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Determination of nutritional properties of honey from *Apismellifera*

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Abstract
Honey is a natural sweet viscous liquid product produced by various honey bees from the nectar of blossoms or from the secretion of living parts of plants that has been consumed since early humans. Honey is noted for its high nutritional values and beneficial roles in human health. The purpose of this study was to evaluate the nutritional properties of honey from *Apis mellifera* by determining proximate, micro and macro mineral compositions using the standard method of Association of Analytical Chemist (AOAC). Proximate results obtained in the honey sample revealed the values of moisture content, ash content, crude protein, crude lipid, crude fiber, dry matter and nitrogen free element to be 21.31±0.30%, 1.43±0.03%, 0.58±0.04%, 0.25±0.03%, 1.41±0.05%, 78.69±0.30% and 96.36±0.005% respectively. Micro mineral contents were Iron (1.32±0.03%), Manganese (0.03±0.001%), Zinc (0.24±0.01%), and Copper (0.14±0.01%) and macro mineral contents were 5.06±0.09% for calcium, 0.28±0.02% for Magnesium, 2.46±0.02% for Sodium, 6.78±0.06% for Potassium and 2.87±0.04% for Phosphorus. This study reveals that honey possesses nutritional quality and supports their utilization in various food products that can be used as supplement for the need of human.

Keywords: nutritional properties, honey, proximate, mineral, human health

1. Introduction
Honey is a natural sweet viscous liquid product produced by various honey bees (*Apis mellifera, Apis cerana indica* and *Apis mellipodae*) from the nectar of blossoms or from the secretion of living parts of plants that has been consumed since early humans. Honey is noted for its high nutritional values and beneficial roles in human health. The mechanism of honey synthesis by these bees is same all over the world but the differences in honey observed in their physical and chemical properties are basically on geographical and botanical origins. The variation in taste, flavor, aroma and colour determines that honey is produced from many different flora substances majorly from plants [1]. This substantial variation is ably observed in the composition and nutritional values of honey.
Chemical components of honey are of great importance as they influence the keeping quality, granulation, texture, as well as the nutritional and medicinal efficacy [2]. The major constituents of honey are nearly the same in all honey samples, however, the chemical composition and physical properties of natural honeys varies greatly according to the plant species on which the bees forage [3, 4, 5]. Furthermore, the properties of natural honeys also vary depending on the differences in climatic conditions and vegetation of the areas. Buba *et al.* [6], reported that natural honey is one of the most widely sought products due to its unique nutritional and medicinal properties, which are attributed to the influence of the different groups of substances it contains.
The production of quality honey to assure food safety and hygiene depend on the variation in the active components of the honey which is base on the plant species differences. Commercial samples of honey available in various parts of the world are of highly different quality, on the basis of factors like geographical conditions, production season, processing, and source of nectar, packaging and storage period. Given the importance of honey as a nutrient full of energy and prebiotic compounds and its usage in disease treatment [7]. The purpose of this study therefore was to evaluate the nutritional properties of honey from *Apis mellifera.*

2. Materials and Methods
2.1 Collection of Honey sample
Honey sample was bought from local market in Yenagoa, Bayelsa state, Nigeria.

2.2 Methods for Proximate Analysis
The dry matter, moisture, ash, lipid, crude protein (nitrogen x 6.25) and crude fibre contents were analyzed in powdered brown mustard seed using the standard methods of the Association of Official Analytical Chemists [8] while Dry Matter and Nitrogen Free Element contents was calculated based on the net difference between the other nutrients and the total percentage composition.

Estimation of moisture content
Fresh sample materials were taken in a flat bottom dish and kept overnight in a hot air oven at 100-110°C and weighed. The loss in weight was regarded as a measure of moisture content.

Estimation of ash
About 2g of the sample was weighed and taken in a vitreosil basin. The basin was heated in a low flame at the beginning till no fumes were given off by the charred mass. It was broken by a glass rod carefully and burnt in a muffle furnace at 550- 600°C for 4-5 hrs. The muffle was allowed to cool to 150°C. The basin was then cooled in a desiccator and the ash content was then weighed. The total ash was calculated as follows:

% of total ash = weight of the ash ×100 / weight of the sample

Estimation of crude protein (Micro-Kjeldahl Method)
Digestion: About 2gm of sample was taken in a Kjeldahl flask, 10gms of sodium sulphate and 0.5 gm of copper sulphate was added and mixed well. A few glass beads were added into the flask to prevent spurting while heating. Then 25 ml of concentrated H_2SO_4 was added and then heated atleast for 15-20 mins in inclined position. The solution was boiled until a greenish colour was obtained. It was allowed to cool.

