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Physico-chemical properties and mineral content of *Apis Mellifera* L. honey samples sourced from different localities in Anambra and Enugu States, South-eastern, Nigeria

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Abstract

Six honey samples were collected from various locations in Anambra and Enugu states in southeastern Nigeria and analyzed for physicochemical characteristics and mineral composition. pH, moisture, protein, fats, ash, polyphenol, free acidity, hydroxymethylfurfural (HMF), and sugar were among the physicochemical parameters studied. Minerals such as potassium, calcium, zinc, magnesium, sodium, cadmium, and lead were also investigated. The samples had pH values ranging from 4.00 - 4.40. Moisture content ranged from 8.95% - 14.30%, ash 0.21 - 0.54%, protein 0.21-0.74%, fat 0.00 - 0.50%, polyphenol 2.75 - 12.00%; free acidity 33.60 - 89.890 meq kg-1 and HMF 18.70 - 75.43 mg/kg. The sugar assays revealed that all of the honey samples contained the appropriate quantity of sugar for acceptable quality honey, albeit there were substantial variances in the values recorded across the locations. The mineral composition revealed that potassium was the most abundant element, followed by zinc, calcium, magnesium, and sodium. In the samples, no cadmium or lead was found. The results of the evaluated honey samples revealed that the majority of the measured parameters recorded met international standards, indicating that they were safe for human consumption.

1. INTRODUCTION

Honey's use dates back to the dawn of time, and its nutritional, medicinal, and preventive properties have made it a valuable medicine and sustenance. Natural honey is a sweet liquid made by bees from nectar collected from a variety of high-energy flowering plants. It's the only sweetening agent that does not require processing before being consumed by humans. It is reported to contain around 200 substances in addition to its components and flavour (Ramanauskiene *et al.,* 2012). Bee species, meteorological conditions, soil type, colony physiological state, nectar source, and honey maturity all influence the chemical composition of honey (Carvalho *et al.,* 2005). Antioxidant and antibacterial activity, as well as specialized therapeutic characteristics, are among their medical qualities. Honey, according to Bogdanov (2009), contains trace amounts of minerals, proteins (mostly enzymes), amino acids, and other acids such as lactic, formic, acetic, pyruvic, and others. Honey's positive effects are attributed to a variety of minor components found in honey, including polyphenols, enzymes, free amino acids, proteins, minerals, and vitamins (Bogdanov, 2017). It's antibacterial, anti-inflammatory and antioxidant qualities have all been proven to be useful. Antimicrobial action is critical in the treatment of infections, especially when the body's immune response is insufficient to eliminate the infection.

Heavy metals were identified near particular industrial sites, traffic-related pollution, and chemical-intensive agriculture, according to Buba *et al.,* (2013), contaminating the air, water, and soil. Certain factors may also play a role in the rising levels of these components in honey. Honey, like food used for healing, must be free of contaminants and toxins that enter the product as a result of beekeeping operations, anthropogenic sources, and honey processing. Because minerals are transferred into plants through the roots and eventually to other sections of the plant, including the nectar, and then to the honey, the amount of different minerals in honey is primarily dependent on the soil composition and the various types of floral plants. Except for chlorine, which was only identified in honey samples from Spain (Gonzalez Paramas *et al.,* 2000), practically all macro minerals are typically present in honey from all nations. When microelements are present in optimal concentrations, they are beneficial to people, especially if they come from an organic source. If they come from an inorganic source, however, they will have a specific gravity of at least 5 times that of water and will be poisonous. The inability of the heavy metal to be digested by the organism causes toxicity, resulting in a build-up in human or animal soft tissues without being completely inactivated or eliminated (Ajibola *et al.,* 2012). Metabolic

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irregularities, nausea, migraines, respiratory diseases, and vomiting are just a few of the health problems induced by heavy metals. Minerals and heavy metals are key constituents of honey and have unique functions in human health. As a result, the goal of this research is to look into the physicochemical properties and mineral content of honey samples collected from various locations in southeastern Nigeria.

