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QUALITY ASSESSMENT OF SELECTED HONEY BEE SPOT AND BRANDED HONEY PRODUCTS IN ENUGU METROPOLIS

Kolawole Oladimeji Olubusayo[1], Ekele Dinneya-Onuoha[2], Vivian Chinekwu Onwujiogu[3], Okorafor-Nwosu Adaku[1], Anigbogu, Chinenye Bibiana[4], Ogburubi Orieoma Ibeuro[5]

[1]Projects Development Institute (PRODA), 01609, Enugu State

[2]Department of Pure and Industrial Chemistry, University of Nigeria, Nsukka, Enugu

[3]Alnak Paints and Chemicals, Emene, Enugu State

[4]Department of Food Science and Technology, Nnamdi Azikiwe University, Awka, Anambra State

[5]Department of Food Technology, Akanu Ibiam Federal Polytechnic, Uwana, Ebonyi State.

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Abstract*: Honey is a natural, sugary, sticky and nutritious liquid product produced from the nectars of flowers by honey bees. Its composition depends highly on the flowers utilized by the bee as well as the climatic conditions. This study was intended to evaluate the physicochemical and selected concentration of heavy metals in ten honey samples. The physicochemical properties of the samples (moisture, pH, Ash, Sucrose, Insoluble material, acidity (free, total and lactone), electrical conductivity, reducing sugars and hydroxymethyl furfural) were determined. The concentration of heavy metals (Zn, Cu, Mn, Cd, Cr and Pb) were also determined using on atomic absorption spectrophotometer. The moisture shows value range from 13.90 to 19.04%. The water insoluble material content ranged from 0.014 to 0.710g/100g. The reducing sugars were between 66.83 to 88.48%, mean percentages of apparent sucrose ere 8.96%, Honey samples showed an appropriate HMF (hydroxymethy furfural content ranging from 6.77 to 36.42 mg/kg. The ash content ranged from 0.099 to 0.600%. Conductivity values ranged from 15.0 to 536µ/cm. All honey samples were acidic, having a pH in the range 3.75 to 4.78. The average values for free acidity were between 10.0 to 26mg/kg. Lactonic acidity ranged were 2.5 to 5.3meg/kg. The total acidity ranged from 12.10 to 30.0 meg/kg. The concentration of heavy metals Zn, Cu, and Mn became the highest values than the other metals and the concentration of Pb and Ni in all samples were below detection limits.

***Keywords:* Honey bee, Metals, Enugu honey, Physico-chemical properties, FAAS.**

1. INTRODUCTION

Honey is the natural sweet substance produced by honey bees from the nectar of plants or from honey dew (CAC, 2001). Honey is one of the most widely required products due to its unique nutritional and medicinal properties (Afzal *et al.*, 2014). Honey bees gather the material, transform by combining with particular substances in their own, deposit, dehydrate, save and leave within the honey comb to grow up and mature. Extracted honey is a gelatinous liquid food stuff containing a member of nutritiously important complementary elements (Birhanu, 2015), such as carbohydrates, maltose, sucrose, fructose, glucose, trace metal, organic and inorganic substances and water (Oana and Hortensia, 2016). High concentrations of these trace toxic elements in honey may result to minimized quality set by regular control of food

(Reshma *et al.*, 2016). In ancient time, the golden yellow liquid honey is processed for its medicinal properties (Razzagh and Bahare 2015). The heavy metals found in the environment can be deposited at the hairy bodies of bee hive, flower, herb and water (Ibrahim *et al.*, 2011). The toxic metals in the human body causing side effects, so honey quality is determined by the specific elemental content (Marcovecchio *et al.*, 2007). Honey consists essentially of sugars, predominantly fructose (40-50%) and glucose (32-37%, small amounts 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 (Alwarez-Suaresz *et al.*, 2013). In order to produce and improve the quality of honey to meet the demands of international markets and quality criteria (ACA, 2001), information about the quality of honey produced in the area is important.

The aim of the present work was to determine the quality of honey from Enugu in terms of physicochemical properties and heavy metals, and verify their compliance with national and international standards.