Distillation
About 100 ml of distilled water was added to the Kjeldahl flask, shaken properly and transferred it into a 250 ml volumetric flask. Then the final volume was made up to 250 ml by adding distilled water. In a conical flask, 10-15 ml of 2% Boric acid was taken and the flask was placed below the condenser of the distillation apparatus. Thereafter, 5 ml of aliquot was transferred to the Micro Kjeldahl steam distillation apparatus and added 1 drop of phenolphthalene and 10-15 ml 40% NaOH. The distillation was carried out at least for 5-10 mins until ammonia was free from aliquot. Titration: The distillation product was then titrated against N/10 H_2SO_4
Calculation is done as follows:

$$\% \text{ of Nitrogen} = \frac{\text{ml of N/10 } H_2SO_4 \text{ used up} \times 250 \times 0.0014 \times 100}{\text{Volume of aliquot} \times \text{gm of the substance taken}}$$

% of crude protein=% Nitrogen ×6.25

Estimation of crude Lipid (Ether extract)
Five gm of dry sample was weighed on a piece of glazed paper and transferred into an extraction thimble. The thimble was introduced into soxhlet extractor over a pad of cotton wool, so that top of the thimble is well above the top of the siphon. A clean dry flask was taken, weighed and was fitted with the extractor. Ether was poured along the side of the extractor until it begins to siphon off. Then another half-a siphonful of ether was added. The equipment thus assembled with the flask was placed on a water bath at 60-80°C and the extractor was connected with the condenser. Cool water circulation was started in the condenser and allowed the extraction for 8 hr. Then the thimble with the material was removed from the extractor. The apparatus was assembled again and heated on a water bath to recover all the ether from the receiver flask. The receiver flask was disconnected and dried it in a hot air oven at 100°C for 1 hr, cooled and weighed.

$$\% \text{ of Ether extract} = \frac{(\text{Wt. of oil flask with ether extract-Wt. of the oil flask}) \times 100}{\text{gm of the substance taken}}$$

Determination of crude fibre
About 2 gm of moisture and fat free sample was weighed and transferred to the spout less one litre beaker. Thereafter, 200 ml 1.25% H_2SO_4 was added. The beaker was placed on hot plate and allowed to reflux for 30 mins, timed from onset of boiling. The content was shaken after every 5 min. The beaker was removed from the hot plate and filtered through a muslin cloth using suction. The residue was washed with hot water till it was free from acid. The material was transferred to the same beaker and added 200ml of 1.25% NaOH solution and refluxed for 30 mins. Again filtered and the residue was washed with hot water till it was free from alkali. The total residue was transferred to a crucible and placed in hot air oven, allowed to dry to a constant weight at 80-110°C and weighed. The residue was ignited in muffle furnace at 550-600°C for 2-3 hrs, cooled and weighed again. The loss of weight due to ignition was the weight of crude fiber.

$$\% \text{ of Crude Fiber} = \frac{(\text{Wt of the crucible with dry residue -Wt of crucible with ash}) \times 100}{\text{gm of the substance taken}}$$

2.3 Procedure for Mineral analysis
For this study, 0.5 gm of powdered dried sample was taken in a crucible and converted to ash in the muffle furnace at 580°C for 3 hrs. After cooling in a desiccators 10 ml of concentrated Nitric acid, 4 ml of Perchloric acid and 1ml of Sulphuric acid was added and digestion at high temperature was carried out until the content became clear, then the tube was cooled and the solution was transferred quantitatively to 50 ml volumetric flask and the final volume was adjusted to 50 ml by adding distilled water. The solution was used for determination of Fe, Zn, Mg, Mn, Na, K and Cu through the atomic absorption spectrometry (AA203D). Calcium and Phosphorous estimation were done as per method described by Odangowei *et al* [9].

3. Results

3.1 Proximate result

Proximate results obtained in the honey sample revealed the values of moisture content, ash content, crude protein, crude lipid, crude fiber, dry matter and nitrogen free element to be 21.31±0.30%, 1.43±0.03%, 0.58±0.04%, 0.25±0.03%, 1.41±0.05%, 78.69±0.30% and 96.36±0.005% respectively, Table 1.

Table 1: Proximate Compositions of Honey

Proximate parameters (%)	Honey sample
Moisture	21.31±0.30
Ash	1.43±0.03
Protein	0.58±0.04
Lipid	0.25±0.03
Fibre	1.41±0.05
Dry Matter	78.69±0.30
Nitrogen Free Element	96.36±0.005

Data are mean values of triplicate determinations ± standard deviation

3.2 Micro and Macro Mineral results

Micro mineral contents were Iron (1.32±0.03%), Manganese (0.03±0.001%), Zinc (0.24±0.01%), and Copper (0.14±0.01%) and macro mineral contents were 5.06±0.09% for calcium, 0.28±0.02% for Magnesium, 2.46±0.02% for Sodium, 6.78±0.06% for Potassium and 2.87±0.04% for Phosphorus, Tables 2 & 3.

