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PHYSICOCHEMICAL PROPERTIES AND MINERAL COMPOSITION OF HONEY (*Apis mellifera*) FROM NORTH CENTRAL NIGERIA

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Abstract

This study evaluated the nutritional quality and potential adulteration of six honey samples obtained from Benue and Kogi States by analyzing their moisture content, ash content, fat content, protein content, hydroxymethylfurfural (HMF), free acidity, pH, polyphenol content, heavy metals, and mineral composition. Standard analytical methods were used for evaluation. The pH values of the samples ranged from 3.95 to 4.30, with free acidity between 2.72±0.10 and 57.40±0.81 meq/kg. The ranges of values of the other tested parameters in the samples are as follows: moisture content (8.35±0.33 to 10.34±0.02%), ash content (0.19±0.01 to 0.86±0.01%), fat content (0.00±0.00 to 0.54±0.03%), protein content (0.28±0.01 to 0.56±0.02%), polyphenol content (3.75±0.72 to 90.12±0.07%) and HMF (16.13±2.62 to 66.77±6.78 mg/kg). Cadmium and Lead were not detected. The mineral concentrations were as follows: sodium (1.80±0.06 to 9.75±0.14 mg/100 g), potassium (12.89±0.03 to 27.62±0.02 mg/100 g), calcium (3.55±0.20 to 9.50±0.23 mg/100 g), magnesium (2.00±0.06 to 7.60±0.17 mg/100 g), and zinc (2.15±0.09 to 4.10±0.06 mg/100 g). The nutritional quality of these honey samples is supported by their high carbohydrate content, metabolizable energy, low crude fat content, and significant levels of essential minerals, which make them suitable for use in various food products.

Keywords: North central; heavy metals; honey; minerals; nutritional quality

Introduction

Honey, a golden sweet substance produced by bees, serves a purpose beyond that of natural sweeteners. Its complex chemical composition, which varies according to the

floral sources visited by bees, influences its color, flavor, and aroma. The primary constituents of honey are approximately 80% carbohydrates, comprising 35% glucose, 40% fructose, and 5% sucrose, along with approximately 20% water. Minor components include minerals, proteins, vitamins, organic acids, flavonoids, phenolic acids, enzymes, and other phytochemicals (Afroz *et al.,* 2016). The African honeybee (*Apis mellifera*) is the most extensively reared honeybee and the principal honey producer in Nigeria (Ayansola and Davies, 2012). In addition to nectar, bees occasionally collect exudates from succulent plant parts, such as leaves, stems, trunks, or fruits, whether living or dead, as well as secretions from plant-sucking insects. Bees combine these substances with intestinal enzymes to produce honey, contributing to their diverse health and medicinal properties (Kadri *et al.,* 2017). Honey has multiple functions including wound healing, treatment of respiratory tract infections, relief from colds and coughs, and as a source of nutrition and natural medicine. It is also highly valued as a commercial product (Haider, 2022). According to Adeonipekun *et al.* (2016), honey has antibacterial, antioxidant, and anti-inflammatory properties. Rico *et al.* (2009) observed that honey does not spoil. However, its chemical and biological composition can vary in quality and quantity depending on its botanical and geographical origins (Bogdanov *et al.,* 2008). Its complex biochemical makeup, which includes proteins, carbohydrates, enzymes, and amino acids, significantly contributes to its taste and aroma (Kaur *et al.,* 2015). Honey also contains trace amounts of minerals derived from nectar and pollen collected by bees and the soil in which host plants grow (Vanhanen *et al.,* 2011). These minerals offer health benefits when consumed in moderation. However, at high concentrations, they may pose health risks owing to the potential presence of toxic metals (Aghamirlou *et al.,* 2015). Therefore, while honey is widely consumed owing to its nutritional value, there is also a risk of contamination with harmful elements.