2. MATERIALS AND METHODS

Six honey samples were collected from colonized hives of *Apis Mellifera* L. honey in Anambra and Enugu state. All the honey samples were stored in containers with tight-fitting lids at an ambient temperature to avoid moisture absorption.

2.1. Proximate analysis

Physicochemical analyses including polyphenol, free acidity, pH level, moisture, ash, fat, protein, and sugar types were determined according to the protocols as detailed in A.O.A.C (2012) whereas Hydroxymethylfurfural (HMF) was determined using spectrophotometric method (White, 1979).

2.2. Mineral analysis

The pH of the samples was determined with a pH meter, while the moisture content was determined using the drying oven method, by drying a representative 5 g sample in an oven at 105 °C for 3 hrs until the weight was constant. The ash content was determined by the incineration of a 4 g sample in a muffle furnace at 600 °C for 6 h until the ash turned whitish-grey colour. The fat content was determined by petroleum ether extraction in a Soxhlet apparatus. A representative 3 g of sample was extracted for 6 h. Crude protein was estimated by the Kjeldahl method. Total protein was calculated by multiplying the evaluated nitrogen by 6.25. Each of the trace mineral elements was read at its respective wavelength with their respective cathode lamps using appropriate fuel and oxidant combination (AOAC, 2010). The colour of the honey samples were determined by comparing the samples with Munsel colour chart. The fructose, glucose and sucrose contents of the honey samples were analysed according to the methods of (AOAC, 2012).

2.3. Statistical analysis

The data were presented as means ± standard deviations. Analysis of variance (ANOVA) was used to compare the quantified variables in the samples of honey. Significant differences were separated using Duncan New Multiple Range Test (DNMRT) at $P < 0.05$.

3. RESULT AND DISCUSSION

The physicochemical composition and proximate analysis of six honey samples collected from six different locations within Enugu and Anambra State, as shown in Table 1, revealed that all of the samples contained the appropriate amount of moisture, though the values varied significantly across the locations. Fats was not present in most of the samples. The majority of the samples were devoid of fats. The protein content ranged between 0.21% - 0.74 %. The moisture content of the honey sample from Ndiagu was the greatest, and it differed significantly from the values collected from the other locations. The protein level of honey from Njikoka was not considerably different from that of Obollo Afor honey. Similarly, there was no significant difference in protein content between Ayamelum and Njikoka. Apart from the Ekusigo sample, the ash concentration of the honey sample taken from Njikoka was the greatest, and this differed substantially at $p< 0.05$ from all the other locations. Polyphenol was found in every sample. The honey sample from Abolo Afor had the lowest polyphenol content which differ from the value obtained from all the other locations.

Table 1: Proximate composition of honey samples across different Local Government Areas in Enugu and Anambra States of Nigeria

Locations	Moisture	Fat	Protein	Ash	Polyphenol
Ayamelum	11.84 ± 0.96^{b}	0.00 ± 0.00^{c}	0.61 ± 0.03^{b}	0.37 ± 0.04^{b}	11.00 ± 0.58^{ab}
Ekwusigo	9.93 ± 0.13^{c}	0.00 ± 0.00^{c}	0.57 ± 0.02^{bc}	0.54 ± 0.02^{a}	5.00 ± 0.00^{c}
Njikoka	7.62 ± 0.08^{d}	0.00 ± 0.00^{c}	0.22 ± 0.01^{d}	0.55 ± 0.03^{a}	12.00 ± 1.15^{a}
Eziani	14.34 ± 0.14^{a}	0.30 ± 0.06^{b}	0.48 ± 0.06^{c}	0.20 ± 0.00^{c}	10.00 ± 0.00^{b}
Obollo-Afor	8.95 ± 0.02^{c}	0.50 ± 0.06^{a}	0.21 ± 0.01^{d}	0.21 ± 0.03^{c}	2.75 ± 0.14^{d}
Ndiagu	9.08 ± 0.19^{c}	0.00 ± 0.00^{c}	0.74 ± 0.04^{a}	0.35 ± 0.03^{b}	5.00 ± 0.00^{c}

Means with different alphabet on each column represents significant differences separated using Duncan New Multiple Range Test (DNMRT) at $P < 0.05$.

