contents using Infrared and atomic absorption spectroscopy. pH, ash and moisture contents, protein, lipid, reducing sugars, total sugar contents, colour, taste and refractive index were also determined. They all had characteristic honey smell and taste. pH and the refractive index of the samples were similar and resembled the quality of standards. The pH ranged from 6.50 to 6.59. The refractive index ranged from 1.480±0.01 to 1.498±0.02. Moisture content in samples was significantly higher in Sokoto and Kogi samples, while colours of the samples were shades of light to dark brown. Fe and Ca contents were higher (538.04 - 194.64 versus 399.03 - 155.18) in Lagos sample compared to the standards while Na, K and Mg contents were lower in the samples (4.92 -69.47 vs 5.32- 28.92)compared to the standards. Ash content ranged from 0.0135 ±0.002 to 0.8251±0.01 in the samples. Infra-red spectroscopy values of the two standard honey samples had maximum peaks (2360.96-2398.65) cm^{-1} for Kanye (Adamawa) and (2360.84- 2398.75) cm^{-1} for Makurdi (Benue) samples. The seven samples had significantly higher maximum peaks compared to the standards. Infrared spectrophotometry showed the honey samples analyzed to be mixtures of compounds such as carboxylic acids, aldehydes, alkynes, nitrites, alkynes and ethers. Samples from 6 out of 9 states analyzed showed purity quality indicating quality orientation among cultivators of honey that may be on the increase in Nigerian cities. The infra-red and atomic absorbance spectroscopy values from this study suggest their possible use in honey fingerprinting which may be helpful in detecting honey adulteration. Some peaks from the spectra did not correspond to known constituents in pure honey and were likely contaminants introduced into the honey samples in during processing not necessarily due to deliberate adulteration of the samples.

Keywords: *Honey, physico-chemical, infra-red, spectroscopy, atomic absorption*

INTRODUCTION

Honey is a natural sweet substance which is produced by honey bees from nectar of blossoms or from secretions of living parts of plants or excretions of plant sucking insects on the living part of plants, which honey bees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature (Mendes *et al*., 1998, Aydin *et al*, 2008). The nutritional benefits of quality honey have been studied by many researchers (Canini *et al*., 2009, McKibben and Engeseth, 2002; Wang and Li, 2011). The benefits include therapeutic and medicinal values such as uses for treating ulcers, kidney problems, asthma, and wound healing among others**.** (Aparna and Rajalakshmi, 1999). Antibiotic properties of honey have also been observed (Tambekar and Rathod, 2007; Hussein *et al*., 2003; Rozaini *et al*., 2005). It is a natural sweetener and a healing agent having a wide range of applications in the food industry in many parts of the world with potential to become a major foreign exchange earner by making value added product of honey (Krell, 1996). Works have been carried out in analyzing different varieties of honey as well as value added products (Khalil *et al*., 2001; Kamal *et al*., 2002; Adebiyi *et al*., 2004; Ahmed *et al*., 2007; Dibyakanta and Mishra, 2011. Some studies on the healing effects and antimicrobial activity of Nigerian honey on burns and wounds have been reported (Adesunkanmi and Oyelami, 1994). Studies had shown honey to contain approximately 80% carbohydrates (35% glucose, 40% fructose, and 5% sucrose) and 20% water, serving as an excellent source of energy, more than 180 substances, including amino acids, vitamins, minerals, enzymes, organic acids and phenol compounds. It is essentially a highly concentrated water solution of two sugars, dextrose and levulose, with small amounts of 22 other complex sugars. Many other substances also occur in honey, but the sugars are by far the major components (Adebiyi, *et al*., 2004). The principal physical characteristics and behavior of honey are due to its sugars, but the minor constituents – such as flavoring materials, pigments, acids, and minerals – are largely responsible for the differences among individual honey types. It is one of the most easily assimilated foods used in baking, cooking, candies, cosmetics and in medicine. In Nigeria honey has different names among different tribes where it is a popular substance. However, as at 2000 at least 171 million people worldwide representing 2.8% of the world population suffer from diabetes (WHO 2000, Wild *et*