Honeybee colonies and their products are vulnerable to various pests, parasites, and diseases (Tessega, 2009). Honey is produced and consumed on a national scale in Nigeria. To determine honey varieties and authenticity, some pollen, physicochemical, microbiological, and sensorial analysis must be taken into account. Numerous studies have investigated the pollen content, physicochemical properties, and microbiological characteristics of honey, both in Nigeria and internationally (Aina *et al.,* 2010; Kavapurayil *et al.,* 2014; Aina *et al.,* 2015; Adeonipekun *et al.,* 2016; Ikegbunam *et al.,* 2022). However, in North Central Nigeria, research has predominantly focused on pollen analysis (Ige and Modupe, 2010; Aina and Owonibi, 2011; Agwu *et al.,* 2013; Aina *et al.,* 2015; Essien *et al.,* 2022; Ikegbunam *et al.,* 2023), with limited investigation on the physicochemical composition of honey (James *et al.,* 2009; Aina, 2016; Aina and Esien, 2016). Physicochemical and mineral analyses are essential for quality control and health risk assessment. Unfortunately, data regarding the mineral and physicochemical properties of honey from this region are scarce. Consequently, this study presents preliminary findings from an investigation of six honey samples collected from north-central Nigeria.

Materials And Methods

Collection Of Samples

Six honey samples were collected from various locations within the north-central region of Nigeria, specifically from the Benue and Kogi States (Figure 1). The sampling sites in Benue State included Jato-Aka, Naka, and Otukpa, while those in Kogi State comprised the Bassa, Omala, and Ofu Local Government Areas. These locations

were selected to ensure a broad representation of honey produced across the different ecological zones within the region. Following collection, each honey sample was carefully transferred to clean, dry containers with tight-fitting lids to prevent contamination and moisture absorption, which could compromise the physicochemical integrity of the samples. The containers were then stored at ambient room temperature under stable conditions to preserve the natural quality and composition of honey.

