Baseline Analysis of Physico - Chemical Parameters and Anti-Bacterial Activity of Honey obtained from Kano state, Nigeria

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Abstract

Apiculture is now a growing industry in Kano state. Analysis of honey aids our understanding of its properties and applications. Physico-chemical characteristics and antibacterial activity of honey obtained from seven different locations of Kano state were determined using AOAC procedure and agar diffusion method respectively. The results of elemental analysis indicate that honey is quite rich in minerals. K, Na, Ca and Mg have high mean concentrations of 549.96(±0.03); 82.97(±0.05); 236.9(±87); 10.5(±2.8) respectively. The results of physical properties show that honey has high acidity (pH 4.01 ±0.31) and low water content (17.1±1.95). Analysis of glucose, sucrose, total reducing sugar and protein gave mean values (%) of 20.8 (SD ±4.9); 38.5(SD ±8.8); 64.3 (SD ±13.4) and 1.0 (SD±0.3) respectively. This indicates that honey has high sugar content. The samples were assayed for anti- bacterial activity against some bacteria isolated from human feacal samples; P*seudomonas spp, E.coli and S. aerus* and the result shows a mean zone of inhibition of 12mm in both cases. The result however, indicates highest activity at undiluted honey (100%). The indices obtained were within their respective ranges recorded for honey in other regions, with little variations. The honey obtained from the market has some evidence of adulteration.

Keywords: *honey, physico-chemical characteristics, antibacterial activity.*

INTRODUCTION

Honey, a sweet, thick, viscid fluid of agreeable taste and aromatic odor, is collected by the honeybees (genus: *millipera*) from the nectarines of flowers, swallowed, assimilated in their honey-stomachs (crops), regurgitated, stored and thoroughly ripened in the cells of the combs. (Adebiyi *et al.,* 2004; info@honey-health.com, 2005).

Honey is a pure and natural product that does not include any other substance such as water or sweeteners (Wikipedea.com, 2008). Honey has been used by man for many purposes since the dawn of history. It was found in the writings of Hindu many centuries before Christ. It is used in food systems, religious and magical ceremonies as well as in human and veterinary medicines (Tchoumboue *et al.*, 2007). Freshly collected honey is a viscous liquid, has a greater density than water, a strong hygroscopic character, relatively low surface tension and various colors that are basically amber (Wikipedea.com, 2008). It has also been claimed to have therapeutic properties in the treatment of digestive, respiratory, cardiac and rheumatic disorders (info@honey-health.com 2005) and inhibits browning of fruits (Lazaridou *et al.,* 2004). Liquid honey has been found to kill most bacteria by plasmolysis and impedes the development of air borne yeasts. This is attributed to its low water content (Tchoumboue *et al.,* 2007; Anonn, 2003)

For at least 2,700 years, honey has been used by humans to treat a variety of ailments through topical applications, but only recently have the antiseptic and antibacterial properties of honey been chemically explained (www.honey.com).

At a concentration of 40%, honey has a bactericidal effect on various gut bacteria known to cause diarrhoea and dysentery such as *Salmonella*, *Shigella*, enteropathoganic *Escharichia coli* and *Vibrio cholerae*. Undiluted honey inhibits the growth of bacteria such as *staphylococcus aureus,* certain gut pathogens and fungi such as *Candida albicans*. Honey is of value in treating burns, infected surgical wounds and decubitus ulcers. Honey is very viscous, enabling it to absorb water from surrounding inflamed tissues.

High quality honey can be distinguished by fragrance, taste and consistency. Ripe, freshly collected, high quality honey at 20°C (68°F) should flow from a knife in straight stream without breaking into separate drops. After falling down, the honey should form a bead. The honey when poured should form small, temporary layers that disappear fairly quickly, indicating high viscosity. If not, it indicates excessive water content (over 20%) of the product (www.bee-hexagon.com).

The composition and quality of honey depend on several environmental factors during production such as weather and humidity inside the hive, nectar conditions and treatment of honey during extraction and storage. (White, 1975) There are however great variations in the composition and characteristics of honey reflecting its geographical and botanical origin (Tchoumboue *et al.,* 2007). Honey is farmed and used widely in Kano state. Apiculture is now a growing industry in the state. There is however growing concern of adulteration of the by retailers of the product. This is largely in an effort to maximize profit.

