



(RESEARCH ARTICLE)



Physicochemical Analysis of Honey Produced in and Around Anambra State Polytechnic (ANSPOLY), Mgbakwu, Nigeria

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International Journal of Science and Research Archive, 2024, 12(02), 1926–1932

Publication history: Received on 25 July 2024; revised on 06 August 2024; accepted on 08 August 2024

Article DOI: <https://doi.org/10.30574/ijrsra.2024.12.2.1444>

Abstract

Physicochemical properties of honey produced in and around Anambra State Polytechnic, Mgbakwu, Nigeria were studied with the aim of determining their physical properties, biochemical properties and trace element content. Honey samples were characterized by pH, colour, temperature, viscosity, density, specific gravity, conductivity, moisture content and ash content measurements, and spectrophotometric methods. Honey samples collected from ANSPOLY, Ugbene, Ebenebe, Urum and Achala showed respective conductivity values of 24.557 ± 0.793 , 34.67 ± 0.480 , 23.67 ± 0.315 , 24.80 ± 0.09 and $34.029 \pm 0.43 \mu\text{s}/\text{cm}$, with observed ash contents of 0.247 ± 0.011 , 0.554 ± 0.020 , 0.289 ± 0.012 , 0.295 ± 0.001 and $0.589 \pm 0.03\%$. Moisture content ranged from 25.018 ± 0.040 to 24.063 ± 0.025 which are attributable to floral origin of the honeys. Sucrose was found to range from 0.868 ± 0.016 to 0.954 ± 0.002 , while pfund readings of 6.202 ± 0.417 to $7.090 \pm 0.112\text{mm}$ affirm the "water white nature" of the honey samples. All honeys studied are nectar honey with good composition of trace elements.

Keywords: Nectar honey; Trace elements; Sucrose; Ash content; water content

1. Introduction

Honey is the natural sweet substance produced by honeybees from the nectar of blossoms or from the secretion of living parts of plants or excretion of plant sucking insects on the living parts of plants, which honeybees collect, transform and combine with specific substances of their own, store and leave in the honeycomb to ripen and mature (Codex Alimentarius Commission, 1994). Different kinds of bee honey vary considerably in their physical, chemical and organoleptic properties, so the composition of honey is rather variable, depending on its geographical floral origin, season and other external factors, such as environmental factors and treatment of beekeepers (EL-Metwally, 2015). Honey consists essentially of sugars, predominantly fructose (40-50%) and glucose (32-37), small amount of sucrose (<2%) and mineral constituents (ash less than 0.1%). Honey also contains water (13-20%), other substances such as organic acids, enzymes, vitamins in small amounts, trace elements (Fe, Cu, Zn, Sn etc.), and solid particles, mainly consisting of pollen (Alvarez-Suarez *et al.*, 2013). According to Bastos and Alves, (2003), honey flavor and aroma are determined by volatile compounds includes alcohols, aldehydes, ketones, esters and acids. Nevertheless, depending on the source(s) of nectar, geographical origin, seasonal and environmental factors and handling techniques, honey can have a variable chemical composition (Alvarez-Suarez *et al.*, 2010). Moreover, research finding indicate that honey-making processes are profoundly related to enzymes included by the bees so that composition of honey is additionally influenced by the sorts of bee species (Kek *et al.*, 2017). Based on its entomological derivation, honey may be from the species *Apis mellifera*, which is the most broadly known and commercially accessible around the world, or from meliponines, which are commonly known as stingless bees without sting, or native bees (Temaru *et al.*, 2007). This work is aimed to study the physicochemical properties of honey produced in and around Anambra State Polytechnic, Mgbakwu, Nigeria.

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2. Material and methods

2.1. Study Area

This study was carried out in selected communities in Awka North Local Government of Anambra State, Nigeria. Awka North Local Government is located on Latitude: 6.0333 ° N and Longitude: 7.0667 ° E

2.2. Collection of Samples

Five honey samples were purchased directly from different farmers in and around Anambra State polytechnic, Mgbakwu in Awka North LGA, Anambra State, Nigeria. The samples were filtered in the laboratory with sterile seitz filter with a pore size of 0.02mm connected to an electronically operated vacuum pump. This was done to remove particles. The honey filtrates whose temperatures were already taken at the point of collection were stored in brown bottles at room temperature prior to use.

2.3. Determination of Physicochemical Parameters

The physicochemical parameters will be determined in accordance with the procedures outlined in the standard method for the examination of water and waste water (AOAC, 1999; APHA, 1995).

2.3.1. Determination of Temperature

50mL of each of the samples was measured and separately transferred into beakers; a laboratory thermometer was inserted to determine the temperature of the individual sample. This was determined at the point of sampling, with readings on the thermometer appropriately recorded.

