BENIN JOURNAL OF PHYSICAL SCIENCES BJPS Vol. 2(1), June, pg. 45-64 (2025) ISSN 3043-6931(Print) 3043-694X(Online) www.bjps.org.ng

Quality assessment of honey obtained from apiaries and markets in Edo State, Nigeria

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Abstract

This research evaluated the quality of honey samples collected from artificial and natural apiaries and the surrounding markets in three municipal locations in Edo State, Nigeria. *The physicochemical properties, proximate and heavy metal composition of honey samples* from five farms and five nearby markets were analyzed using standard methods. The results were compared with the Codex Alimentarius General Standards and the European Union Council Directive. A narrow pH range of 3.43±0.03 to 3.87±0.00 was obtained, while the free acidity varied between 27.00±0.10 to 83.70±0.30 meg/kg. Colour (58.22±0.04-125.08±0.02 mm Pfund), total solid (73.20±0.10-84.00±0.10%), Brix $(71.00\pm0.01-82\pm0.01\%)$ and refractive index at 20° C (1.4965-1.4847) were also evaluated. The proximate analysis showed moisture content (16.03±0.13-26.80±0.20%), ash content $(0.24\pm0.01-1.44\pm0.03\%)$, crude fat $(0.06\pm0.02-4.47\pm0.02\%)$, crude protein $(0.30\pm0.09-4.47\pm0.02\%)$ 1.06±0.03%) and carbohydrate (73.05-82.58%) while crude fibre was not present. The most abundant elements were K (7.62±0.11-31.40±0.15 mg/L) and Ca (14.00±0.12- $30.60\pm0.10 \, \text{mg/L}$), followed by Na (2.41±0.13-5.27±0.09 mg/L) and Mg (0.42±0.10-0.83±0.09 mg/L). In most of the samples, Pb, Ni, Cd, Cr and Cu were not detected but occurred in very low concentrations in a few samples while Zn (0.04±0.01-2.55±0.06 mg/L), Fe (0.02±0.01-1.03±0.03 mg/L) and Mn (0.02±0.01-0.23±0.06 mg/L) were detected in most of the samples. The characteristics of the apiary honeys were comparable to the ones from the respective nearby markets, suggesting relative purity and wholesomeness. Because the majority of the parameters determined for the honey samples complied with international standards, they may be consumed or used therapeutically.

Keywords: Honey bee, Stingless bee, Adulteration, Moisture content, Total carbohydrate, Dietary fibre, Heavy metal

An Official Journal of the Faculty of Physical Sciences, University of Benin, Benin City, Nigeria.

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1. INTRODUCTION

Honey is a complex natural product with a rich history as food and medicine (Hossain *et al.*, 2021). It is primarily composed of sugars, peptides, enzymes, organic acids, water-soluble vitamins, micro and macro-elements, and phenolic compounds. This is complemented by various bioactive compounds that contribute to the wide range of health benefits of honey (Kostić *et al.*, 2024). Despite its ancient origin, honey remains a valuable natural remedy in modern medicine (Yousuf *et al.*, 2022).

Honey is produced by mainly two species of honey bees (Apis mellifera and Apis dorsata) from the nectar of blossom, plant sugary secretion or fecal matter from sap-feeding insects on the active parts of plants followed by water evaporation, regurgitation and enzymatic activity (Satarupa and Subha, 2014). Bumble bees, stingless bees as well as other insects that are hymenopteran such as wasps, also produce honey. Honey bees make a larger quantity of honey with a slight difference in its properties. Bees store honey in wax structures called honeycombs, inside the beehive (Abeshu and Geleta, 2016), and it is gathered from natural bee populations or hives, a practice known as beekeeping or Apiculture. The primary properties of natural honey are predominantly determined by the geographical location and environmental conditions of the bee species, which are influenced by the specific flora, vegetation, and ecosystem in which the bees were nurtured (Kayode and Oyeyemi, 2014). Additionally, the physiological state of the colony, nectar source, weather conditions, processing, storage conditions, and methods involved in extracting and storing the honey can also determine the characteristics of different natural honey samples (Sereia et al., 2017). It is gathered from natural bee populations or hives, a method known as beekeeping or apiculture

Physicochemical and quantitative properties such as moisture, ash content, pH, colour, viscosity, sugar content, titrable acidity, and proximate composition of honey are basically used to characterize a sample of honey (Al-Kafaween *et al.*, 2023).