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al 2004) and many are Nigerians. Its incidence is increasing rapidly, and it is estimated that by 2030, this number will almost double (WHO 2000, Wild *et al* 2004). This is suggesting an environmental (i.e., dietary) effect, but there is little understanding of the mechanism(s) at present, though there is much speculation, some of it most compellingly presented (WHO 2000, Wild *et al* 2004). One of these is the probability that intake of adulterated honey or processed sugar supposedly consumed as pure honey by people that have metabolism related diseases or those using honey as therapeutic agents might be a contributory factor to the incidence of diabetes in Nigeria, where it is difficult to differentiate between quality and adulterated honey by looking at the honey sample bottle or studying its food and nutrition labels which rarely exist. Also, there is paucity of data on the chemical and physical characterization of varieties in the Nigerian honey (Adebiyi, *et al*., 2004). In Nigeria, natives rely on improvised devices in determination of quality in honey samples. These include insertion of match stick in honey sample, striking the match in order to observe if it lightens, spotting honey gently in a water filled glass cup to observe if it remains at a spot or disperses, allowing a drop of honey to be at a spot where ants gather and observing if the ants avoid the honey spot or not and by simply visualizing its viscosity under gravity. These crude methods are however unreliable. There is a need for characterization of honey in Nigeria, as this may aid our understanding of its chemical composition and applications (Adebiyi et al, 2004)

Therefore, this study was aimed at determining the physicochemical parameters of honey samples on sale as pure honey in major Nigerian States by evaluating their physicochemical parameters and comparing these with two internal standards obtained from reliable local sources which were already matched with the standards prescribed by the Codex Alimentarius (2001) and the European honey directive (Council directive 74/409/EEC, 1974 and standardized by the German Institute of Norms, DIN. 10751-10759) (Bergit *et al*; 2002)..

MATERIALS AND METHODS

Samples: The honey samples were collected from nine major cities across nine States in Nigeria namely- Ondo (Ondo state), Ilorin (Kwara state), Lokoja (Kogi state), Kano city (Kano state), Asaba (Delta state), Mushin (Lagos state), Sokoto (Sokoto state), Makurdi (Benue state) and Kanye (Adamawa state).

Reagents used were Methanol, Folin-ciocalteu's reagent, potassium ferric cyanide, thiobarbituric acid (TBA), trichloroacetic acid (TCA), Biuret reagent, ammonium molybdate, Iron (III) chloride ($Fecl_3$), sodium trioxocarbonate (Na_2CO_3), CH_3COOK, aluminium chloride ($AlCl_3$), hydrochloric acid, dichloroindophenol, dichloroacetic acid, standard glucose, phosphate buffer, acetone, potassium sodium tartarate, copper sulphate (CuSO4), sodium hydroxide (NaOH), chloroform and alkaline copper reagents that were analytical grades an obtained from Sigma, U.S.A. The experiments were carried out at the Biochemistry laboratory, Pharmaceutical-chemistry laboratory in the College of Medicine, University of Lagos, Nigeria and Chemistry laboratory in the University of Lagos, Akoka, from May-August, 2011.

Physicochemical analysis

The honey samples were purchased from popular locations in the 9 States sampled and carried in 75cl plastic bottles. They were stored in the refrigerators

Statistical analysis: All values were expressed mean ± standard error of mean and the statistical significance were analyzed by one way analysis of variance (ANOVA) and Tukey's *pos-thoc* test using the SPSS statistical package (Version 17.0) and Microsoft excel window vista.

RESULTS

The results of the physicochemical analysis of the honey samples evaluated in this study are as presented in Tables 1-4 and Tables 5a- 5i while Figures 1-2 are the infrared spectroscopy of honey samples from Kanye, Adamawa State and Makurdi, Benue State indicating quality standard expected. The analyzed honey samples had characteristic smell and honey taste by organoleptic test while the colours ranged from dark brown, ember, light orange, chocolate to light orange. The trends in the values of other evaluated physicochemical parameters have correlations with Codex Alimentarius accepted standards. For the infrared spectroscopy, Lagos samples had 6 recognizable peaks (1645.44- 3796.06cm^{-1}), Ondo, 10 (911.22-2361.98 cm^{-1}),), Kwara, 5(688.74-2971.98 cm^{-1}),), Kogi, 9 (620.02-3794.2 cm^{-1}),), Kano 10, (813.05-2397.16 cm^{-1}),), Sokoto, 10 (768.59-3794.55 cm^{-1}),), Delta 8, (909.51-2961.74 cm^{-1})), while the two standards- Adamawa 16, (768.75-3796.15 cm^{-1}),) while Benue has 17 (773.57-3795.74 cm^{-1}),) respectively

Table 1.
Methods of analysis of some physicochemical parameters in samples of honey from nine Nigerian states**.**

