

## Biochemical Diversity of Honeybee (*Apis Mellifera*) Population in Commercial Apiaries in Minna, Nigeria

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### ABSTRACT

Honeybees (or honeybee) are member of the genus *Apis*, primarily distinguished by the production and storage of honey and the construction of perennial, colonial nests from wax. Honey is used for nutritional, medicinal and industrial purposes and it is an important commodity in the international market, serving as foreign exchange earner for many countries. In Nigeria, honey production (beekeeping) has the potential to develop as a prime agro-horticultural and forest-based industry which can be a major foreign exchange earner if international standards are met. The bee's chemical composition and physical properties of natural honeys differ according to the plant species on which the bees forage. Differences in climatic conditions and vegetation are also important factors that can affect the various properties of honey. Thus, analysis of the biochemical components of honeybee samples obtained from five different hives in Minna, Nigeria was carried out. The results obtained showed a significant difference in the proximate analysis from the different hives. Moisture content ranged between 17.19 $\pm$ 0.02 and 49.70 $\pm$ 0.01g/100g, Ash contents of the samples had mean values ranging from 0.47 $\pm$ 0.00 and 0.86 $\pm$ 0.01g/100g. The protein contents ranged between 18.59 $\pm$ 0.01 and 27.62 $\pm$ 0.05g/100g while lipid content ranged between 0.20 $\pm$ 0.00 and 4.93 $\pm$ 0.00g/100g. Total carbohydrates contents and values of 6.68 $\pm$ 0.03 and 45.79 $\pm$ 0.01g/100g and 12.13 $\pm$ 0.01 and 23.91 $\pm$ 0.01g/100g/100g, respectively. In conclusion, the study highlights significant variability in the biochemical composition of honey from different hives in Minna, Nigeria, emphasizing the influence of environmental factors on honey's nutritional and commercial value.

**Keywords:** *Apis mellifera*, Biochemical Assay, Honeybee.

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### INTRODUCTION

The study of honeybees is known as apiology. Honeybees are the only extant members of the tribe *Apini*, all in the genus *Apis*. Currently, only seven species of honeybee are recognized, with a total of 44 subspecies (Malaka and Fasasi, 2022) though historically, from six to eleven species have been recognised.

Honeybees represent only a small fraction of the roughly 20,000 known species of bees. Some other types of related bees produce and store honey, but only members of the genus *Apis* are true honeybees.

The original distribution range of honeybee, *Apis mellifera* L. included Africa, Europe (except the northern part)

and the Near East. Within this large area the species was differentiated into several evolutionary branches. Up to 29 subspecies have been distinguished on the basis of morphological traits (Sulaiman and Erejuwa, 2021) and these were originally grouped into five evolutionary lineages: (M) from northern and western Europe and northern Africa, (A) from southern and central Africa, (C) from the northern Mediterranean region and eastern Europe, (O) from the eastern Mediterranean and the Near and Middle East region, and (Y) from the east African country of Ethiopia (Ruttner and Ruttner, 2020). Based on various phylogenetic parameters the speciation event that produced *A. mellifera* has been estimated to have occurred between 0.7 to 1.3 million years ago (Zhang and Wang, 2021). The nutritional requirements of individual honeybees within the hive vary with their life-stage, with larvae primarily requiring protein (Khalil and Sulaiman, 2020) and adult honeybees requiring greater carbohydrate and less protein (Nwoko *et al*, 2010). In the same way that nectar is converted to honey in the hive, so pollen is converted to "beebread" (Osho and Osho, 2020). The nutritional content of beebread has rarely been examined and previous studies have been limited in sample size so fail to capture the potential variation in beebread nutritional composition (Udeh and Nwafor, 2021). However, given that the main ingredient of beebread is pollen, it seems likely that beebread will vary in nutritional composition depending on the local and seasonal availability of pollens from different plant species.

Beekeeping is an age-old tradition in Nigeria, but it is not considered as a

profit-making venture in most parts of the country. Thus, while beekeeping has been part of normal agricultural enterprise among some communities in the country (Oguleye and Adebayo, 2022), honey production has largely been at a subsistence level (Malaka and Fasasi, 2022). However, honey is found in beehives in large quantities in Nigeria (Dandago *et al*, 2021) and it has been recognized that honey production (beekeeping) has the potential to develop as a prime agro-horticultural and forest-based industry which can well become a major foreign exchange earner if international standards are met in the area of food, medicine and agriculture but we are not benefiting maximally from these areas because the practice of beekeeping is not popular. Also, variation in genetic and biochemical attributes affects the performance of bees. These variations and how they affect bees in their productivity is not known. That is what necessitated this study. Bees and their products are of high economic value to mankind as they aid in pollination.

Their products serve as medicinal as well as food especially for diabetic patients. Productivity of honeybee depends greatly on environmental factors as well as biological factors such as genetic makeup of the bees. Bee products are in high demand now in Nigeria and apiculture is fast becoming a serious business venture for many people. However, optimum production of bee product from apiculture is hampered by inadequate knowledge of bee species in the country. Therefore, this research will attempt to bridge the gap in the paucity of knowledge and available literature especially on the genetic and biochemical bases of bee products. The aim of the research is to evaluate the genetic and

biochemical variability in honeybee populations in Minna, Niger state, Nigeria.

## MATERIALS AND METHODS

### Study Area

The study was carried out in Minna, the Capital of Niger State, North-central Nigeria. Minna is located within longitude 6°33'E and latitude 9° 37'N, covering a land area of 88km<sup>2</sup> with an estimated human population of 1.2 million. The area has a tropical climate with mean annual temperature, relative humidity and rainfall of 30.20°C, 61.00% and 1334.00mm, respectively.