Processing methods, such as homogenization, can improve honey texture and quality (Radtke and Lichtenberg-Kraag, 2018). Storage conditions, particularly temperature and humidity, significantly affect honey's physicochemical temperatures (above properties. Higher 21°C) lead to increased hydroxymethylfurfural (HMF) content and decreased invertase activity of honey (Radtke and Lichtenberg-Kraag, 2018). In tropical climates, storage at 35-40°C and 50-60% humidity can reduce honey's shelf life to one year (Nombré et al., 2010). Improper handling and storage can lead to non-conformity with quality standards (Buba et al., 2013). Despite these variations, honey maintains its nutritional value, antibacterial properties, and health benefits, making it a valuable functional food

(Pavlova *et al.*, 2019). Honey produced by stingless bees contains more moisture than honey bee honey, even though its water activity, ash content, and free acidity are higher. However, its pH and total soluble solid content are low (Gela *et al.*, 2021).

Honey has high glucose and low sucrose content. It thus functions as an efficient immediate energy source and contributes to muscle fatigue attenuation. Its lower glycemic index than sucrose makes it a suitable sugar substitute for better blood sugar management, particularly in athletic and diabetic contexts (Abdulrhman et al., 2011; Abdulrhman et al., 2013; Jimoh and Ummi, 2015). Honey contains other minor nutritive compounds such as amino acids, enzymes, vitamins, as well as flavours, minerals, and volatile compounds (Schievano et al., 2013). It also contains small amounts of Zn, Ca, Mg, Fe, and Cu, as well as niacin and riboflavin. Many elements such as potassium, chlorine, sulphur, phosphorus, magnesium, silicon, iron, and copper pass through the plant sap, which the bee feeds on, into the honey (Solayman, 2016). The nurturing of honey in areas with heavy industrial activity and the post-harvest treatment given to it can result in a high level of heavy metal contamination (Squadrone et al., 2020). Elements such as zinc, manganese, selenium, copper, nickel and iron are important for regular metabolism but excessive amounts could lead to pollution and health hazards (Kumar et al., 2010). Trace metals including Cd, Cr, Pb, and Hg are regarded as poisonous and can affect metabolism in humans due to their carcinogenic and cytotoxic characteristics (Adesetan et al., 2023).

The nutritive and medicinal value of honey has inevitably raised its demand substantially. This has unfortunately led to the adulteration of the product (Omode and Ademukola, 2008). Adulteration refers to the intentional degradation of food quality either by dilution or substitution with lower-quality substances or by removing valuable ingredients (Woldemariam and Abera, 2014). Honey adulteration happens when sucrose syrups, such as those made from sugar beet, high-fructose corn syrup (HFCS), or maltose syrup, are directly added, or when industrial sugars such fructose and glucose are blended with honey. Adulterants can be classified as deliberate, accidental, metallic or microbial, depending on the type of contaminant in the honey (Tura and Seboka, 2020). Honey adulteration is an internationally recognized problem with economic and nutritional consequences. Chromatography, spectroscopy, and isotope analysis are key methods for detecting food adulteration, each with unique advantages and limitations. Chromatography and spectroscopy offer detailed chemical profiling, while isotope analysis provides specific insights into adulterant origins (Mantha et al., 2024). Recent research has explored physical and chemical composition analysis to identify markers of adulteration (Gün and Karaoğlu, 2024). Adulteration of honey with sugar syrups and other substances significantly alters

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its physicochemical properties, including proline content, pH, moisture, and HMF levels (Brar et al., 2023). Principal Component Analysis (PCA) has also shown promise in differentiating pure and adulterated honey samples based on physical characteristics (Singh and Barman 2021).

Most of the honey sold in Nigeria is made with caramelized sucrose (Omode and Ademukola, 2008). This caramelized sucrose does not add any medicinal or nutritional value to honey (Tosun and Keleş, 2021; Wang *et al.*, 2023; Hu *et al.*, 2024).

This research was conducted to compare the physicochemical properties and proximate composition of honey samples procured from honeybee farms and their surrounding markets, in order to assess compliance with international standards.

2. MATERIALS AND METHOD

Analytical grade reagents were used in this research and were used directly without subjecting them to further purification. Triplicate replications were done for each analysis to enhance the reliability of the results. The data obtained were presented as the mean ±standard deviation (SD) of the three replicate experiments.