Test	Methods
Color	Lovibond comparator scale (WEDC, 1980)
Organoleptic	(AOAC, 2000 adapted by Horowitz, 2000)
Ash content (%)	Muffle furnace (AOAC, 2000 adapted by Horowitz, 2000)
Moisture content(%)	(AOAC, 2000 adapted by Horowitz, 2000)
Acidity (%)	(AOAC, 2000 adapted by Horowitz, 2000)
Protein content (%)	Modified Kjeldahl / Dumas (AOAC, 2000 adapted by Horowitz, 2000)
Reducing sugars (%)	(AOAC, 2000 adapted by Horowitz, 2000)
Total sugar content(%)	(AOAC, 2000 adapted by Horowitz, 2000)
Lipid content (%)	(AOAC, 2000 adapted by Horowitz, 2000)
pH	Digital pH meter model HI 8519, Hanna Instruments, U.S.A
Refractive index (%)	(AOAC, 2000 adapted by Horowitz, 2000)
Infrared spectroscopy (%)	IR machine, Buck Model M500 , Buck Scientific, U.S.A
Mineral element (%)	AAnalyst 2000 Agilent Technologies, Model 20000

Table1 2
pH, percentage ash, moisture content and refractive index in honey samples from nine Nigerian States

State	colour	Organoleptic test	pH	Ash content (%)	M moisture Content (%)	R refractive inde x(%)
Lagos	black	characteristic honey smell and taste	6.58±0.01	0.19±0.02	12.40±0.02	1.49±0.01
Ondo	dark brown	characteristic honey smell and taste	6.58±0.02	0.34±0.01	13.90± 0.01	1.49±0.01
Kano	chocolate	characteristic honey smell and taste	6.54±0.00	0.83±0.01	11.30±0.02	1.50±0.02
Kwara	ember	characteristic honey smell and taste	6.58±0.01	0.48±0.02	11.15± 0.03	1.50±0.01
Kogi	light orange	characteristic honey smell and taste	6.59±0.00	0.01±0.00	16.16±0.01	1.48±0.00
Sokoto	light brown	characteristic honey smell and taste	6.55±0.01	0.24±0.02	19.05±0.01	1.48±0.01
Delta	ember	characteristic honey smell and taste	6.55±0.00	0.07±0.01	12.98±0.02	1.49±0.02
Adamawa (Standard)	dark yellow	characteristic honey smell and taste	6.51±0.01	0.20±0.03	13.69± 0.01	1.48±0.01
Benue (Standard)	light brown	characteristic honey smell and taste	6.50±0.01	0.23±0.03	13.53± 0.02	1.49±0.02

Table 3.
Content of lipid, protein, reducing sugars and total sugar content in honey samples from selected nine Nigerian states.

sample	Lipid content (mg/g)	protein content(mg/g)	reducing sugar (mg/g)	total sugar(m g/g)
Lagos	9.0 ±0.00	25.82 ±0.01	33.78 ±0.01	86.99 ±0.02
Ondo	0.85 ±0.01	27.63 ±0.01	83.42 ±0.02	109.12 ±0.00
Kano	4.160 ±0.02	29.54 ±0.00	5.37 ±0.01	34.35 ±0.01
Kwara	7.7 ±0.03	34.13 ±0.01	43.61 ±0.01	101.75 ±0.01
Kogi	6.34 ± 0.02	26.47 ±0.01	159.89 ±0.00	166.30 ±0.01
Sokoto	5.58 ± 0.00	43.91 ±0.01	92.32 ±0.01	80.11 ±0.01
Delta	3.82 ± 0.01	30.84 ±0.02	159.89 ±0.001	178.74 ±0.00
Adamawa (Standard)	4.83 ± 0.03	26.35 ±0.01	132.33 ±0.01	95.62 ±0.01
Benue (Standard)	2.99 ± 0.01	26.65 ±0.00	159.89 ±0.01	178.74 ±0.01

Table 4.
Mineral composition of honey samples from selected states in Nigeria using atomic absorbance spectroscopy.

Sample	K (mg/Kg)	Ca (mg/Kg)	Mg (mg/Kg)	Fe (mg/Kg)	Na (mg/Kg)
Lagos	36.39	194.64	16.62	538.04	32.44
Ondo	13.86	100.93	12.79	210.03	31.99
Kano	12.68	140.35	13.13	218.22	45.73
Kwara	9.13	170.95	4.56	163.48	8.30
Kogi	21.66	209.71	12.80	273.28	4.92
Sokoto	22.59	224.50	13.41	297.22	43.06
Delta	14.50	225.97	12.21	299.25	69.47
Adamawa (Standard)	20.24	155.18	10.12	399.03	28.92
Benue (Standard)	16.43	203.45	29.48	208.77	5.32