2. MATERIALS AND METHODS

Sample collection

Samples (250g) of honeys in Enugu were collected from beekeepers while the remaining five were bought from store as branded products. The samples were stored in a refrigerator in airtight plastic containers until analysis.

Physicochemical Analysis

Moisture content

A Sartorius moisture analyzer MA-300000V3 (Gottingen, Germany) was used to determine the moisture content of the honey samples following the procedure of the instruction manual. The moisture analyzer was warmed up for at least 30 minutes; approximately 2 gram of sample was evenly spread on the tarred aluminum pan. Analysis was performed in the fully automated mode at 105^{o}C.

HMF

The determination of hydroxylmethylfufural in honey was based on the original method of Winkler (Bogdanor, 2009). To aliquot parts of a honey solution, solutions of p-toluidine and barbituric acid were added and the resultant colour was measured against a blank in 1cm cuvettes at 550nm.

Apparent Reducing Sugars and Apparent Sucrose

Apparent reducing sugars are defined as the sugars that reduce a Fehling' solution under specified conditions. Reducing sugar content was determined by titration of a modified Fehling's solution at its boiling point against a solution of honey. Methylene blue was used as the internal indicator. The apparent sucrose content was calculated by multiplying difference of percent invert sugar values by 0.95 and expressed as grams, apparent sucrose per 100 g honey (Bogdanor, 2009).

pH, Free Acidity, Lactones and Total Acidity

The pH was measured on a 10% honey solution. The free acidity was obtained by plotting the neutralization curve with a sodium hydroxide solution and determining the pH of the equivalence point (pHe). The acidity of the lactones was obtained by adding an excess of sodium hydroxide to the honey solution and plotting the neutralization curve of the excess sodium hydroxide by a back titration with sulphuric acid (Bogdanov, 2009).

Total Ash

Ash determination was carried out according to AOAC (2011) procedure. Two grams of sample was placed in silica dish had been ignited for 6 h, cooled in a dessicator and weighed. The dish and sample was ignited first gently and then at 550 ^{o}C in a muffle furnace (Thermotec TIC-400) for 3 h, until a white or grey ash was obtained, the dish and content was cooled in a desiccator and weighed.

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Insoluble Ash

The insoluble ash was collected on a crucible of specified pore size and the dried residue was weighed after being washed from of soluble material (Lord *et al.*, 1988).

Conductivity

The electrical conductivity of the samples was carried out with the aid of conductivity meter

Heavy metal analysis

Honey samples were heated at 65^{o}C on a water bath until liquefy to allow easier handling and have more uniform distribution (Adane, 2015). The samples were then cooled and weighed for the wet digestion. Exactly 12mL of acid mixture (3:1 ratio of perchloric and Nitric acid) were added in 1000 mL conical flask containing 1g of honey samples. The flasks were heated until the manufacturing of red nitrous gas ceased. The content was brought down to room temperature and 20 mL of deionized water was added. After cooling, the mixture was made up with deionized water and filtered with Whatman Number 1 fitter paper and stored for AAS analysis.

Statistical analysis

Statistical analysis was performed using. Excel Software, all the experiments were operated in triplicate. Values are expressed as mean ± standard deviation.

3. RESULTS

The measured values of physicochemical properties of honeys are shown in Table 1; while the concentration of heavy metals in honey samples are shown in Table 2 below.