Sample Collection

The honey samples were purchased from ten (10) municipal locations (Table 1) in Edo State, Nigeria. These include Benin City, Ekpoma and Okada. A total of ten samples were procured: five (FA1 to FA5) from farms and five (MB1 to MB5) from markets located near the corresponding honey farms. Each sample was labelled based on its source and transported to the laboratory for analysis.

Table 1: *Geographical coordinates of sampling locations*

| - | Collection Point | Location | Coordinates |
|-------|------------------|-----------------------------|--------------------|
| Codes | | | |
| FA1 | Honey farm | Okada | 6° 38′06.72″ N, 5° |
| | | | 19′59.52″ E |
| FA2 | Farm settlement | Ukhun, Ekpoma | 6° 52′27.89″ N, 5° |
| | | | 19'59.59" E |
| FA3 | Honey farm | Upper Sakponba | 6° 10′45.48″ N, 5° |
| | · | | 32′48.37″ E |
| FA4 | Private farm | Ugbor, Benin City | 6° 15′51.54″ N, 5° |
| | | | 36′23.04″ E |
| FA5 | Private Farm | Oluku Primary School Lane | 6° 20′44.16″ N, 5° |
| | | · | 38'00.60" E |
| MB1 | Market | Okada | 6° 48′54.09″ N, 3° |
| | | | 50'44.26" E |
| MB2 | Market | Ukhun, Ekpoma | 6° 45′19.44″ N, 6° |
| | | • | 09'15.12" E |
| MB3 | Supermarket | Jehis, Off Benin Lagos Road | 6° 20′07.45″ N, 5° |
| | _ | - | 35′39.78″ E |
| MB4 | Market | Uselu, Benin City | 6° 23′12.48″ N, 5° |
| | | | 36′34.92″ E |
| MB5 | Supermarket | Ugbowo, Benin City | 6° 20′06.18″ N, 5° |
| | | · · | 37′38.96″ E |

Determination of pH

The pH was measured using a 10% solution according to the AOAC 1990, method 962.19. Five grams of the honey was homogenized in 50 mL of distilled water and a digital pH meter was used to measure the pH directly. The pH meter was earlier calibrated using pH buffers4 and 7. To maintain accurate pH readings, the meter was recalibrated every 4 hours to account for any decrease in sensitivity.

Determination of free acidity

The free acidity test was done by using the A.O.A.C. 1990 official method 962.19. In the procedure, 10% solution was made with 10 g of the honey and distilled water. The solution was then titrated with a 0.1M NaOH solution until a pH of 8.3 was achieved, with phenolphthalein used as the indicator. The results were reported as milliequivalents per kilogram of honey by applying the following equation:

$$Free \ acidity = V \times 10 \tag{1}$$

Where: V = Titre value, i.e. the volume of NaOH (in mL) which neutralized the 10g of the honey sample. 10 = the dilution factor of the honey sample.

Determination of colour

The colour of the honey sample was determined by measuring its absorbance using a UV-Visible spectrophotometer according to the method described by White (1984) and modified by Smetanska *et al.*, 2021. The honey was first warmed up to 50°C in a thermostat water bath and cooled rapidly to room temperature. After cooling, a 50% (w/v) solution was homogenized and centrifuged at 3200 rpm for 5mins. Afterwards, the absorbance was read at 635nm using a spectrophotometer. The color intensity was measured using the Pfund scale in Table 2, after converting the absorbance values according to Equation 2.

$$mm\ Pfund = -38.70 + (371.39 \times Abs)$$
 (2)

Where:

Abs= absorbance at 635nm

Table 2: Scale (mm Pfund) established by the USDA, for the determination of the colour of honey

| Colour | Pfund unit (mm) |
|-------------------|-----------------|
| White water | 0 - 8 |
| Extra white | 8 – 16 |
| White | 16 – 34 |
| Extra Light amber | 35 – 50 |
| Light amber | 51 – 84 |
| Amber | 85 – 114 |
| Dark amber | 115 - 140 |

(Source: Guede et al., 2022)

Determination of moisture content

The A.O.A.C, 1990 method was used to estimate the amount of moisture in the honey. The analysis was done in triplicate for each sample. Clean and dried crucibles were weighed, and two grams of honey were weighed into previously weighed crucibles. The crucibles were put in the oven set at 105°C and heated for

3 hours and then transferred to a desiccator to cool. Equation 3 was then used to calculate the moisture content.