The study of the physical and chemical properties of honey aids our understanding of its properties and applications. This study investigated some physical and chemical parameters as well as anti-microbial activity of honey on some selected human bacteria pathogens at various concentrations. The study also compares the properties of honey produced in beekeeping areas in Kano State with honey obtained from the market.

ANALYTICAL PROCEDURE

Collection of Honey Samples

Five honey samples each were collected using pre cleaned glass containers sterilized using Tyndalization method (Mesquita *et al.*, 1998), the samples were collected directly from beehive keepers from 6 locations in Kano state. One sample was obtained from the market (as control). The samples were sealed and preserved by refrigeration at 4°C in laboratory condition to prevent fermentation and possible oxidation. The samples were labeled A to G as follows;

A - Buram village located at Rano LGA
B - Santa Village located at Rimin Gado LGA
C - Kaokiyobawa Village located at Shanono LGA
D - Dan Dabino village located at Bagwai LGA
E - Gawo village located at Gezawa LGA.
F - Shimar Farm (KNARDA) located at Bunkure LGA
G - Sabon Gari Market.

Study Area

The study area is Kano state (figure 1) covers a perimeter extending between latitudes of 120 40' and 100 30' and longitudes of 70 40' and 90 30' (Ahmed, 2008).

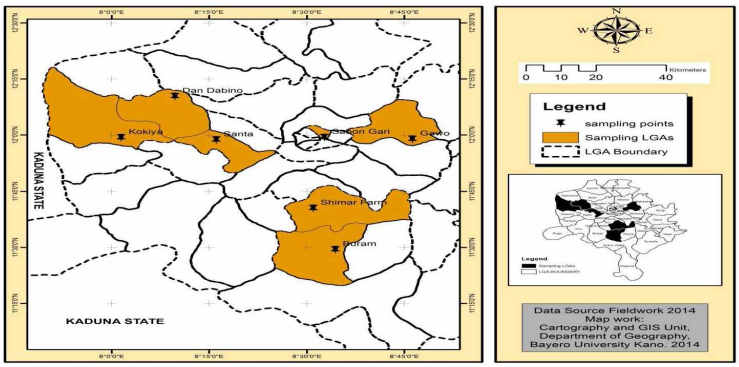

Figure 1. Map of Kano State

Physico - chemical Analysis

Conductivity was measured using conductivity meter (model MC - 1). The density was measured using a mass to volume ratio, by measuring a known volume of the honey in a pre-weighed density bottle Adamu and Jimoh (2008) while viscosity was measured at 25^{o}C using viscosity meter (model DV - E). Surface tension was determined by measurement of capillary pull and capillary rise using molecular weight apparatus (James, and Prichard, 1974) while pH was measured using pH meter (Jenway model 3505). Ashing was done by incinerating the sample in a muffle furnace for 6 hours at 550^{o}C following (AOAC, 1990) procedure. The water content was determined by drying the content at 110^{o}C in an oven for 3 hours, then cooled, weighed and re-dried at 1 hour interval until a constant weight was obtained (Atrooz, *et al.,* 2008).

Mineral Composition

The mineral composition was determined by dissolving the ash residue in 5mL HCl (50%v/v) (AOAC, 1984) and analyzed using Atomic Absorption Spectrophotometer and flame photometer (FAAS and FES). The result was obtained using a calibration curve procedure (Arnold *et al.,* 1985).

Determination of Sugar Content

2g of each sample was diluted with 250 ml water and total reducing sugar determined gravimetrically by drying and weighing method (Owoso *et al.,* 2000). The percentage yield was calculated as follows:

$$\% \text{ Yield} = \frac{\text{Final Yield} \times 100}{\text{Initial Yield}}$$

Fructose and glucose were estimated iodimetrically as explained by Owoso *et al.,* (2000).

$$(\%) \text{ Glucose} = \frac{(\text{cm}^3 \text{ of the thiosulphate} - \text{cm}^3 \text{ of the blank}) \times m \times 100\ 25\text{cm}^3}{25\text{cm}^3}$$

(%) Fructose = % Total Reducing Sugar - % Glucose (AOAC, 1984)

Determination of Protein

Protein content of the honey samples was determined by the formol titration method used for the rapid determination of protein in honey (Pearson, 1976). The protein content of the honey (equivalent to % N x 6.25 from the Kjeldahl method) was obtained as follows: (Pearson, 1976).

$$\% \text{ N} = \frac{1.95 \times (\text{cm}^3 \text{ NaOH sample titre} - \text{cm}^3 \text{ NaOH titre of blank}) \times \text{MNaOH} \times 100}{\text{Volume of sample used} \times \text{volume of water used}}$$

% Protein = % Nitrogen x 6.25 (AOAC, 1990)

Microbiological Analysis

Three different clinical isolates namely, *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* were collected from the Pathology Department, Murtala Muhammad Specialist Hospital Kano and verified at Microbiology Laboratory, School of Technology, Kano state Polytechnic.