Table 1: Physicochemical properties of honey samples

Sample Code	Mc (%)	Ash (%)	Insoluble material (g/100g)	Reducing sugar (%)	Apparent Sucrose (%)	HMF (mg/kg)	Conductivity (µS/cm)	Free acidity (meq/kg)	L/acidity (meq/kg)	T/acidity meq/kg)	pH
UNN Vendor	19.04 ±0.51	0.099 ±0.03	0.051 ±0.02	72.63 ±0.31	6.20 ±0.14	8.90 ±0.26	15.0 ±0.57	21.0 ±0.64	5.3 ±0.67	17.3 ±0.84	3.75 ±0.07
CAN	13.90 ±0.61	0.598 ±0.05	0.710 ±0.04	76.21 ±0.31	5.61 ±0.23	13.72 ±0.11	24.0 ±0.62	16.0 ±0.61	2.0 ±0.14	12.4 ±0.37	3.93 ±0.03
SCORL	14.05 ±0.33	0.100 ±0.01	0.056 ±0.01	66.83 ±0.35	9.40 ±0.20	7.43 ±1.3	21.0 ±0.52	10.0 ±0.22	2.8 ±0.32	15.1 ±0.42	4.78 ±0.05
PPM	14.00 ±0.20	0.099 ±0.02	0.014 ±0.06	71.64 ±0.41	9.03 ±0.31	6.77 ±0.18	301.0 ±1.13	13.0 ±0.10	4.0 ±0.12	21.6 ±0.23	4.33 ±0.02
OGM	15.65 ±0.11	0.316 ±0.03	0.480 ±0.01	69.20 ±0.30	2.73 ±0.41	28.22 ±0.43	18.0 ±0.53	26.0 ±0.53	2.4 ±0.21	28.8 ±0.39	4.16 ±0.03
AFO	15.25 ±0.29	0.299 ±0.05	0.036 ±0.02	88.48 ±0.26	6.18 ±0.11	23.48 ±0.33	113.0 ±0.75	15.0 ±0.27	5.0 ±0.15	24.1 ±0.62	3.84 ±0.03
SIL	18.14 ±0.54	0.600 ±0.03	0.059 ±0.03	75.03 ±0.47	9.06 ±0.33	31.62 ±0.12	70.0 ±0.34	14.0 ±0.36	2.7 ±0.26	30.0 ±0.65	3.65 ±0.02
ZOZ	19.04 ±0.15	0.600 ±0.01	0.040 ±0.06	82.11 ±0.29	8.35 ±0.36	14.93 ±0.16	290.0 ±0.81	18.0 ±0.41	4.3 ±0.21	12.1 ±0.31	4.29 ±0.05
NSK	18.15 ±0.15	0.598 ±0.04	0.330 ±0.03	78.55 ±0.10	8.69 ±0.26	36.42 ±0.23	484.0 ±1.48	13.0 ±0.10	3.0 ±0.18	26.3 ±0.39	4.11 ±0.03
UNLB	19.04 ±0.33	0.300 ±0.02	0.671 ±0.01	75.31 ±0.22	6.48 ±0.31	14.03 ±0.17	536.0 ±2.41	24.0 ±0.18	3.2 ±0.17	18.0 ±0.29	3.90 ±0.02

Values are means ± standard values across the column with different superscripts are insignificantly different (p>0.05).

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Table 2: Concentration of heavy metals in honey samples (ppm)

Sample	Zn	Cu	Mn	Cd	Cr	Pb	Ni
UNN vendor	2.11±0.01	2.70±0.01	0.93±0.00	0.03±0.00	0.28±0.02	ND	ND
CAN	1.72±0.01	2.01±0.01	1.04±0.00	0.08±0.00	0.41±0.01	ND	ND
SCORL	2.02±0.00	1.84±0.05	1.00±0.01	0.09±0.04	0.45±0.01	ND	ND
PPM	2.07±0.03	1.92±0.01	0.96±0.01	0.03±0.01	0.33±0.01	ND	ND
OGM	1.96±0.01	1.96±0.02	0.89±0.01	0.09±0.01	0.43±0.01	ND	ND
AFO	1.93±0.03	2.06±0.03	0.91±0.02	0.10±0.01	0.26±0.03	ND	ND
SIL	1.61±0.06	1.93±0.01	1.30±0.03	0.02±0.01	0.20±0.02	ND	ND
ZOZ	2.04±0.03	1.72±0.04	1.08±0.01	0.04±0.02	0.49±0.03	ND	ND
NSK	2.14±0.01	1.63±0.03	1.43±0.03	ND	0.27±0.02	ND	ND
UNLB	2.24±0.07	2.91±0.01	1.66±0.02	ND	0.40±0.01	ND	ND

Values are means ± values across the column with different superscripts are insignificantly different.