%Moisture content =
$$\frac{(M_1 + M_2)}{(M_1 + M_0)} \times 100$$
 (3)

Where: M_0 = Weight of the crucible

 M_1 = Weight of the fresh honey sample + crucible

 M_2 = Weight of the dried sample + crucible

Determination of total solids

The total solids in the honey sample were determined using Equation 4 as applied by Kayode and Oyeyemi, (2014).

$$Total \ solids \ (\%) = 100 - Moisture \ content \ (\%) \tag{4}$$

Determination of ash content

The ash content was measured following the procedure outlined by Williams *et al.* (2009). 2 g of honey were placed in a clean and dry crucible, and transferred to the oven set at 105°C. The honey was allowed to char and dry. It was thereafter ashed in a muffle furnace at 550 °C. After 4 hours, the sample was removed from the oven, cooled in a desiccator and weighed. Ash percentage was calculated as follows:

$$\%Ash = \frac{(M_1 + ash) - M_1}{M_0} \times 100 \tag{5}$$

Where: M_1 = weight of crucible

 M_0 = weight of the sample

Determination of crude protein, fibre and fat

The crude protein, crude fibre and crude fat were determined according to the AOAC (2005) methods.

Crude protein

The Kjeldahl method was employed to evaluate the crude protein in the honey. 1 g of the honey was measured into a digestion flask and 10 mL of concentrated sulphuric acid was added alongside 8 g of the digestion mixture (K₂SO₄ and CuSO₄, in a ratio of 8:1). A selenium catalyst was included to elevate the boiling temperature. After thoroughly mixing the contents, it was then boiled on a digestion block until it became clear or pale green. After cooling to room temperature, the digest was poured into a 100 mL volumentric flask and made up to the mark with distilled water. 10 mL of the digest were distilled with 0.5 M

NaOH in a distillation apparatus. The distillate, containing NH₃, was collected as NH₄OH and titrated with 0.1 N HCl using phenolphthalein as an indicator, until a pink color appeared. The percentage of crude protein in the sample was calculated using the following formula:

Crude protein (%) = % Nitrogen
$$\times$$
*Correction factor (6)

*Correction factor = 6.25

$$\% Nitrogen = \frac{(S-B) \times N \times 0.014 \times D \times 100}{Weight of sample \times V}$$
 (7)

Where:

S =Sample titration reading

B = Blank titration reading

N = Normality of the HCl

D = Dilution of the sample after digestion

V = Volume taken for distillation 0.014 = Milli equivalent weight of Nitrogen

Crude fibre

Two grams of honey sample was weighed into a 1000 mL beaker, and 200 mL 0.128M H₂SO₄ was added. The beaker was placed on a heater and allowed to boil for 30mins. The beaker was agitated after every 5minutes. The beaker was removed from the hot plate and the content was filtered through a muslin cloth. Excess acid was rinsed off the residue with hot water. 200 mL of 0.313 M NaOH was added to the residue in a beaker and boiled for 30 mins. The content of the beaker was filtered once more and residue was rinsed off excess alkali with hot water. The final residue was then placed in a crucible and dried in an oven set to 105°C. The dry residue was then placed in a muffle furnace and heated at 550°C for 3 hours. The residue was then cooled in a desiccator and reweighed. The weight loss due to ashing was recorded as crude fibre.

$$\%Crude\ fibre = \frac{W_1 - W_2}{W_0} \times 100 \tag{8}$$

Where: W_0 = weight of sample

 W_1 = weight of crucible +dry residue

 W_2 = weight of crucible with ash

Crude fat

Twenty grammes of honey sample were placed into a separatory funnel, and 20 mL of a chloroform-methanol mixture in a 2:1 ratio was added. The mixture was shaken thoroughly while removing the stopcock intermittently to reduce the

pressure. The mixture was left to stand, and the non-aqueous substance was transferred into a pre-weighed beaker. The procedure was repeated twice. The beaker containing the non-aqueous substance was transferred to an oven to dry to a constant weight, cooled in a desiccator and weighed. The crude fat was calculated by using equation 9.

%Crude
$$fat = \frac{B_2 - B_1}{B_0} \times 100$$
 (9)

 B_0 = weight of sample taken

 B_1 = weight of empty beaker

B₂= weight of beaker + dry non-aqueous substance

Determination of carbohydrate

Total carbohydrate was obtained by using the mathematical expression of Charrondiere *et al.* (2004) and modified as equation 10.