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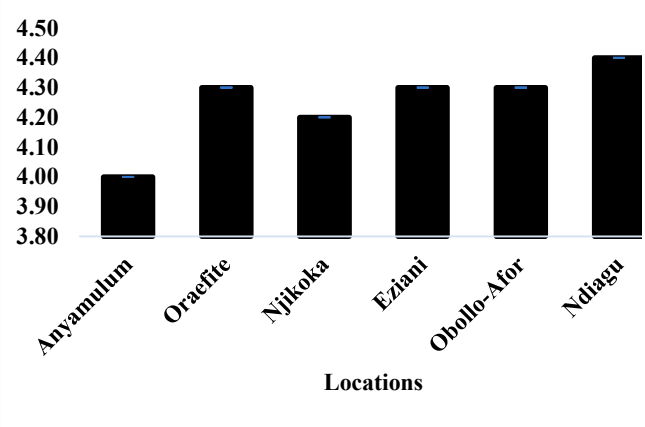

Figure 1. pH level of honey samples across different Local Government Areas in Enugu and Anambra States of Nigeria

Except for a few samples where the values of the measured parameters were slightly higher than the suggested norm, the biochemical composition of all the studied honey samples revealed that the majority of the samples were quality honey. The moisture content recorded from the samples was less than the regulatory standard of 21% specified by the Codex Alimentarius Commission for quality honey (2001) (Figure 1). These could be due to the low relative humidity and dry weather conditions that prevailed during the collecting period, as honey is more prone to ferment during the rainy season than during the dry season. Furthermore, the bulk of the honey samples were collected in the bee farm alongside the beekeeper, eliminating the possibility of adulteration. Adulteration is indicated by honey with a high moisture content (Nyau *et al.,* 2013). The beekeeper's operation, floral origin, processing techniques, and suitable storage facilities were other important variables. Honey's low moisture content helps to protect it from microorganism deterioration. Honey with a high water content is more likely to ferment, making preservation and storage more difficult (Iglesias *et al.,* 2012).

Honey's protein level recorded were within the range 0.21 - 0.74%. Njikoka and Obollo-Afor recorded the least protein content. Honey contains a trace amount of protein usually originated from pollen which is a plant source. The variability in protein content of different types of honey might be as a result of the origin of honey and the type of pollen present. The findings of this study are similar with Adeniyi et al. (2014) analysis on the protein content of Nigerian honey from *Apis mellifera*. The high intake of pollen grains and other variables could explain the abundance of protein in honey.

The ash concentration of honey samples from both states was within the normal range for natural nectar honey. The ash values found in this study were similar to those found by Ayansola and Banjo (2011) in honey samples collected in southwestern Nigeria, where the ash level ranged from 0.14 to 0.71 %. The findings of this study back up previous research on multiple Nigerian honey samples from various regions, which indicated that the ash percentage of honey samples ranged from 0.05 to 0.79 %. Except for a few samples where the analysed parameters had low values, the biochemical makeup of all the honey samples studied revealed that the bulk of them were slightly higher than the required standard. The total amount of inorganic minerals in a sample is represented by ash content after burning. The Codex Alimentarius Commission (2001) stipulated that the level of ash in a sample should not exceed 0.6 % for grade honey.

The findings of the HMF revealed considerable variances across honey samples from various places. HMF concentrations ranged from 20.9 mg/kg to 54.7 mg/kg. With the exception of a couple of samples that were a little closer to or above the Codex Alimentarius Commission's suggested HMF threshold value of 40 mg/kg, some honey samples had lower HMF readings (2001). Anambra and Ebonyi honey samples had levels that were within the required grade honey threshold. The honey samples are still fresh, as seen by this. HMF readings in honey samples collected in Enugu State were slightly higher than the permissible range. Because HMF levels in honey rise throughout storage, this could be due to the amount of time the honey has been stored.