4. DISCUSSION

The results of Table 1 revealed the physicochemical composition of the ten different honey samples. The moisture content, a parameter related to the maturity degree, was between 13.90% to 19.04%, which indicates optimum harvesting and a good degree of maturity. The moisture in this study are in the range with those reported by Makhloufi *et al.*, (2010). Ouchemoukh *et al.*, (2007) found in eleven honey samples from Bejaia (Algeria) values from 14.6% to 19.0%. Nanda *et al.*, (2003) stated that MC is affected by climate and the Mc of original plant. The MC was also influenced by the time of extraction from the comb in relation to ripening process by the bees. The values obtained were all below 20.0%, which is the maximum allowed by the European Union Directive (2003).

The ash content of the studied honey samples differs widely. The values ranged from 0.099% to 0.600% with the lowest recorded in PPM and UNN vendor. These differences in the total mineral content are depended on the type of soil in which the original nectar bearing plant was located (Anklam, 1998). Similar findings were reported by Ouchemouka *et al.* (2007).

Water insoluble matter content of the honey sample ranged between 0.014 to 0.710 g/100g with sample PPM and CAN having the lowest and highest values, respectively. According to Codex Alimentarius Commission, the maximum amount should be below 0.1 %. Rebial *et al.* (2015) made mention of 0.1 to 0.19 % range of water insoluble solid contents in the honey from different regions of South Algeria. Water insoluble solids, represents suspended particle, debris and particulate matters in the honey.

The major sugars present in honey are fructose and glucose. The reducing sugars as shown in Table 1 ranged from 66.83% to 88.48%. These values were not in consonant with 65%, the minimum limit set by EC regulations (2002) in all samples. The values obtained in this study are above the values, 55.20 to 84.30% reducing sugars by Makhloufi *et al.* (2010); however; however Ouchemoukh *et al.* (2007) reported similar values as in this study; the values were 77.75% in eleven Algerian honeys.

The mean percentage apparent sucrose as shown in Table 1 were 8.76%. All the samples were below 10%, which is the maximum legal limits set by Booquet (1993). The reducing and sucrose represent the major constituents of honey.

Hydroxymethyl furfural content of the honey samples ranged from 6.77 to 36.42 mg/kg with PPM and NSK having the lowest and highest values, respectively. makhloufi *et al.* (2010), Benaziza-Bouchema and Schweitzer (2010) have reported that HMF values were between 0 and 598.8mg/kg in 140 Algerian honeys.

Electrical conductivity varies significantly. This variation is dependent on the botanical origin of the honey (Terrab *et al.* 2003). The values obtained from this study ranged from 15.0 to 536 µS/cm with samples UNN vendor and UNLB having the lowest and highest values, respectively; this again suggests that honey collected in this work were of floral origin. The international standards prescribe a maximum limit of 800 µ/cm for most nectar honeys (CAC, 2001). Ouchemoukh *et al*, (2007) mentioned 0.21 to 1.61µ/cm range of electrical conductivity in the honey from different regions of Bejaia (Algeria).

All honeys were acidic, having a pH in the range 3.75 to 4.78. The pH of honey at the point of harvesting is usually between 4.5 to 5.5 (Gonnet, 1986). Those pH values indicate that the honeys tested were most likely of floral origin. These results agreed with reported data by Ouchemoukh *et al.* (2007).

Acidity in honey is usually calculated as free, lactonic and total. European Commission specifies a free acidity of not more than 50 meq/1000g (meg/kg) (EC, 2002). The average values for free acidity in the studied samples were between 10.0 to 26 meq/kg. These values are within limit (below 50 meq/kg) indicating an absence of undesirable reaction (fermentations).

Lactonccacidity ranged from 2.0 to 5.3 meq/kg. Total acidity ranged from 12.10 to 30.0 meq/kg; the results obtained in this study are in agreement with ouchemoukh *et al.* (2007); though not in consonant with the reported average values 39.82 meq/kg by Rebial *et al.* (2015).

The concentration of essential heavy metals – Zn, Cu and Mn became the highest value than the other metals and the concentration of Pb and Ni in all samples were below the detection limits.