DISCUSSION

The characteristic smell and honey taste by organoleptic test indicates that all the samples contain at some quantity of honey although adulterated while gradation in colours observed might be due to weather conditions in sampled states which influence quality. Honey with variety of dark colors such as obtained in Ondo, Lagos and Adamawa are known to be rich in minerals more than the light ones (Tolga et al., 2010) . The pH range indicates that the honey samples on sale in major Nigerian cities are moderately acidic with values agreeing with previous findings (Molan, 1992) and are comparable to the pH values of honeys from U.S (range 3.4-6.1) (Adebiyi et al., 2004). The honey samples having pH in the range obtained in this study therefore, may be safe for consumption since they compared favourably with known quality honey. Apparently, physico-chemical properties of honey differ from one region of the world to another and they are determined by climatic factors and mode of cultivation.

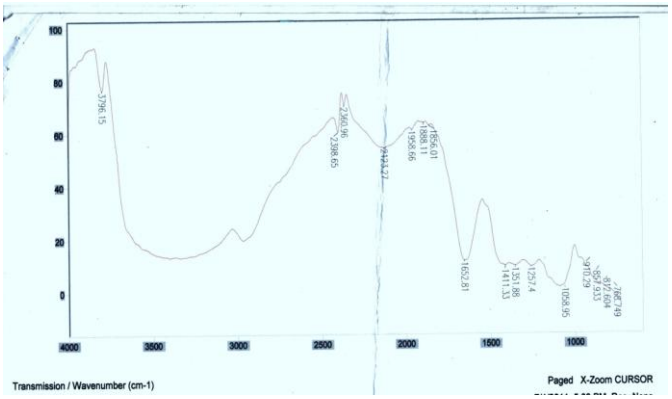

Figure 1:
Infrared spectroscopy of locally sourced pure honey sample from Kanye (Adamawa) Nigeria

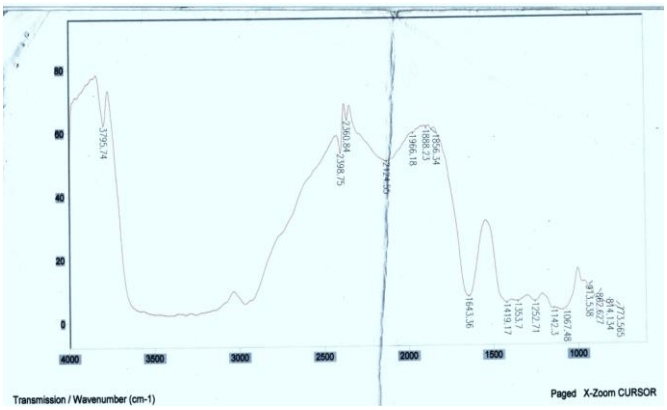

Figure 2:
Infrared spectroscopy of locally sourced pure honey sample from Makurdi (Benue state) Nigeria.

Table 5
Assignment of possible Functional Groups in Honey samples

Honey source	Wave No.	Possible Assignment(s)
Lagos	3600-2500	OH (Hydroxyl group)
	3796.06 (broad)	Carboxylic acids, sugars
	2964.83	CHO , C-H
	2361.59 (broad)	C=C (Alkenes), Alkynes and NO_2) Nitrites
	1645.44	C═O, N-H
Ondo	2361.98	C=C (Alkenes)
	1854.77 (broad)	C═C (str), Alkenes
	1640.91	C═C (str), N-H, C=O
	1418.64-1100	C-C (bending), C-N, C-O (carbonyl group)
	1084.04	C-O (str), Ethers
Kwara	2971.98	CHO (str), Aldehydes
	2403.42	C. C and C-N (str)
	2398.65	Alkynes and Nitrites
	1108.7	C- O (str), Ethers
Kogi	3794.2 (broad)	C. C and C. N (str)
	2400.3	C- O (str), ethers
	1143.99	C – C (bending), C-O (ethers)
	1076.7	C- O (str), ethers
Kano	2397.16	C. C and C. N (str)
	2398.65	Alkynes and Nitrites
	1857.39	C= C (str), Alkenes
	1358.39	C - C (bending)
	1078.54	C- O (str), Ethers
Sokoto	3600-2500	OH (hydroxyl group)
	2360.22	C. C and C. N (str)
	1855.99	C=C (str), Alkenes
	1251.06	C –C (bending)
	1084.69	C-O (str), Ethers
Delta	2961.74	CHO (str), Aldehydes, sp^3 C-H
	2398.09	C. C and C. N (str), Alkynes and Nitrites
	1424.02	C-C (bending), C-O
	1063.54	C- O (str), Ethers
Adamawa	3796.15 (broad)	OH, Carboxylic acids
	2398.65	C. C and C. N (str), Alkynes and Nitrites
	1652.81	C=O (str), Alkenes
	1411.33	C –O (ether)
	1058.95	C- O(str), Ethers; finger print region
Benue	3795.74 (broad)	OH, Carboxylic acids
	2124.55 (broad)	C. C and C. N (str)
	1643.36	C=O (str), Alkenes
	1419.17	C – C (bending)
	1067.48 (broad)	C-O (str), Ethers