2.3.2. Determination of Electrical Conductivity

20g of honey was dissolved in 100mL of distilled water and mixed thoroughly to form solution. Clean electrode of electrical conductivity meter (HANNA instrument USA made in Romania. HI 98129) was immersed and the readings taken. The instrument was calibrated using potassium chloride (KCl) dissolved in de-ionized water.

2.3.3. Determination of Density

The pycnometry method was used to determine densities of the honey samples using the expression below

$$P = \frac{(W_2 - W_1)g}{V(cm^3)} \dots\dots\dots (i)$$

Where: W_1 = mass of the pycnometer when empty; W_2 = mass of the pycnometer filled with honey sample; V = volume of the pycnometer.

2.3.4. Determination of pH

pH values of the honey samples were measured using a pH meter (HANNA instruments HI 96107 model, made in Italy), from a solution containing 20g of honey in 75mL of de-ionized water. The readings were recorded for each and every honey sample.

2.3.5. Determination of Moisture Contents

5g of each sample was placed into a pre-weighed aluminum drying dish. The sample was dried to constant weight in an oven at 105°C for four hours under vacuum. The moisture content was calculated for each honey sample

$$\text{Moisture content} = \frac{M_1 - M_2}{M_1 - M_0} \dots\dots\dots (ii)$$

Where: M_0 = weight of aluminum dish; M_1 = weight of the fresh sample + dish; M_2 = weight of the dried sample + dish

2.3.6. Determination of Colour

Determination of colour was carried out using a colour comparator. Clear blanks were placed in compartments, 1, 3, 5 of the comparator, the honey samples will also be placed in compartment 2 or 4 of the comparator. The comparator was held at a convenient distance from the eye and viewed through diffused light. The samples were moved from compartment to compartment until the samples matched the standard.

2.3.7. Determination of Specific Gravity

The specific gravity was obtained as the ratio of weight of sample to that of equal volume of water.

$$SG = \frac{W_{sb} - W_b}{W_{wb} - W_b} \dots\dots\dots (iii)$$

W_{sb} = weight honey + bottle, W_{wb} = weight of water +bottle, W_b = weight of bottle

2.3.8. Determination of Viscosity

The viscosity of the honey samples was determined using viscometer (Brookfield viscometer (model RVDVE serial No.8488113). The viscosity was recorded in centipoises (CP).

2.3.9. Ash content determination

20mL of each honey samples were weighed into crucible. The honey samples were charred on a Bunsen flame until samples turned black, dried and with no trace of foam. The sample were then ashed in a furnace at 600°C and cooled in desiccator until constant weights were obtained (AOAC, 2000).

$$\% \text{ Ash} = \frac{(\text{Weight of crucible} + \text{ash}) - (\text{Weight of empty crucible}) \times 10}{\text{Sample weight}} \dots\dots\dots (iv)$$

2.3.10. Citric Acid Estimation

0.5 mL of honey sample was be mixed in 50ml distilled water and titrated with 1M NaOH using 0.5 mL of dilute phenolphthalein as indicator.

$$\text{Factor} = \frac{\text{Titre value} \times \text{equivalent weight} \times 10}{\text{Weight take}} \dots\dots\dots (v)$$

2.3.11. Lactic Acid Estimation

1mL of honey sample was mixed in 10mL distilled water and 20ml of 1M NaOH in a volumetric flask. This was stocked with a stopper and allowed to stand for 30min. 0.5ml dilute phenolphthalein solution was added as indicator and titrated with 1M HCl.

$$\text{Factor} = \frac{\text{Titre value} \times \text{equivalent weight} \times \text{factor} \times 10}{\text{Weight take}} \dots\dots\dots (vi)$$

2.3.12. Determination of riboflavin (Vitamin B₁₂)

The honey samples were diluted in buffer solution of pH 4 in order to obtain between 10 and 20 µg/ml B₂. The sample was read in a spectrophotometer at 445 nm with A₁ % 1cm = 308

$$\text{Riboflavin concentration (mg/ml)} = \frac{(\text{Absorbance} \times 100)}{(\text{A}_1 \% 1\text{cm})} \dots\dots\dots (vii)$$

2.3.13. Sucrose and Glucose

1.0 mL of 1.0mg/mL of honey solutions in water were mixed with 1.0mL of 3,5-dinitrosalicylic acid (DNSA) (Sigma Aldrich, USA), the solutions were then warmed in water bath for 10 minutes, and the absorption of resulting reddish-orange colour solutions were measured spectrophotometrically at 540nm. Glucose (Merck, Germany) was used as standard. Sucrose content in each honey sample was measured by refractometric method using 20% (w/v) solution.