The results in Table 2 shown that the concentration of Cu is the highest (1.63 to 2.91 ppm), followed by Zn (1.61 to 2.48 ppm), Mn (0.89 to 1.66ppm), chromium (0.20 to 0.49 ppm), cadmium (0.02 to 0.10 ppm) whereas Pb and Ni were below the detection limit for all the honey samples.

Comparing the results of this study with the results of the studies done so far; the obtained concentration level of heavy metals for cadmium was higher (0.020 to 0.10 ppm) when compared to the reported values 0.03 ppm by Gebru *et al.* 2022 in heavy metals analyzed with flame atomic absorption spectroscopy (FAAS). Cadmium is a cumulative toxic agent with half-life of several years and their burden of the body increases with age. Eating food or drinking water with high cadmium concentration irritates the stomach causing vomiting and diarrhea. It accumulates in the kidney and liver causing kidney dysfunctioning and liver failure, in addition to being a teratogen and carcinogenic agent (Abera, 2014).

The concentration of Zn values ranged from 1.61 to 2.48 ppm with sample SIL and UNLB being lowest and highest, respectively. The values obtained in this study are higher than the reported values 0.062 to 0.335 ppm by Ezeh *et al.* (2018) in Nsukka honey samples. Zinc has numerous functions in the body and it is essential element for human health. At the same time, zinc function as a co-factor for many enzymes of the body. Excess intake of zinc into the body through food, water or dietary supplements can affect health. If large doses of zinc by mouth even for a short time, stomach cramps, nauses and vomiting may occur. Ingesting high levels of zinc for several months may cause anemia, damage the pancreas and decrease levels of high-density lipoprotein (HDL) cholesterol (Ogbabiela *et al.* 2011).

Chromium concentration ranged from 0.20 to 0.49 ppm; the lowest and highest values were observed on sample SIL and ZOZ, respectively. These values obtained from this investigation were in consonant with the reported values 0.25 to 0.45 mg/l by Dessie *et al.* (2022) in heavy metal assessment using FAAS. Chromium is a trace element that are needed by humans in trace amount. It is found primarily in two forms: Trivalent (Chromium III), which is biologically active and found in food and hexavalent (Chromium VI) a toxic form that results from industrial pollution. Chromium produces significant increase in enzyme activity and serves an important function in carbohydrate metabolism, stimulation of fatty acid and cholesterol synthesis from acetate in the liver and improved sugar metabolism through the activation of insulin (Wolde *et al.* 2018).

The concentration of Cu in the ten honey samples ranged from 1.63 to 2.91 with sample NSK and UNLB having the lowest and highest values, respectively. The results obtained from this study are at par with the reported values 1.92 to 2.01 mg/L by Dessie *et al*. (2022) in heavy metal assessment. Copper is essential for a vanity of biochemical processes and is needed for enzymes in the body. It is also involved in the functioning of nervous system and in maintaining the balance of other useful trace metals in the body. Although copper homeostasis plays an important role in the prevention of

copper toxicity, exposure to excessive levels of copper can result in a number of adverse health effects including liver and kidney damage, anemia, immunotoxicity, and developmental toxicity. Many of these effects are consistent with oxidative damage to membranes or macromolecules (Goyer, 1983).

The concentration of Mn ranged from 0.88 to 1.66 ppm with sample OGM and UNLB having the lowest and highest values, respectively. The values obtained in this study is in accordance with the reported value s0.83 to 101 ppm by Dessie *et al.* (2022) in selected heavy metals in honey samples using FAAS at Ethipia province. Manganese (Mn) is a trace mineral that is present in tiny amounts in the body. It is one of the most important nutrients for human health. The average human body contains about 12 mg of Mn. Manganese helps the body to form connective tissue, bones, blood clotting factors and sex hormones. It also plays a role in fat and carbohydrate metabolism, calcium absorption and blood sugar regulation (Goyer, 1986).

5. CONCLUSION

The studies of the physicochemical and heavy metal compostions of honey are important for the certification process that determines honey quality. In this study, the physicochemical and heavy metal properties of honeys collected from different honey bee spot and branded at stored was investigated. It can be concluded that the samples were found to meet all major national and international honey specifications and that they were generally of floral origin.

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