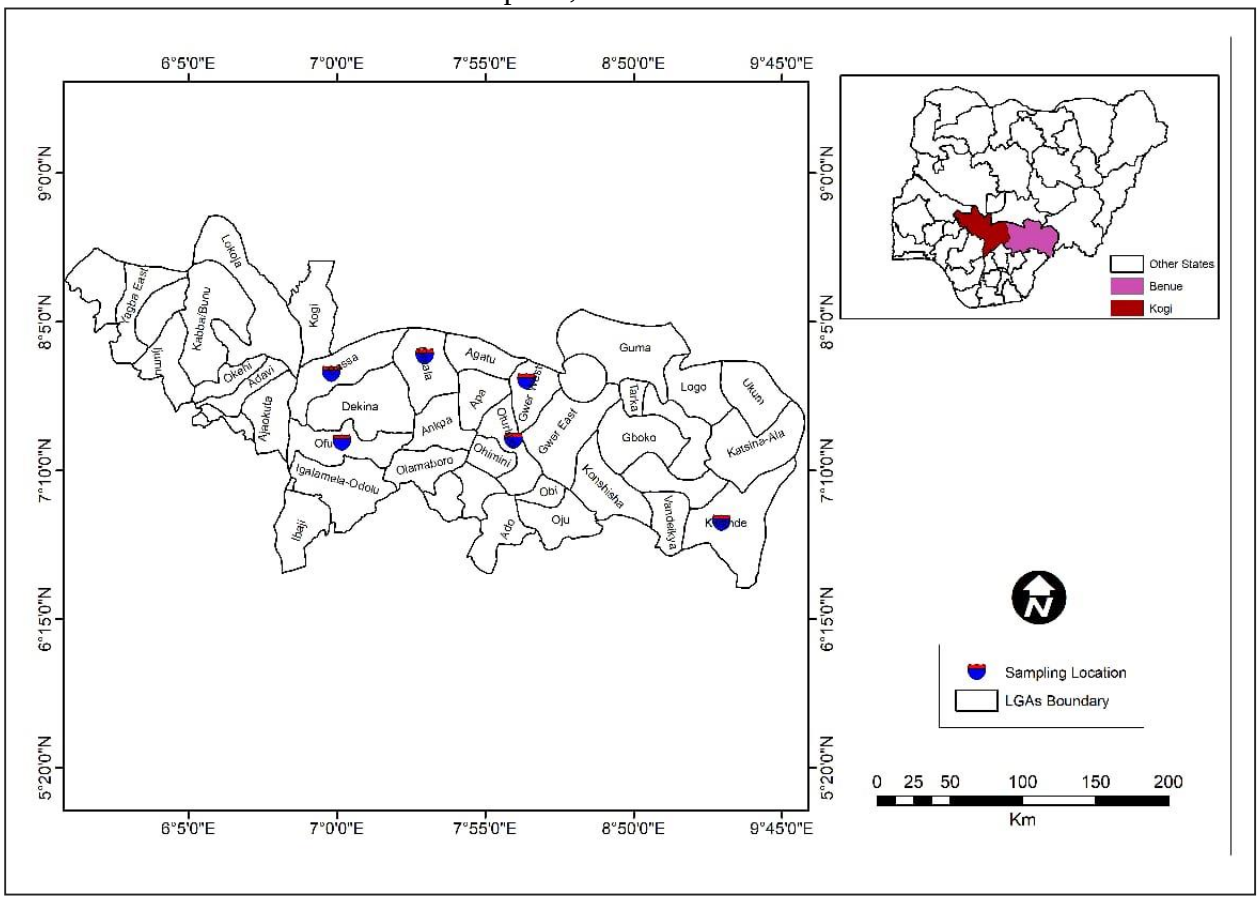


Figure 1: The study area (Adapted from Ikegbunam *et al.*, 2023)

Physicochemical analysis

The moisture content of the honey samples was assessed using the hot oven method outlined by the Association of Official Analytical Chemists (AOAC, 2010). Specifically, ten grams (10 g) of each honey sample was placed in a pre-weighed silica dish and dried in a hot-air oven (Heraeus 064054, Hanau, Germany) at 100°C until a constant weight was achieved. Protein content was quantified using the Kjeldahl method (AOAC, 2010). Two grams (2 g) of honey was measured using an analytical balance (Mettler Toledo PL 303, Colmworth, UK) and combined with 5 g of anhydrous sodium sulfate, 1 g of copper sulfate, and a trace amount of selenium. This mixture was digested and diluted to 250 ml with distilled water in a volumetric flask. A 10 ml aliquot was distilled, and the released ammonia was absorbed in 10 ml of a boric acid indicator solution. Then, the flask was removed and titrated with 0.01N hydrochloric acid. Fat content was extracted using the Soxhlet extraction method (AOAC, 2010), with samples undergoing continuous extraction with petroleum ether for four hours. Ash

content was determined by weighing 5 g of honey in a previously ignited and weighed crucible. Pre-ashing was conducted using an electric heater (Model QCP-100D, China) within a fume hood (Labe 1286, Delhi, India), followed by incineration in an oven (Heraeus 064054, Hanau, Germany) at 100°C (AOAC, 2010). The pH of honey was determined using a digital pH meter (Jenway, Model 3510, Staffordshire, UK). A solution comprising 1 ml of honey and 10 ml of distilled water was prepared, and the pH was measured using a digital display (AOAC, 2010). The total polyphenol content was quantified using a spectrophotometer (AE–IID, Ningbo, Zhejiang, China), with absorbance measured at 490 nm, in accordance with standard AOAC (2010) procedures. The free acidity was evaluated by titration using phenolphthalein as an indicator. The volume of alkali used in the titration was recorded and subsequently used to calculate free acidity (AOAC, 2010). The (HMF) content was assessed spectrophotometrically following the method of White (1979), with absorbance recorded at 284 nm and 336 nm, and the HMF content was calculated by comparing the test sample with a reference. The concentrations of fructose, glucose, and sucrose were determined using a colorimeter (Jenway 6050, Staffordshire, UK), with absorbance readings taken at 520, 549, and 620 nm, respectively (AOAC, 2010).