The microbial analysis was carried out using agar diffusion method as reported by Harrigan and McCance (1976): 100%, to 0% honeys were used. Five colonies of clinical isolates were inoculated into the test-tubes. Holes of about 8mm wide and 3-4mm high were made after the nutrient agar solidified. 0.5ml of the solutions of the honey were added to each of the hole and allowed to diffuse for 2 hours and then incubated at 37^{o}C for 24-48 hours. The samples were observed for zone of growth inhibition and measured with the aid of transparent ruler in millimeter (mm), (Allen and Molan, 1997).

RESULTS AND DISCUSSION

Elemental Analysis

The result of the elemental analysis of the honey samples indicates that honey is quite rich in minerals with potassium, calcium and sodium being the most abundant with mean values (mg/kg) 549.96$\pm$0.03; 236.90$\pm$87; and 82.97$\pm$0.005

respectively in agreements with the previous reports (Anonn, 2003; White, 1975) (Table 1) except for Iron which has a mean value (mg/kg) of 0.09±0.03 lower than (mg/kg) 3.7-9.2 as earlier reported. This may be due to mixed floral or nectar sources, soil composition and fluctuating environmental conditions. The mineral content of honey is dependent upon its floral and nectar sources as well as soil composition (Anonn, 2003 and info@honey-health.com, 2005). The values for Mg, Mn, Cu and Zn were smaller than values earlier reported (Anonn, 2003; White, 1975). The element with highest content is potassium. This is due to the high levels of potassium in plant tissue. Nutritionally, the presence of these minerals makes honey an excellent food for humans especially for children.

Table 1. Concentration of Minerals in the Honey Samples (mg/Kg)

SAMPLE	Na	K	Ca	Mg	Cu	Mn	Zn	Fe
A	82.98	549.90	255.1	8.14	0.011	0.42	1.12	0.13
B	82.98	549.97	382.5	14.82	0.011	0.46	1.30	0.11
C	82.98	549.99	127.6	7.95	0.011	0.14	1.80	0.11
D	82.97	549.96	255.1	11.57	0.006	0.22	1.21	0.11
E	82.97	549.98	255.1	12.90	0.006	0.28	1.35	0.06
F	***82.97***	549.97	255.1	10.36	0.006	0.33	1.21	0.06
G	82.97	549.98	127.6	7.65	0.006	0.33	1.12	0.11
MEAN	82.97	549.96	236.9	10.50	0.008	0.31	1.30	0.09
SD	±0.005	±0.03	±87	±2.8	±0.03	±0.11	± 0.24	±0.03
Previous reports	11.8-278	296-1935	47.7-341	10.3-136	0.002-3.0	0.5-2.1	1.3-7.8	3.7-9.2

(Anonn, 2003 and White, 1975)

Physical Parameters

Seven physical parameters were analyzed for each of the honey samples; pH, water content, ash content, conductivity, density, viscosity and surface tension (Table 2).

The pH of the honey samples ranged from 3.72- 4.45 with a mean value of 4.01±0.31 and are found to be within the US honey range 3.2-4.5 (White, 1975). Honey is characteristically quite acidic and this quality imparts anti-fermentative, antiseptic and conserving quality to honey and is found to promote healing of wounds by causing oxygen release from hemoglobin. The water content ranges from 14-20% with a mean value of 17%±1.9 in agreement with an earlier report of (14-20.9%) by White, (1975). The water content in sample G is higher than the previously reported values (Anonn, 2003). This may be due to some physical processes such as storage and handling. It is however worth mentioning that honey with excessive water content (above 20%) is not suitable for preservation (info@honey-health.com, 2005).

The ash content ranged from 0.4- 0.8% with a mean value of 0.6±0.2 and is within the value of US honey (White, 1975). Ash represents the direct measure of the inorganic residue after honey carbonization. The variability in the ash content can be explained by the floral origin (Tchoumboue *et al.,* 2007). The conductivity ranged from 0.0046-0.008 µOhms/cm with a mean value of 0.0056-0.0012 µOhms/cm the range is within the literature values reported (Anonn, 2003) for a good quality honey its conductivity must be below 1, that is approaching zero (Tchoumboue *et al.,* 2007).