Total carbohydrate (%) =
$$100 - (ash\ content + protein + lipids)$$
 (10)

Refractive index

Abbe refractometer (NAR-IT, Japan), thermostat at 20°C, was used to measure the refractive index of the honey. The soluble solids (°Brix) were measured using the same device. The values of the refractive index and degree brix were read directly on the display board of the instrument.

Heavy metal analysis

The honey sample was digested by weighing 1 g of the sample into a Kjeldahl digestion tube and adding 10 mL of *aqua regia* reagent [Nitric acid: Hydrogen peroxide (3:1)]. It was then digested for 20 minutes to obtain a clear solution and allowed to cool to room temperature. Deionized water was used to dilute the digest and then filtered into a 100 mL volumetric flask. It was made up to the mark with more deionized water. The concentrations of heavy metals were measured by directly aspirating the solution from the 100 mL volumetric flask into the Atomic Absorption Spectrophotometer (AAS) Buck Scientific VGP210, U.S.A., 2005.

3. RESULT AND DISCUSSION

Physicochemical Properties

The results of the physicochemical properties of the honey samples from selected markets and apiaries in Benin City, Ekpoma, and Okada in Edo State, Nigeria, are shown in Table 3.

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Table 3: *Physiochemical properties of honey samples obtained from apiaries and markets in Edo State*

| Samples | рН | Free Acidity (meq/kg) | Colour (mm Pfund) | Total Solids (%) | Brix (%) | Refractive Index at 20°C |
|---------|-----------|-----------------------------|-------------------------|------------------------|------------|--------------------------------|
| FA1 | 3.59±0.03 | 53.70±0.06 | 65.28±0.01 | 82.10±0.11 | 80.30±0.03 | 1.4918±0.001 |
| FA2 | 3.72±0.00 | 41.70±0.02 | 104.28±0.06 | 80.20±0.03 | 78.30±0.08 | 1.4871±0.001 |
| FA3 | 3.76±0.02 | 83.70±0.03 | 108.36±0.03 | 81.90±0.04 | 79.70±0.02 | 1.4912±0.001 |
| FA4 | 3.51±0.04 | 65.00±0.07 | 111.71±0.01 | 81.80±0.03 | 80.00±0.01 | 1.4911±0.001 |
| FA5 | 3.66±0.01 | 27.30±0.06 | 122.85±0.01 | 81.20±0.06 | 79.00±0.03 | 1.4896±0.001 |
| MB1 | 3.71±0.02 | 36.70±0.06 | 61.94±0.03 | 73.20±0.10 | 71.00±0.01 | 1.4696±0.001 |
| MB2 | 3.62±0.03 | 27.00±0.10 | 69.74±0.02 | 84.00±0.10 | 82.00±0.01 | 1.4965±0.001 |
| MB3 | 3.76±0.01 | 52.00±0.10 | 58.22±0.04 | 83.97±0.12 | 81.30±0.01 | 1.4957±0.002 |
| MB4 | 3.87±0.00 | 29.30±0.05 | 117.28±0.07 | 81.30±0.10 | 79.00±0.03 | 1.4897±0.004 |
| MB5 | 3.43±0.03 | 62.70±0.06 | 125.08±0.02 | 79.30±0.06 | 77.30±0.01 | 1.4847±0.001 |

values reported as mean±SD, n=3

The pH of the honey samples analysed ranged from 3.43 to 3.87, indicating that it is acidic. The average pH values were within the 3.40-6.10limit set by the Codex Alimentatrius Commission (CAC, 1998). The mean pH value was similar to previously reported values for Nigerian honey (Adesetan et al., 2023; Ndife and Maarfi, 2014; Kayode and Oyeyemi, 2014). The acidity is low enough to prevent the growth of many bacteria, which implies an extended shelf life for the honey. The acidity of honey is mainly due to organic acids such as gluconic acid, lactic acid, acetic acid and some quantities of citric succinic acids (Cedillo et al., 2024). These acids contribute significantly to the characteristic flavour and antimicrobial properties of honey (Al-Kafaween et al., 2023). Acidity can also be an indicator of the freshness and authenticity of honey. The free acidity values of the honey samples analysed in this study were within the maximum limit of 50 meq/kg set by Codex Alimentarius, (CAC, 2001). However, samples FA3 (83.70 meg/kg), FA4 (65.00 meg/kg) and MB5 (62.70 meg/kg) were above the standard limit. However, these results are similar to those reported by Loza et al. (2020). According to Garcia-Chaviano et al., (2022), the acidity of honey varies with its flora origin.