Mineral and Heavy Metal Analysis

Calcium and magnesium concentrations were determined by titration according to AOAC (2010) guidelines. Twenty-five milliliters of the digested sample (25 ml) were used. A pinch of the Calcine indicator was added for calcium determination, and the Eriochrome Black T (EBT) indicator was used for magnesium determination. Titration was performed using a 0.01N EDTA solution. Sodium and potassium levels were measured via flame photometry using a Jenway PFP 7 flame photometer (Staffordshire, UK). Two grams (2 g) of the sample were digested and dissolved in 10 ml of 30% hydrochloric acid (HCl). Aliquots were analyzed and concentrations were calculated using calibration curves prepared from standards (AOAC, 2010). Pb and Cd concentrations were measured using a spectrophotometer (AE–IID, Ningbo, Zhejiang, China). Ten milliliters of the digested sample (10 ml) were used. Absorbance was read at 520 nm for lead and 518 nm for cadmium (AOAC, 2010), and concentrations were extrapolated from the standard calibration curves. Zinc concentration was determined following the method of Pearson (1976). Ten millilitres (10 ml) of the digested sample were mixed with 5 ml of acetate buffer, 10 ml of sodium thiosulfate solution, and 10 ml of dithizone solution (II). Absorbance was measured at 535 nm using a spectrophotometer (AE–IID, Ningbo, Zhejiang, China).

Results

The results of the proximate analyses are presented in (Table 1). From the results, it was observed that the least value (8.08 ± 0.02%) for moisture was recorded in sample from Bassa. Also, all the samples contained the required amount of moisture for quality honey. For fats content, samples from Omala, ofu, Bassa and Otukpa had no fats in them. There were no significant differences in the value of free acidity recorded in samples from Omala, Ofu and Bassa. However, significant differences exited in the recorded values from Jato-aka, Naka and Otukpa at $P \leq 0.05$. For protein, the highest value (0.56 ± 0.02%) was recorded in sample collected from Ofu whereas the least value (0.28 ± 0.01%) was observed in Naka sample. There were significant differences in the values recorded for ash across most of the locations though samples from Bassa and Ofu (0.86 ± 0.01% and0.81 ± 0.05%) had similar values. The highest value (90.12 ± 0.07%) for polyphenol was observed in samples from Bassa and this was significantly different from all other locations at $P \leq 0.05$(Table 1).

The results of sugar composition showed that samples from Ofu and Omala had similar values (28.35 ± 0.14 mg/100g and 28.25 ± 0.09 mg/100g) which were significantly different from the recorded values in other samples. The least values (21.20 ± 0.00 mg/100g and 21.20 ± 0.00 mg/100g) for glucose were observed in samples from Jato-aka and Otukpa while Bassa sample had the highest value (30.35 ± 0.09 mg/100g). There were significant differences in the recorded values (5.50 ± 0.29 mg/100g, 4.75 ± 0.14 mg/100g and 2.95 ± 0.03 mg/100g) for sucrose in samples from Jato-aka, Naka and Omala at P≤0.05. The HMF values (17.47 ± 0.26 mg/kg and 16.13 ± 2.62 mg/100g) for samples from Jato-aka and Bassa were similar and significantly different from values from other locations. The pH values for all the samples were acidic. However, the highest value (4.30 ± 0.17) was observed in sample from Jato-aka (Table 2).

Mineral analysis revealed the following concentration ranges (mg/100 g): sodium (1.80 ± 0.06 to 9.75 ± 0.14), potassium (12.89 ± 0.03 to 27.62 ± 0.02), calcium (3.55 ± 0.20 to 9.50 ± 0.23), magnesium (2.00 ± 0.06 to 7.60 ± 0.17), and zinc (2.15 ± 0.09 to 4.10 ± 0.06). Potassium was the most abundant mineral (Table 3). The samples from Omala and Ofu had similar potassium levels, which were significantly different from those at other locations. Magnesium levels varied significantly among Naka, Omala, and Ofu samples ($P \leq 0.05$), while the highest calcium concentration (9.50 ± 0.23 mg/100g) was recorded in the Naka sample. Cadmium and lead were absent in all the samples.