The mean density, viscosity and surface tension values for all the samples were in agreement with earlier reports (Anonn, 2003, White, 1975). Samples D and F having densities (g/cm^3) 2.02 and 1.80 respectively and are above the reported literature values (1.3-1.58 g/cm^3). The variations in density could be attributed to the botanical and geographical differences in the origin of the honey. Sample G (collected from market) has viscosity of 2.82 Ns/m^2 which is by far below the reported values for tropical honey 5.0 - 6.9 Ns/m^2, (Anonn, 2003; White, 1975). This low value could have been due to effects of dilution which is a form of adulteration (Codex, 1989). The surface tension values (dynes/cm) 45 and 46 obtained for sample D and F respectively were below the reported values (White, 1975). This may be attributed to the differences in the botanical and geographical origin of the honey.

Table 2. Some Physical Parameters for the Honey Samples obtained from Different Areas in Kano State

Sample	pH	Water content %	Ash content %	Conductivity µOhms/cm	Density g/cm^3	Viscosity Ns/m^2	S/tension dynes/cm
A	3.72	18	0.8	0.0048	1.42	4.65	59
B	4.09	16	0.6	0.0050	1.34	6.47	52
C	4.04	16	0.4	0.0080	1.33	4.49	56
D	3.83	14	0.6	0.0052	2.02	5.54	45
E	4.45	18	0.4	0.0055	1.34	4.91	52
F	4.32	**18**	**0.6**	0.0060	1.80	5.13	46
G	3.60	20	0.8	0.0046	1.36	2.82	62
MEAN	4.01	17	0.6	0.0056	1.52	4.86	53
SD	±0.31	±1.9	±0.2	±0.0012	±0.28	±1.12	±6.3
Previous reports	3.2-4.5	14-20.9	0.39-0.9	0.001-0.008	1.36-1.58	5.0-6.9	58-65

(Anonn, 2003 and White, 1975)

Analysis of Sugar

The results of glucose content (Table 3) ranged from 20–35% with a mean value of 25.8 ± 4.9. All the honey samples analyzed were in agreement with US honey standard 22 - 42% (White, 1975). The least value obtained was for samples A and G with a value of 20.8% and 20% which are below the US honey standard 22– 42% (White, 1975). These variations depend upon the floral and botanical origin of honey.

The results of fructose content (Table III) range from 25% – 55% with a mean value of 38.5 ± 8.8%. Samples F and G with values 55% and 25% are between the upper and lower values earlier reported 48% - 27.3% respectively (White, 1975). This may be a possible indication of adulteration. The results of total reducing sugar (Table 3) range from 45% - 90% with a mean values of 64.3% ± 13.4%. Samples F and G have values (90% and 45%) above and below previous reports (60%-75.9%) respectively (White, 1975) (Table 3) which may also be as a result of adulteration. The total reducing sugars content of 90% (sample F) clearly indicates addition of sugar.

Table 3. Concentration of Glucose, Fructose, Total Reducing Sugar and Protein Expressed in (%)

Samples	Glucose (%)	Fructose (%)	Total Reducing Sugar (%)	Protein (%)
A	20.8	39.2	60	1.2
B	26	39	65	1.3
C	25	35	60	1.0
D	27	38	65	1.2
E	27	38	65	0.9
F	35	55	90	1.0
G	20	25	45	0.4
Mean	25.8	38.5	64.3	1.0
S.D	±4.9	±8.8	±13.4	±0.3
Literature Reports	22.0-42	27.3-48	60-75.92	0.2-1.0

ANNON, (2003) and White, (1975)

Microbiological Analysis

Undiluted honey showed zone of growth inhibition of 18mm. 50% concentration showed moderate activity with a zone of growth inhibition of 9mm. whereas there was no any zone of growth inhibition at 0% honey (Table 4). This is in conformity with the work by Musa, (1994) and Jacob, (1992), who also found that bacterial sensitivity to honey, was more in the highest concentration (absolute honey).