2.3.14. Trace Elements Analysis

5g of each honey sample was separately weighed in 100ml beaker. 25ml of nitric, 10ml of perchloric acid was added and heated to mineralize organic materials. The mixtures were heated to near dryness. The acid clear solutions were transferred to 50ml volumetric flasks and diluted with deionized water. Three replicates were analyzed per sample. Elements; P, Al, Fe, Mn, Zn, Cu, Ca, Mg, Na and K were determined by emission measurements obtained from direct nebulization in inductively coupled plasma optical emission spectrometer.

2.4. Statistical Analysis

All statistical analysis were performed use Excel and all experiments were carried out in triplicate. Results were expressed in the form, mean \pm standard deviation.

3. Results and discussion

pH values obtained for the honey samples tested ranged from 3.940 \pm 0.218 to 4.633 \pm 0.153. This is fairly within the standard pH range for natural honey (Codex Alimentations, 2001). Honey sample obtained from Achala proved to be most acidic, with honey sample produced in the demonstration farm of Anambra State polytechnic (ANSPOLY) having highest pH value (table 1). Exceptionally high acidity of the honey sample collected from Achala could be attributed to fermentation of sugars into organic acids (Bogdanov *et al.*, 2008). The honey samples collected from ANSPOLY, Ugbene, Ebenebe, Urum and Achala showed respective conductivity values of 24.557 \pm 0.793, 34.67 \pm 0.480, 23.67 \pm 0.315, 24.80 \pm 0.09 and 34.029 \pm 0.43 μ S/cm, which correlates the observed ash contents of 0.247 \pm 0.011, 0.554 \pm 0.020, 0.289 \pm 0.012, 0.295 \pm 0.001 and 0.589 \pm 0.03% as were also obtained for the honey samples (Vorwhol, 1984a). Observed electrical conductivity of all samples falls within standard limit for nectar honey (Bogdanov *et al.*, 2017). While ash contents of ANSPOLY, Ebenebe and Urum honey samples fall within the standard limit, ash contents of Ugbene and Achala honey samples were found to be slightly higher than the standard limit (Codex Alimentations, 2001). This variation in ash content could be attributed to differences in composition, botanical and geographical origin of honey (Buba *et al.*, 2013). The observed moisture content of analyzed honey samples ranged from 25.018 \pm 0.040 to 24.063 \pm 0.025. These values are well above the standard limit as reported by Codex Alimentation, (2001). These high values aside the effects of geographical floral origin on honey quality, could be attributed to the prevalent humid weather condition in the season of harvest (El Sohaimy *et al.*, 2015). The specific gravity and density of the honey samples were also to fall within the standard limit (Codex Alimentation, 2001). The measured colours of the honey samples ranged from pfund readings of 6.202 \pm 0.417 to 7.090 \pm 0.112mm. From these values, the honey samples could be categorized “water white” honeys (Salas and others, 1993). The water white nature of honey produced in and around Anambra State Polytechnic, Mgbakwu could be attributed to its mineral content and morphology (Terrab *et al.*, 2004b).

Table 1 Physical Properties of Honey

S/N	Physical Properties	ANSPOLY	Ugbene	Ebenebe	Urum	Achala
1	Temperature($^{\circ}$ C)	32.7 \pm 2.138	30.710 \pm 0.08	34.0 \pm 1.840	30.4 \pm 3.038	32.6 \pm 0.18
2	Colour	6.733 \pm 0.305	6.400 \pm 0.132	6.202 \pm 0.417	5.902 \pm 0.231	7.09 \pm 0.112
3	pH	4.633 \pm 0.153	4.52 \pm 0.021	4.271 \pm 0.111	4.304 \pm 0.160	3.940 \pm 0.218
4	Density(g/ml)	1.404 \pm 0.012	1.334 \pm 0.02	1.000 \pm 0.014	1.411 \pm 0.002	1.452 \pm 0.01
5	Viscosity(mpa.S)	47.20 \pm 1.117	41.52 \pm 4.120	48.09 \pm 0.048	47 \pm 0.005	46 \pm 0.047
6	Specific Gravity	1.179 \pm 0.017	1.361 \pm 0.005	1.287 \pm 0.011	1.399 \pm 0.044	1.454 \pm 0.007
7	Conductivity(μ S/cm)	24.557 \pm 0.793	34.67 \pm 0.480	23.67 \pm 0.315	24.80 \pm 0.09	35.34 \pm 0.060
8	Moisture Content%	24.063 \pm 0.025	24.029 \pm 0.146	25.018 \pm 0.040	24.034 \pm 0.200	24.029 \pm 0.43
9	Ash Content%	0.247 \pm 0.011	0.554 \pm 0.020	0.289 \pm 0.012	0.295 \pm 0.001	0.589 \pm 0.03