Table 1: Proximate composition of honey samples collected from different Local Government Areas of Kogi and Benue State, Nigeria

Locations	Jato-aka	Naka	Otukpa	Bassa	Omala	Ofu
Moisture (%)	9.27 ± 0.23^{b}	10.34 ± 0.02^{a}	8.35 ± 0.33^{cd}	8.08 ± 0.02^{d}	8.85 ± 0.09^{bc}	8.54 ± 0.07^{cd}
Fats (%)	0.54 ± 0.03^{a}	0.30 ± 0.06^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Free Acidity (Meq/kg)	52.40 ± 4.39^{b}	57.40 ± 0.81^{ab}	61.60 ± 3.23^{a}	2.72 ± 0.10^{c}	3.00 ± 0.12^{c}	3.10 ± 0.06^{c}
Protein (%)	0.48 ± 0.01^{de}	0.28 ± 0.01^{gh}	0.30 ± 0.01^{fg}	0.50 ± 0.06^{c}	0.45 ± 0.02^{e}	0.56 ± 0.02^{bc}
Ash (%)	0.41 ± 0.01^{c}	0.29 ± 0.02^{d}	0.19 ± 0.01^{e}	0.86 ± 0.01^{a}	0.72 ± 0.01^{b}	0.81 ± 0.05^{a}
Polyphenol (%)	3.75 ± 0.72^{c}	5.00 ± 0.00^{c}	5.25 ± 0.43^{c}	90.12 ± 0.07^{a}	83.50 ± 0.87^{b}	81.60 ± 0.92^{b}

Data are presented as mean ± Standard error. Means with different alphabets on each row represents significant differences separated using Fisher Least Significant Difference (F-LSD) test at $P \leq 0.05$.

Table 2: Physicochemical composition of honey samples collected from different Local Government Areas of Kogi and Benue States, Nigeria

Locations	Jato-aka	Naka	Otukpa	Bassa	Omala	Ofu
Fructose (mg/ 100g)	48.90 ± 0.17^{bc}	48.60 ± 0.00^{c}	49.50 ± 0.29^{a}	30.35 ± 0.09^{d}	28.35 ± 0.14^{e}	28.25 ± 0.09^{e}
Glucose (mg/100g)	21.20 ± 0.00^{d}	23.20 ± 0.35^{c}	21.20 ± 0.00^{d}	30.35 ± 0.09^{a}	28.35 ± 0.14^{b}	28.25 ± 0.09^{b}
Sucrose (mg/100g)	5.50 ± 0.29^{a}	4.75 ± 0.14^{bc}	4.50 ± 0.29^{c}	4.65 ± 0.09^{c}	2.95 ± 0.03^{d}	2.55 ± 0.20^{e}
HMF (Mg/Kg)	17.47 ± 0.26^{d}	43.27 ± 4.36^{c}	59.47 ± 0.12^{b}	16.13 ± 2.62^{d}	66.77 ± 6.78^{a}	46.37 ± 1.18^{c}
pH	4.30 ± 0.17^{a}	3.95 ± 0.03^{d}	4.00 ± 0.00^{cd}	4.07 ± 0.07^{bc}	4.00 ± 0.00^{cd}	4.13 ± 0.07^{b}

Data are presented as mean ± Standard error. Means with different alphabets on each row represents significant differences separated using Fisher Least Significant Difference (F-LSD) test at $P \leq 0.05$.