Table 2: Micro mineral contents of honey sample

Micro-mineral content (ppm)	Honey sample
Iron (Fe)	1.32±0.03
Manganese (Mn)	0.03±0.001
Copper (Cu)	0.14±0.01
Zinc (Zn)	0.24±0.01

Data are mean values of triplicate determinations ± standard deviation

Table 3: Macro Mineral Contents of Honey sample

Macro Mineral content (ppm)	Honey sample
Calcium (Ca)	5.06±0.09
Magnesium (Mg)	0.28±0.02
Sodium (Na)	2.46±0.02
Potassium (K)	6.78±0.06
Phosphorus (P)	2.87±0.04

Data are mean values of triplicate determinations ± standard deviation

4. Discussion

4.1 Proximate Compositions

Proximate analysis is usually carried out to determine the nutritional values of foods and food based products. The nutrient content is essential not only for health promotion, but also for metabolic energy. The proximate properties of the honey sample used for evaluation in this study are depicted in Table 1. The moisture content was 21.31%. This value was similar to the results of Ajao *et al.* [10] who previously reported a range of 19.26% to 22.09% for honey samples in Nigeria. Moisture content is practically the most important parameter that determines quality of honey, since it affects storage life and processing characteristic. The strong interaction of sugar in honey with water molecules may decrease the water available for microorganisms. It was found that the honey sample would be prone to granulation because of high moisture content (> 20%) [11]. Honey Regulation Council Directive stipulated honey moisture content should not be more than 21%. It is apparent that the water content varies greatly and may range widely. The amount of moisture is a function of factors involved in ripening, including, among others, the original moisture of the nectar. According to the United States Standards, extracted honey may not contain more than 18.6% moisture. Moisture level of about 17% has been found to be optimum. The moisture content of honey is one of the criteria that determine the shelf stability of honey [12, 13]. Thus the higher the moisture, the higher the probability that honey will ferment upon storage by osmotolerant yeasts [14]. A high moisture content of honey is also an indicator of adulteration [15]. Moisture content also play an important role in honey viscosity and savour [16]. Our result are within the limit (21%) recommended by the Codex Alimentarius Commission [17].

The result of the ash content recorded in this study was 1.43%. The value of our findings corroborated with previous work of Ndife Joel *et al*. [18] who reported the range of 1.18% to 1.73%. Codex Alimentarium Commission Standard [17] proposed not more than 0.6% ash content for normal honey. The result for the honey sample was higher than the acceptable ash content range.

The Lipid content recorded in this study was 0.25%. Several literatures reported that honey has little or no lipid [19, 20]. The low lipid content in the honey sample indicated that they contain very little quantity and are not considered as adequate sources of lipid. The crude protein content was 0.58%. This value was relatively lower compared with the value range of 1.43-2.72% reported by Agunbiade *et al*. [21] for honey obtained from three states of Nigeria. This is an indication that honey is not an adequate source of dietary protein. Though the result of protein contents obtained in this research work were in agreements with the work of Buba *et al*. [6] who reported that the protein content of honey in north-east of Nigeria ranged between 0.35 and 1.08. The results were also in conformity with an average amount of 0.70mg per 100g reported by National Honey Board.

4.2 Micro and Macro Mineral Compositions

The results of micro and macro mineral contents of the honey sample analyzed were presented in Table 2 and 3. The concentration of the micro minerals found in the honey sample was in order of Iron> Zinc> Iron> Copper>Manganese while macro minerals were Potassium> Calcium> Phosphorus> Sodium>Magnesium. The results of this work showed that the honey sample were quite rich in macro minerals. In comparison with other studies, the honey sample having the highest Potassium and Calcium content (6.78% and 5.06%) agreed with the reports of Matinez-Gomez *et al*. [22]. Also, the results were in conformity with the studies of Agbagwa *et al.* [23] and Ndife Joel *et al*. [18] who reported potassium dominance in honey investigated. The mineral contents of honey may vary as a result of the differences in plant species visited by the honey bees during nectar collection, and the types of the soil in which the floral were found. These minerals play a great number of physiological and biochemical functions in human health. Calcium is needed for growth and maintenance of bones, teeth and muscles [24].

Sodium and Potassium found in the intracellular fluid help to maintain electrolyte balance and membrane fluidity. Magnesium protects and manages high blood pressure and cardiovascular diseases [25]. Iron plays an important role in hemoglobin formation, normal functioning of the central nervous system and oxidation of carbohydrate, protein and fats [26]. Copper contributes to iron and energy metabolism. Manganese acts as a cofactor of many enzymes [27].

5. Conclusion

The consumption and uses of honey in Nigeria has increased tremendously over the years. This study reveals that honey possesses some nutritional quality that can be used as supplement for the need of human. The honey sample possesses important nutritional properties such as proximate, micro and macro minerals and of good quality when compared with Codex Alimentarius honey specifications.

6. References

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