The colours of the honey samples ranged between light amber (58.22 mm Pfund) and dark amber (125.08 mm Pfund). The values are comparable to other results obtained from Nigerian honey (Jimoh and Ummi, 2015). The colour of honey is

influenced by a combination of factors. These include the type of nectar and pollen collected by the bees including the climatic conditions and geographical location of the apiary. Dark amber honey is known to contain more minerals than the lighter ones. The colour of honey is one of the quality indicators associated with its flavour, odour, and storage conditions (White, 1984). The colour of the honey obtained from markets in rural communities correlated with that of the farms. However, there was a variation in the colour of the honey sample obtained from urban markets. Whereas colour varies with honey's origin, age, and storage conditions, the quantity of suspended matter (such as pollens) determines how clear or transparent it is. Heat also affects the colour of honey, and its crystallization, taste, and fragrance. Natural honey becomes dark in colour when heated (Smetanska *et al.*, 2021)

The brix content is used to determine the sugar content in a honey sample (Abdulrhman *etal.*, 2011). It is a major criterion of the glycemic index, a concern for diabetic persons (Akinwande, and Oladapo, 2022). Excess sucrose is due to the loss of invertase due to heat (Chirsanova *et al.*, 2021). MB1 showed the lowest Brix % (73.20), while MB2 was the highest (84.00%). Though a higher level of Brix in honey increases the possibility of crystallization, it is not a determining factor of its quality (Cedillo *et al.*, 2024).

The refractive index of the honey samples measured at 20°C ranged from 1.4847 to 1.4947. Refractive index is a measure of the ratio of the velocity of light in free space to that of the medium (honey), in MB1 indicates that light travels fast through them and no change in the light direction travelling through them, while MB2 recorded the highest at 1.4964.

Table 4: Proximate composition of honey samples obtained from apiaries and markets in Edo State

| Samples | Proximate Composition (%) | | | | | | | |
|---------|---------------------------|------------|-----------|-----------------|-------|--------------|--|--|
| | Ash | Moisture | Crude | Crude | Crude | Carbohydrate | | |
| | | | Fat | Protein | Fiber | | | |
| FA1 | 0.78 ± 0.08 | 17.90±0.25 | 1.07±0.01 | 0.36±0.05 | ND | 79.89 | | |
| FA2 | 0.66±0.15 | 19.80±0.35 | 0.57±0.01 | 0.70±0.13 | ND | 78.27 | | |
| FA3 | 0.69±0.11 | 18.10±0.42 | 3.15±0.02 | 0.51±0.08 | ND | 77.55 | | |
| FA4 | 0.68±0.18 | 18.17±0.32 | 0.62±0.01 | 0.60±0.07 | ND | 79.93 | | |
| FA5 | 0.55±0.06 | 18.78±0.06 | 0.22±0.03 | 0.43±0.13 | ND | 80.02 | | |
| MB1 | 0.87±0.13 | 26.80±0.20 | 0.65±0.01 | 1.02±0.05 | ND | 70.66 | | |
| MB2 | 0.89±0.09 | 16.03±0.23 | 0.06±0.02 | 0.44 ± 0.02 | ND | 82.58 | | |

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| MB3 | 0.24±0.02 | 16.40±0.60 | 2.92±0.03 | 1.06±0.03 | ND | 79.38 |
|-----|-----------|------------|-----------|-----------|----|-------|
| MB4 | 1.33±0.12 | 18.70±0.15 | 2.85±0.05 | 0.30±0.09 | ND | 76.82 |
| MB5 | 1.44±0.03 | 20.70±0.06 | 4.47±0.02 | 0.34±0.05 | ND | 73.05 |

values reported as mean±SD, n=3

Proximate analysis

Table 4 contains the results of the proximate composition of the honey samples. The ash content ranges from 0.24 to 1.44%. The standard (maximum) value for ash content in honey is 0.6% (CAC, 2001). Therefore, the ash content of most of the honey samples exceeds the standard value. The ash content of honey is influenced by the mineral elements present in the plant nectar and other different floral sources used by bees during pollination (Gela *et al.*, 2021).