Table 3: Mineral composition of honey samples collected from different Local Government Areas of Kogi and Benue States, Nigeria

Locations	Jato-aka	Naka	Otukpa	Bassa	Omala	Ofu
Na (mg/100g)	4.32 ± 0.00^{a}	4.09 ± 0.01^{c}	4.18 ± 0.01^{b}	8.70 ± 0.12d	9.75 ± 0.14^{d}	1.80 ± 0.06^{e}
K (mg/100g)	27.62 ± 0.02^{a}	12.89 ± 0.03^{e}	24.61 ± 0.05^{b}	21.22 ± 0.12^{c}	17.00 ± 0.58^{d}	17.65 ± 0.20^{d}
Zn (mg/100g)	3.60 ± 0.06^{b}	4.10 ± 0.06^{a}	3.45 ± 0.03^{b}	2.15 ± 0.09^{d}	2.70 ± 0.17^{c}	2.60 ± 0.12^{c}
Ca (mg/100g)	5.35 ± 0.38^{b}	9.50 ± 0.23^{a}	4.85 ± 0.20^{bc}	3.57 ± 0.19^{d}	4.20 ± 0.17^{c}	3.55 ± 0.20^{d}
Mg (mg/100g)	4.30 ± 0.29^{b}	7.60 ± 0.17^{a}	3.90 ± 0.17^{b}	2.15 ± 0.09^{cd}	2.59 ± 0.08^{c}	2.00 ± 0.06^{d}
Cd	ND	ND	ND	ND	ND	ND
Pb	ND	ND	ND	ND	ND	ND

Data are presented as mean ± Standard error. Means with different alphabets on each row represents significant differences separated using Fisher Least Significant Difference (F-LSD) test at $P \leq 0.05$. ND- Not detected.

Discussion

The proximate and physicochemical analyses of all honey samples revealed that most were of acceptable quality, with only a few exhibiting parameter values slightly above the recommended standards. The moisture content across all samples remained within the Codex Alimentarius Commission (2001) regulatory limit of 21%, indicating good quality. Moisture is a crucial quality determinant that influences the shelf life of honey and its resistance to microbial spoilage (Iglesias *et al.,* 2012). The moisture values recorded in this study were lower than those reported by Oyeyemi *et al.* (2015), Kayode and Oyeyemi (2014), and Oshomah and Agbaji (2015), as well as stingless bee honeys studied by Alves *et al.* (2008) and Anacleto *et al.* (2009). Variations in moisture content are often influenced by environmental conditions and harvest practices (Acquarone *et al.,* 2007) and may also be related to the floral origin of honey (Malika *et al.,* 2005). Higher moisture levels suggest adulteration (Nyau *et al.,* 2013).

The detection of trace proteins in honey samples indicates their nutritional richness, supporting their suitability as dietary supplements (Njokuocha and Osayi, 2015).

The protein levels observed were consistent with the findings of Buba *et al.* (2013) and Adeniyi *et al.* (2014) for *Apis mellifera* honeys in Nigeria. Lower protein values were recorded by Ndife *et al.* (2014), whereas higher values were reported by Agunbiade *et al.* (2012) and Oyeyemi *et al.* (2015). Proteins in honey mainly originate from pollen grains in addition to animal and plant sources (Bosnar and Sakac, 2012; Escuredo *et al.,* 2013). The ash content in samples from Jato-aka, Naka, and Otukpa fell within the typical range of natural nectar honeys, which contain less ash than honeydew honeys (Viuda-Martos *et al.,* 2010). These findings are consistent with those reported by Ayansola and Banjo (2011), Agbagwa *et al.* (2011), and Oyeyemi *et al.* (2015). The variability in ash content is often attributed to its floral origin (Molan, 1992). Ash reflects the total inorganic mineral content post-combustion (Vanhanen *et al.,* 2011), and the Codex standard recommends levels not exceeding 0.6%.

The hydroxymethylfurfural (HMF) content varied among samples, ranging from 17.47 66.77 mg/kg. Most samples had HMF levels within the acceptable limits (<40 mg/kg) set by the Codex Alimentarius (2001) and the European Union Commission (2002). Jato-aka and Bassa samples met the standards, indicating freshness (Getachew *et al*. 2014). Elevated HMF levels in Omala, Ofu, Naka, and Otukpa suggest prolonged storage, as HMF content increases over time (Tornuk *et al.,* 2013). HMF is a key indicator of honey degradation (Oshomah and Agbaji, 2015).