Acidity levels in all of the honey samples were found to be high. The pH levels were within the previously specified norms (Kayode and Oyeyemi, 2014; Njokuocha and Osayi, 2015). The fermentation of carbohydrates in honey into organic acid, which is responsible for two major features of honey: flavour and stability against microbial spoilage, and stimulates wound healing by releasing oxygen from haemoglobin, corresponds with the acidity of the honey samples. The Codex Alimentarium Commission (2001), on the other hand, recommends a maximum value of fifty (50 meq/kg). As a result, the honey samples were found to contain levels beyond the statutory limit, which could have been caused by high mineral content (Mohammed and Babiker, 2009).

Fat was not found in Anambra State honey samples, but it was found in trace amounts in Enugu State samples. Ndife et al. (2014) discovered low fat content (0.14 - 0.21%) in four honey samples collected from different floral regions in Nigeria (Abuja, Nsukka, Ibadan, and Kaduna). This means that the honey is low in fat or fat-free. The small amount detected, on the other hand, could be attributable to wax melting in the beehive, most likely during harvest. The honey samples had low polyphenol levels ranging from 4.7-9.3 %, which could be attributed to the honey's bright colour. Eleazu et al. (2013) discovered similar results in a study that examined the effect of colour on physicochemical composition, microbiological purity, and antioxidant activity in five samples. The findings revealed that glucose and fructose were the primary sugars in all of the samples tested (Table 2), which is consistent with previous findings (Ayansola and Banjo 2011; Buba *et*

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al., 2013). Although fructose and glucose are the most common sugar types in honey, there are no established limits for their individual values. However, the Codex Alimentarius Commission's standard limit for quality honey is 60 g/100 g of honey (Fructose + glucose) (2001). The fructose and glucose summation of the honey samples, as recorded in this investigation, suggests that the levels are within the international standard's limit. The apparent sucrose level in the honey samples was also within the accepted international standard for quality honey. High-quality honey should not contain more than 5 g/100 g of sugar, according to the Codex Alimentarius Commission's (2001) global guideline.

The mineral content of honey samples was investigated, and some of the major and trace elements found in the honey were discovered. Mineral concentrations in honey samples were in the following order: K > Ca > Zn > Mg > Na (Table 3). The findings corroborated the findings of Agbagwa et al. (2011) and Ndife et al. (2014), who found potassium dominance in honey samples. Similarly, Obiegbuna et al. (2017) found that potassium and calcium predominated in honey samples collected from several areas in Anambra State. This difference in mineral content could be explained by the composition of the source plants' soil, which is influenced by atmospheric precipitation and the usage of pesticides and fertilizers in crop fields (Marcovecchio *et al.,* 2007). The mineral composition of honey might also vary according to the different plant species visited by honey bees when collecting nectar. In human health, these minerals perform a wide range of physiological and biochemical roles.

The calcium amounts found in honey samples from Enugu state in this investigation were similar to those found in previous studies by (Ndife *et al.,* 2014 and Oyeyemi *et al.,* 2015). The calcium concentration, on the other hand, was lower than that found in Nigerian and Malaysian honey by (Adeniyi *et al.,* 2014; Moniruzzaman *et al.,* 2014 and Njokuocha *et al.,* 2019). Calcium and magnesium are essential for the growth and maintenance of teeth, bones, and muscles, as well as the prevention and management of hypertension and cardiovascular disease. Despite this, the potassium concentration in honey samples was the highest of all the minerals tested. One possible explanation for the honey samples' lower potassium values is the presence of smaller levels of this mineral in the plant leaves, which could be transferred to the nectar.

Sodium (Na) and zinc levels in honey samples from Anambra and Enugu States were lower and higher respectively. The zinc contents were higher than those found by Njokuocha et al. (2019) in their examination of mineral compositions of honey samples collected from various parts of Nigeria, including Nsukka (Enugu State) and Aguata (Anambra).