Biochemicals which include sucrose, vitaminB₁₂, citric acid and lactic acid contained in the honey samples were also determined. Sucrose was found to range from 0.868 \pm 0.016 to 0.954 \pm 0.002, which falls within the standard limit specified by Codex Alimentations, (2001). The slight deviation in the amount of lactic acid, citric acid, glucose and vitaminB₁₂ found in honey samples from ANSPOLY, Ugbene, Ebenebe, Urum and Achala, and from the standard limit for

these biochemicals could be attributed to effects of variation in composition and characteristics of honey due to geographical and botanical origin of the nectar (Joseph *et al.*, 2007).

Table 2 Biochemical Properties of Honey

S/N	Biochemical Properties	ANSPOLY	Ugbene	Ebenebe	Urum	Achala
1	Lactic Acid (%)	2.5±0.002	2.7±0.010	2.9±0.001	2.5±0.003	2.4±0.011
2	Citric Acid (%)	14.3± 0.021	14.5±0.004	14.7±0.014	14.4±0.020	13.9±0.007
3	VitaminB ₁₂ (mg/l)	0.622±0.001	0.656±0.001	0.648±0.004	0.627±0.012	0.610±0.003
4	Sucrose(mg/l)	0.954±0.002	0.942±0.010	0.962±0.003	0.868±0.016	0.874±0.008
5	Glucose(mg/l)	21.0±0.912	23.55±0.576	18.44±1.002	19.75±1.246	22.84±0.872

Trace elements in honey samples studied in this work which ranged from phosphorous(P), Aluminum (Al), Iron(Fe), Manganese(Mn), Zinc(Zn), Copper(Cu), Calcium(Ca), Sodium(Na), Potassium(K) to Magnesium (Mg) differ widely from one location to another around the polytechnic as presented in table 3. This difference in mineral composition of produced in and around the polytechnic could be dependent upon the type of soil in which the nectar bearing plant was located (Anklam, 1998).

Table 3 Trace Elements in the Honey

S/N	Trace Elements (ppm)	ANSPOLY	Ugbene	Ebenebe	Urum	Achala
1	Phosphorous(P)	3.779±0.324	3.288±0.017	3.412±0.142	3.197±0.091	3.518±0.007
2	Aluminium (Al)	0.010±0.003	0.007±0.002	0.021±0.001	0.005±0.000	0.008±0.001
3	Iron (Fe)	2.499±0.013	2.385±0.074	2.281±0.006	2.501±0.040	2.486±0.051
4	Manganese (Mn)	0.089±0.009	0.094±0.005	0.079±0.009	0.101±0.006	0.088±0.004
5	Zinc (Zn)	0.457±0.021	0.340±0.003	0.641±0.041	0.399±0.033	0.446±0.005
6	Copper (Cu)	0.261±0.009	0.176±0.010	0.098±0.050	0.301±0.008	0.274±0.010
7	Calcium (Ca)	10.472±0.882	7.166±0.193	10.271±0.060	8.468±0.100	7.894±0.009
8	Sodium (Na)	14.708±0.587	14.081±0.262	13.942±0.560	15.081±0.44	14.544±0.37
9	Potassium(K)	5.110±0.211	6.095±0.544	6.102±0.420	6.333±0.021	5.112±0.201
10	Magnesium (Mg)	6.370±0.089	6.110±0.049	6.446±0.020	6.780±0.030	5.982±0.050

4. Conclusion

The study focused on determining the physicochemical properties of honey produced in the demonstration farm of Anambra State Polytechnic, Mgbakwu and selected communities in Awka North Local Government Area. The study revealed that honeys produced in this region are nectar honey with good trace element composition. The study concludes that the observed high moisture content of analyzed honey samples, ranging from 25.018±0.40 to 24.063±0.025% were due to the prevailing humid weather condition in the area at the season of harvest. Slight deviations in the amount of lactic acid, citric acid, glucose and vitamin B₁₂ found in the honey samples are due to botanical origin of the nectar. Low ash content values depict the overall quality of honey samples studied.

Compliance with ethical standards

Acknowledgement

The Principal Researcher (Christian Chinedu Achonye) wishes to gratefully acknowledge the funding granted by the Tertiary Education Trust Fund (TETFund), Nigeria for carrying out the research through Institution Based Research (IBR) Interventions.

Disclosure of conflict of interest

Authors hereby declare that no conflicting interests exist

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