There is a linear relationship between the ash content and the electrical conductivity (Albu *et al.*, 2023)

$$C=0.14+1.74A$$
 (11)

Where C is the electrical conductivity in milli Siemens cm-1 (mS/cm) and A is the ash content in g/100 g.

Honey's electrical conductivity is generally low and is influenced by its mineral, organic acid, and protein content. As a result, it is commonly used in routine honey quality control and can act as a reliable indicator of the honey's botanical origin (Guede *et al.*, 2022). Therefore, an increase in ash content leads to a higher electrical conductivity value. The typical values range from 0.39-0.76 mS/cm.

The percentage of moisture in the honey sample ranged from 16.03% to 20.70% for the majority of the samples except MB1, which has 26.80% moisture content. All samples showed no difference with samples from the Southeast region (Oyeyemi $et\ al.$, 2015) and the Northern part of Nigeria (Buba $et\ al.$, 2013). The standard moisture content value for honey given by European Union (2001) and Codex (2001) is \leq 20%. The values obtained show that the honey samples would remain stable, have a longer shelf life during storage and not undergo spoilage by fermentation through osmo-tolerant yeasts. Because honey is hygroscopic, it should be ensured that during processing, alterations and moderations that affect honey quality due to exposure to the environment are excluded. This is because high moisture content is a strong indication of adulteration (Tosun and Keles, 2021).

The fat content values were relatively low (0.06% - 2.92%), except for FA3 (3.15%) and MB5 (4.47%). These samples (FA3 and MB5) may be susceptible to spoilage during storage. Generally, honey is not a good source of fat. The small quantity

obtained in this study may have been from bee wax melting during harvest (Ige and Olugbenga, 2010).

The fibre content analysis gave no visible result, indicating the absence of unavailable carbohydrates also known as dietary fibre (Gela *et al.*, 2021).

Total carbohydrates ranged from 70.66% in (MB1) to 82.58% in (MB2). This is similar to the 91.11% reported by Adesetan *et al*, (2023) as the average value of carbohydrates in honey samples from Southwestern Nigeria. Simple sugars (fructose and glucose), represent 85% to 95% of the total sugar found in honey (Evbuomwa and Ijomah, 2020).

The proximate analysis result also revealed crude protein was in the range of 0.30% to 1.06%. This is similar to the values found by Ikegbunam and Walter, (2021) who reported a protein content of 0.21% - 0.74% for honey samples sourced from Enugu and Anambra States in Nigeria. This is however low compared to the 1.59% obtained by Adesetan *et al*, (2023) for the protein content in selected honey samples from Southwestern Nigeria. Variations in protein content may be due to soil composition, location and floral origin (Osuagwu *et al.*, 2020).

Mineral Composition

The result of the macro element analysis of the honey samples is shown in Table 4. Potassium (7.62 - 31.40 mg/L) and Calcium (14.00 – 30.60 mg/L) were largely present while Na (2.41 -5.27 mg/L) and Mg (0.42 – 0.83 mg/L) showed lower concentrations. Research has shown that potassium helps regulate the acid-base balance in the blood. It also plays a role in nerve impulse transmission, activates the activity of various enzymes, and supports the heart's muscular function. Potassium also plays a role in the proper functioning of the skin and kidneys (Evbuomwan and Ijomah, 2020). Calcium and magnesium are essential for the growth and maintenance of strong and healthy teeth, bones, and muscles, as well as the prevention and management of hypertension and cardiovascular diseases (Ikegbunam and Walter, 2021).

Table 5: Concentration of macroelements in honey samples obtained from apiaries and markets in Edo State

| Samples | (| Concentration of macroelements (mg/L) | | | | | |
|---------|------------|---------------------------------------|------------|-----------|--|--|--|
| | K | Na | Ca | Mg | | | |
| FA1 | 31.40±0.20 | 3.63±0.10 | 16.40±0.10 | 0.76±0.02 | | | |
| FA2 | 29.65±0.20 | 4.45±0.07 | 30.60±0.10 | 0.79±0.05 | | | |
| FA3 | 31.21±0.15 | 5.27±0.09 | 17.20±0.12 | 0.83±0.09 | | | |
| FA4 | 13.08±0.12 | 3.22±0.05 | 14.30±0.08 | 0.60±0.02 | | | |