Table 2: Composition of sugar in honey samples across different Local Government Areas in Enugu and Anambra States of Nigeria

Locations	Free acidity	Glucose	Fructose	Sucrose	HMF
Anyamulum	68.60 ± 0.81^{c}	49.40 ± 0.12^{ab}	27.65 ± 0.09^{c}	5.25 ± 0.14^{c}	22.30 ± 0.38^{d}
Oraefite	69.20 ± 5.31^{c}	49.00 ± 0.35^{a}	31.26 ± 1.44^{b}	7.50 ± 0.29^{a}	21.63 ± 0.43^{d}
Njikoka	33.60 ± 0.00^{d}	49.10 ± 0.06^{a}	23.75 ± 0.72^{d}	4.05 ± 0.09^{d}	18.70 ± 0.20^{e}
Eziani	78.50 ± 3.18^{b}	49.30 ± 0.17^{a}	30.63 ± 1.81^{bc}	5.25 ± 0.43^{c}	75.43 ± 1.43^{a}
Obollo-Afor	66.10 ± 0.64^{c}	49.20 ± 0.00^{a}	23.78 ± 0.01^{d}	5.00 ± 0.00^{c}	26.60 ± 0.15^{c}
Ndiagu	89.80 ± 0.12^{a}	49.20 ± 0.00^{a}	36.88 ± 1.09^{a}	6.70 ± 0.17^{b}	62.17 ± 1.01^{b}

Means with different alphabet on each column represents significant differences separated using Duncan New Multiple Range Test (DNMRT) at $P < 0.05$. Glucose, Fructose and Sucrose at (mg/100g).

Table 3: Mineral composition of honey samples across different Local Government Areas in Enugu and Anambra States of Nigeria

Locations	Na mg/100g	K mg/100g	Zn mg/100g	Ca mg/100g	Mg mg/100g
Anyamulum	1.92 ± 0.00^{e}	26.48 ± 0.05^{c}	3.70 ± 0.00^{d}	2.69 ± 0.18^{b}	2.15 ± 0.14^{d}
Oraefite	3.40 ± 0.01^{b}	30.50 ± 0.05^{b}	4.55 ± 0.03^{b}	2.49 ± 0.05^{b}	2.00 ± 0.06^{d}
Njikoka	2.17 ± 0.01^{d}	13.21 ± 0.04^{f}	3.75 ± 0.09^{d}	4.73 ± 0.00^{a}	3.90 ± 0.06^{b}
Eziani	1.22 ± 0.00^{f}	15.27 ± 0.01^{e}	4.00 ± 0.00^{c}	4.72 ± 0.07^{a}	4.00 ± 0.00^{b}
Obollo-Afor	2.70 ± 0.01^{c}	18.70 ± 0.04^{d}	4.45 ± 0.26^{b}	4.87 ± 0.08^{a}	4.50 ± 0.29^{a}
Ndiagu	4.01 ± 0.00^{a}	34.05 ± 0.02^{a}	5.25 ± 0.14^{a}	4.82 ± 0.05^{a}	3.80 ± 0.06^{c}

Means with different alphabet on each column represents significant differences separated using Duncan New Multiple Range Test (DNMRT) at $P < 0.05$

The observed variance in zinc level in honey samples could be due to botanical factors such as the varied types of plants foraged by bees and the mineral compositions of the soil. Apart from direct excretion via nectar, such components may enter honey via other routes, such as dust or aerosols landing on flower and nectar surfaces, leaf surfaces, honeydew, or the bees themselves. Other studies have shown zinc levels in honey that are

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significantly lower than those found in this study, and these discrepancies in mineral content can be linked to variances in geographical and botanical origins of the honey (Moniruzzaman *et al.,* 2014).

The Earth's crust contains naturally occurring heavy metals that cannot be degraded or removed. Heavy metals like copper, selenium, and zinc play a crucial role in the human body's metabolism. In higher amounts, however, they can be harmful. Because of the toxicological effects of lead (Pb), tests on the level of this element in honey have been carried out (Oyeyemi *et al.,* 2015). Cadmium and lead were not found in any of the honey samples tested. However, trace quantities of these heavy metals have been found in honey samples (Moniruzzaman *et al.,* 2014).

4. CONCLUSION

In general, the honey from Nigeria's Southeastern region was of good quality. With a few exceptions, the average values of the physicochemical characteristics and minerals identified in the honey samples examined were in accordance with international standards.

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