Table 4. Antimicrobial Activity of Honey at 0%, 50%, 100% Concentrations of Different Clinical Isolates

Bacteria Isolates	00%	50%	100%
S. aureus	0mm	5mm	10mm
Pseudomonas spp	0mm	9mm	18mm
E. Coli	0mm	8mm	9mm
Key	Diameter ≥ 15mm sensitive	Diameter 3-14mm Intermediate	Diameter ≤ 2mm Resistance

CONCLUSION

The result of the elemental analysis indicates that honey is quite rich in minerals and can be a good source of K, Ca, Na and Mg. The physical parameters analyzed shows that the result of honey samples obtained from Kano state was comparable to equivalent values of honey obtained from other parts of the world. There is however evidence of adulteration for honey sample obtained from the market. Honey should therefore be purchased directly from beehive keepers to avoid adulteration.

Antimicrobial Activities of three clinical isolates revealed that honey has anti-bacterial property which is more pronounced at 100% (absolute) honey concentration. However, the result of the sugar analysis indicates that there is evidence of adulteration in sample G (obtained from the market).

References

Adamu GA(2010). The Use of Larva Oils for the Eradication of the Malaria Vector. Chemsearch Journal of the Chemical Society of Nigeria, Kano State Chapter, Bayero University, Kano. 1(1): 16-18

Adebiyi FM, Akpan I, Obiajunwa EI, Olanyi HB(2004). Chemical/Physical Characteristics of Nigerian Honey. Pak. J. Nutrition. 3 (5): 278-281.

Ahmed K(2008). The Kano Physical Environment. http://www.kanostate.net/physical.html

Allen KL, Molan PC(1997). The sensitivity of Mastits-causing Bacteria to the Antimicrobial Effect of Honey. New Zealand J. Agric Res. 40: 537-540.

ANONN(2003). Honey Research: Honey–Health and Therapeutic Qualities. The National Honey Board. Http://Www.Nhb.Org/Download/Factsht/Compendium.

AOAC(1984). Official Method of Analysis, Association of Official Analytical Chemist, Washington D.C. 2(44): 22 - 33

AOAC(1990). Official Method of Analysis, Association of Official Analytical Chemist, Washington D.C. Pp. 51 -53

Arnold EG, Rhodesr TL, Clescerilenores WPCF(1985). Standard Methods for the Examination of Water and Waste Water 16th Edition. Pp. 211 – 213.

Atrooz OM, Al- Sabayleh MA, Al-Abbadi SY(2008). Studies on Physical and Chemical Analysis of Various Honey Samples and their Anti Oxidant Activities. Pak. J. Biol. Sci. Pp. 1338 – 1342.

Codex Alimentarius Commission (1989). Codex Standards For Sugars (Honey). Supplement 2 To Codex Alimentarius Volume III. Food and Agriculture Organization of the United Nations and World Health Organization, Rome.

Dustmann JH(1979). Anti Bacterial Effect of Honey. Apiacta 14, 7-11. Honeybee and Uses of Honey: Retreated From Http:En.Wikipedia.Org/Wiki/Honey.

Harigan WF, McCance ME(1976). Method for the Determination of Antibacterial Activity of Honey. Pp. 65-70.

info@honey-health.com (2005). Healthful Honey, Michigan USA Downloaded 23rd August, 2010.

James AM, Prichard PE(1974). Practical Physical Chemistry, Third Edition Longman Group Ltd Essex. Pp. 220-310

Mesquita JAM, Teixeira MA, Brandao SCC(1998). Tyndalization of Honey "Sample Containers". J. Anim. Sci. Pp. 11-21.

Musa WD(1994). Further Investigation of Antibacterial Effects of Honey. Pp. 1-23.

Owoso OO, Aluko A, Banjoko OI(2000). Manual of Food Analysis and Quality Control, Concept Publication Lagos. Pp. 16-29

Pearson D(1976). The Chemical Analysis of Food. Edinburgh, Churchill living stone. Pp. 30-33.

Tchoumboue J, Awah-Ndukum J, Fonteh FA, Delphine PJ, Mvondo ZA(2007). Physico-chemical and Microbiological Characteristics of Honey from the Sudano-Guinean Zone of West Cameroon. Afr. J. Biotechnol. 6 (7): 908-913.

White JW(1975). "Composition of Honey" in: Honey: A Comprehensive Survey (Ed. Crane E); Heinemann, London. Pp. 157-206.

www.bee-hexagon.com Last accessed June, 2010.

www.beesource,2001 Last accessed August, 2010.

www.honey.com,2008 Last accessed July, 2010.

www.wikipedea.com, (2008) – Downloaded 20th July, 2008.