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| FA5 | 16.64±0.21 | 3.22±0.06 | 14.90±0.05 | 0.52 ± 0.03 |
|-----|------------|-----------|------------|-----------------|
| MB1 | 8.99±0.14 | 3.22±0.05 | 21.50±0.03 | 0.59±0.05 |
| MB2 | 8.01±0.16 | 2.41±0.13 | 18.10±0.04 | 0.47±0.03 |
| MB3 | 15.03±0.20 | 2.41±0.13 | 16.10±0.12 | 0.42±0.10 |
| MB4 | 7.62±0.25 | 2.82±0.10 | 14.00±0.12 | 0.50 ± 0.08 |
| MB5 | 24.39±0.15 | 3.22±0.05 | 17.10±0.05 | 0.59±0.06 |

values reported as mean±SD, n=3

Heavy metal content

The heavy metal concentration of the honey sample is shown in Table 6. Zinc (0.04-2.55 mg/kg) and Fe (0.02-1.03 mg/kg) were detected in all the samples while Cu, Cr, Ni and Pb were found in a few samples and Cd was only detected in one sample. This compares well with the findings of Adesetan *et al.* (2023) who did not detect Cd, Cr and Pb in selected honey samples from Southwestern Nigeria.

Table 5: Concentration of heavy metals in honey samples obtained from apiaries and markets in Edo State

| Samples | Heavy metal concentration (mg/L) | | | | | | | |
|---------|----------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| | Pb | Ni | Cu | Zn | Cr | Fe | Mn | Cd |
| FA1 | 0.02±0.01 | ND | 0.02±0.01 | 1.18±0.03 | ND | 1.03±0.03 | 0.10±0.01 | ND |
| FA2 | ND | 0.04±0.01 | ND | 2.55±0.03 | 0.03±0.01 | 0.37±0.15 | 0.10±0.00 | ND |
| FA3 | ND | 0.02±0.01 | 0.03±0.02 | 0.95±0.01 | 0.02±0.01 | 0.23±0.04 | 0.20±0.00 | ND |
| FA4 | ND | ND | 0.01±0.00 | 1.25±0.02 | 0.02±0.00 | 0.33±0.02 | 0.23±0.03 | 0.02±0.01 |
| FA5 | 0.01±0.00 | ND | 0.01±0.00 | 1.20±0.03 | ND | 0.16±0.02 | 0.20±0.01 | ND |
| MB1 | ND | 0.06±0.03 | 0.02±0.01 | 1.25±0.01 | ND | 0.05±0.00 | ND | ND |
| MB2 | ND | 0.08±0.01 | ND | 0.04±0.01 | 0.02±0.00 | 0.02±0.00 | 0.01±0.00 | ND |
| MB3 | 0.02±0.00 | 0.12±0.01 | 0.01±0.00 | 0.05±0.01 | ND | 1.00±0.03 | 0.02±0.01 | ND |
| MB4 | ND | 0.04±0.01 | ND | 0.05±0.02 | ND | 0.40±0.01 | 0.02±0.01 | ND |
| MB5 | 0.03±0.02 | 0.05±0.01 | ND | 0.09±0.01 | 0.02±0.01 | 0.77±0.02 | 0.02±0.01 | ND |

values reported as mean±SD, n=3

Laaroussi *et al.* (2020) also detected Cd in only one sample but found zinc (1.09-4.02 mg/kg) in all the honey samples from the Middle Atlas in Morocco. Zinc is a vital trace element with diverse roles in DNA synthesis, cell growth, protein building, tissue repair, and immune function. Its deficiency can significantly impact health, highlighting the importance of adequate zinc intake for optimal

physiological functions (Costa *et al.*, 2023). When heavy metals are detected in honey, it may suggest pollution of the plant used by the honey, soil and topographical origin of the honey and the container used in collecting the honey (Omran *et al.*, 2020). While honey provides valuable data on environmental health, its variability necessitates complementary monitoring methods for comprehensive assessments.

4. CONCLUSION

The values of the physicochemical and proximate analysis obtained in the samples studied were found to be in accordance with the *Codex Alimentatrius* standards for honey and similar to other research works for honey quality. However, the moisture of MB1 and MB5 were higher than the standards. There were significant variations in the ash content, free acidity, and crude fat of honey samples from different locations. The types of bees, extraction methods, and storage techniques may have played a vital role in these variations. However, it could not be established whether the variations were natural or induced. It is therefore recommended that these factors be investigated in further studies.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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