Birgit *et al* (2002) noted that the facts that honey is obtainable from variety of floral sources and being a complex product having broad diversity of compounds, color and consistency make the methods of analysis very important on results obtained hence the variations in the many physicochemical parameters analyzed. Ash content in the Kano sample had the highest value with 0.8251±0.01 and this indicates a presence of high mineral content. Kano is located in semi arid region with little annual rainfall which may promote retention of minerals in the soil. The moisture content range of 11.20% to 19.10% in the samples may improve the shelf life. The refractive index ranged from 1.480 -1.498 and this was similar to Adebiyi *et al* (2004). It can therefore be deduced that they have similar flora origin and perhaps same viscosity. The level of acidity was highest in Kano sample (6.9) where mean annual rainfall is low and Kogi sample was the lowest (1.9) and could be due to high mean annual rainfall. The infrared spectrophotometer results showed the honey samples analyzed to be mixtures of many compounds including carboxylic acids, aldehydes, alkynes, nitrites, alkynes and ethers. The Infrared spectra of the samples reveal peaks similar to the two standards except that of Ondo, Kwara and Delta states where carboxylic acids and hydroxyl groups were not detected and this probably due to adulteration rendering these functional groups undetectable.

This result is in accord with many other studies that showed honey to be a mixture of carbohydrates, acids, lipids, proteins, minerals and vitamins (Asif *et al*; 2002; Adebiyi *et al*; 2004). The infrared spectroscopy values are diverse in the samples studied. Some of these peaks represent the impurities present in the honey samples and this might be due to adulteration or impurities introduced into the samples during processing. There was preponderance of hydroxyl group, carboxylic acid group, aldehydes and alkenes in Lagos sample, Ondo has alkenes and ethers as major constituents, Kwara contained aldehydes and ethers, Kogi possess hydroxylgroup, carboxylic acid group, alkynes, nitrites an ethers, Kano sample has aldehydes, alkynes, nitrites, alkenes ethers and othe carbonyl groups, honey sample from Sokoto has hydroxyl group, carboxylic acid group, aldehydes and ethers, Delta honey consist largely aldehyde ethers and other carbonyl compounds, Adamawa indicated presence of hydroxyl group, carboxylic acid group alkenes ethers and other saturated carbon compounds while Benue sample contained hydroxyl group, carboxylic acid group, alkynes, nitrites alkenes, ethers and other saturated carbon compounds. Samples from Benue, Adamawa (standards), Kano, Sokoto and Lagos appeared the closest to pure honey based on the functional group evaluations while Ondo, Kwara and Delta were the foremost inferior honey in quality in the samples analyzed in this study. Interestingly all the three States, Kwara and Ondo are in close proximity to one another the same with Kano and Sokoto, only Lagos is not in close proximity to these states except to Ondo State. The variety of uses to which honey is put makes quality evaluation and control imperative as undertaken in this study.

We conclude that, perhaps, most honey samples on display for sale in Adamawa, Benue, Sokoto and Kano are genuine and of good quality based on their finger prints. Conversely, users of honey from Kwara, Ondo and Delta should be wary of supposedly honey from these States because of the preponderance of adulterated honey from these parts of Nigeria as shown in the study. we assume that the quality of honey samples on sale in Lagos are good largely because it is the main commercial nerve centre in Nigeria, being the former Federal capital, and as such most honey farmers bring their harvest for sale in this city. However further studies on larger scales perhaps on selected local government basis are required to further validate our findings. Furthermore, the use of Infrared spectroscopy with other physicochemical parameters evaluated in this study hold great potentials in the evaluation of honey quality and this can be employed both in the domestic and industrial circles where its products are utilized. This becomes imperative, because long term consumption of adulterated honey containing injurious elements and toxic substances

could be hazardous to health on accumulation and might lead to significant reactions including gastrointestinal disorders, cardiovascular and respiratory problems and allergic reactions all of which are capable negatively affecting the available work force in Nigeria in particular and the World at large.

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