The pH and free acidity influence the texture, palatability, and shelf life of honey (Njokuocha and Osayi, 2015). Organic acids contributing to honey acidity include gluconic acid and citric acid (Mato *et al.,* 2006). All the samples were acidic, with pH values consistent with those reported by Kayode and Oyeyemi (2014) and Njokuocha and Osayi (2015). Free acidity, a parameter indicating honey spoilage, should not exceed 50 Meq/kg (Codex Alimentarius, 2001). Only samples from Bassa, Omala, and Ofu met this standard; elevated levels in others suggested fermentation during storage.

Fat was absent in samples from Otukpa, Bassa, Ofu, and Omala, whereas trace amounts were found in Jato-aka and Naka. This aligns with the findings of Ndife *et al.* (2014), who reported 0.14% to 0.21% fat in honey from various Nigerian regions. Minor fat traces may result from wax melting during harvesting (Tan *et al.,* 1988). Honey contains various minor constituents with antioxidant properties, primarily owing to its phenolic compounds (Gheldof *et al.,* 2003). Polyphenol levels were lower in some samples, potentially because of their lighter color. Darker honey is known to have a higher polyphenol content and antioxidant activity (Anklam and Radovic, 2001). Similar findings have been reported by Eleazu *et al.* (2013).

Carbohydrates form the bulk of the dry matter of honey (95–99%) (Olaitan *et al.,* 2007). Glucose and fructose were the dominant sugars, which is in agreement with the results of Ayansola and Banjo (2011) and Buba *et al.* (2013). Although no standard exists for individual sugar levels, their combined concentration should be ≥60 g/100 g (Codex, 2001). The samples from Omala and Ofu fell short of this threshold. The sucrose content met international standards (<5 g/100 g) in all but the Jato-aka sample. These findings are consistent with those reported by Buba *et al.* (2013), Cantarelli *et al.* (2008), and Vit *et al*. (2009) but were lower than the values reported by Njokuocha and Osayi (2015).

Mineral analysis revealed varying concentrations of major and trace elements, consistent with previous reports from Nigeria and beyond (Adeniyi *et al.,* 2004; Adeniyi *et al.,* 2014; Kambai *et al.,* 2015). The mineral

concentrations followed the order of K > Ca > Na > Mg > Zn. This trend aligns with those reported by Agbagwa *et al*. (2011), Ndife *et al.* (2014) and Obiegbuna *et al.* (2017). Soil type, atmospheric deposition, and agricultural practices can influence mineral levels (Marcovecchio *et al.,* 2007). Mineral variability also reflects the plant species visited by bees (Shah *et al.,* 2014) and these elements are vital for human health (James *et al.,* 2009). Trace elements, such as zinc, were detected at higher concentrations than previously reported by Njokuocha *et al.* (2019) but were consistent with those reported by Oyeyemi *et al.* (2015). Zinc plays a critical role in enzyme function and wound healing (Levenson and Demetriou, 1992). They can enter honey through environmental contamination and plant sources. Cadmium and Lead were not detected in any of the samples, although trace amounts have been found in other studies (Tuzen, 2002; Belouali *et al.,* 2008; Moniruzzaman *et al.,* 2014).

Conclusion

The findings of this study revealed that the honey samples contained all the investigated mineral elements; calcium (Ca), potassium (K), magnesium (Mg), and sodium (Na) in appreciable amounts. The trace element zinc (Zn) was also present in low concentrations, all within acceptable safety limits. Notably, the heavy metals cadmium and lead were not detected in any of the samples, indicating the absence of toxic contamination. These mineral profiles suggest that the honey is a valuable dietary supplement for meeting daily human nutritional needs. The pH values confirmed that all samples were acidic, which contributes to enhanced shelf life and product stability. Overall, the physicochemical characteristics of the honey indicate that the North Central region holds significant potential for producing high-quality honey that meets international standards.

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