The climate presents two distinct seasons: a rainy season between May and October, and a dry season between November and April. The vegetation in the area is typically grass dominated Savannah with scattered trees.

### Honeybees Collection and Preservation and identification

The honeybee samples were collected from five different hives in five containers from Niger State Agricultural and Mechanized Development Authority (NAMDA) apiculturist farm, located in Maitumbi community, Minna, Niger State, Nigeria. All the honeybee samples were collected freshly in sterile containers (labelled with numbers, place and date of collection). The honeybees were preserved using 70-100% alcohol. It was later transported to the laboratory of the Department of Biological Sciences, Federal University of Technology, Minna, and stored at ambient temperature until analyzed.

### Molecular Analysis of Teneral Reserve of Honeybee (Biochemical Compositions)

Proximate analysis carried out on the honeybee samples to determine their composition were: crude protein, lipids, crude fibre, carbohydrate, moisture and ash. All the samples were analysed using standard analytical methods described by Association of Official Analytical Chemists (AOAC). For moisture content, each sample was dried to constant weight in hot air oven at 105°C and the moisture was calculated on dry basis. Protein content was determined using the micro-kjeldhal procedure to estimate the total nitrogen content and the protein content was calculated using the 6.25 conversion factor for protein nitrogen.

Ash content was determined by drying the honeybee samples in porcelain crucibles at 105°C for 3 hrs in hot air oven. The dried samples were ignited in a furnace at 550°C-600°C to constant weight, cooled and weighed. Lipid (Crude fat) content was determined following extraction using Soxhlet extraction apparatus with petroleum ether. The crude fibre consisted of the total, soluble and insoluble fibre of honeybee samples were determined based on AOAC Official Method 991.43, 1991. Carbohydrate contents of the honeybee samples were determined by calculation (by difference) as follows:

$$\% \text{Carbohydrate} = 100\% - (\% \text{Moisture} + \% \text{Crude Fat} + \% \text{Crude Protein} + \% \text{Ash} + \% \text{Crude fibre})$$

### Data analysis

The data were subjected to Analysis of Variance (ANOVA) and Chi-Square test. Differences between mean values were

considered significant at values of  $P < 0.05$ .

### RESULTS

The statistical results obtained shows that there is a significant difference that exist in the proximate analysis of the honeybee's samples among the different hives in Table 1. The highest Carbohydrates content was observed in hive three having (45.79± 0.01) and the lowest Carbohydrates content was recorded in hive one having 6.68±0.03. For the treatment of crude protein content, hive one recorded the highest mean value of 27.62±0.05, however the lowest mean value of 18.59± 0.01 was

recorded in the hive three. The results of the treatment of the lipid content analyzed shows that the highest mean values was recorded in hive four with 4.93± 0.00 while the lowest was recorded in hive two with 0.20± 0.00. The result of the treatment for moisture content was highest in hive five with mean values of 49.70± 0.02 value. For the treatment of the ash content, the highest mean value was recorded the lowest ash content of 0.47± 0.00. the treatment of crude fibre content in the honeybees' samples, hive one recorded the highest with mean value of 23.91± 0.01 and hive recorded the lowest mean value of 12.13± 0.01.

Table 1: Proximate Composition of Honeybees Samples of five different hives in Minna

SAMPLE HIVE	CHO	C.P	LIPIDS	M.C	ASH	C.F
1	6.68±0.03 <sup>a</sup>	27.62±0.05 <sup>d</sup>	0.29±0.00 <sup>a</sup>	40.98±0.01 <sup>c</sup>	0.86±0.01 <sup>c</sup>	23.91±0.01 <sup>d</sup>
2	22.44±0.01 <sup>c</sup>	20.28±0.01 <sup>b</sup>	0.20±0.00 <sup>a</sup>	43.84±0.01 <sup>d</sup>	0.47±0.00 <sup>a</sup>	12.82±0.01 <sup>a</sup>
3	45.79±0.01 <sup>e</sup>	18.59±0.01 <sup>a</sup>	2.91±0.01 <sup>b</sup>	17.19±0.02 <sup>a</sup>	0.54±0.01 <sup>a</sup>	14.50±0.01 <sup>b</sup>
4	35.79±0.01 <sup>d</sup>	20.59±0.01 <sup>b</sup>	4.93±0.00 <sup>c</sup>	18.09±0.01 <sup>b</sup>	0.65±0.00 <sup>b</sup>	20.10±0.10 <sup>c</sup>
5	8.87±0.01 <sup>b</sup>	25.55±0.01 <sup>c</sup>	3.14±0.00 <sup>b</sup>	49.70±0.01 <sup>e</sup>	0.67±0.01 <sup>b</sup>	12.13±0.01 <sup>a</sup>
TOTAL	23.91±5.04	22.52±1.15	2.29±0.60	33.96±4.54	0.63±0.04	16.69±1.52

Values presented are mean ± Standard error of two determinations. \*Mean values followed by different superscript alphabets in the same column are significantly different at ( $P < 0.05$ ). KEYS CHO=Carbohydrate Content C.F=Crude Fibre C.P=Crude Protein M.C=Moisture Content

### DISCUSSIONS

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 results of proximate analysis for all honey Samples are presented in Table 1. The crude protein contents of honeybee samples from the different hives were significantly ( $P < 0.05$ ) different. The protein value was calculated based on the total nitrogen content multiplied by the conversion factor of 6.25 (Akinmoladun and Akinmoladedun, 2022). The values obtained in this study were high ranging

from 18.59± 0.01 to 27.62±0.05. This may be as a result of Keratinized wings of the honeybees and egg carrying honeybees' queens.

Buba *et al*, 2013 reported that the amount of nitrogen in honey is generally low, in average of about 0.04% although it may reach up to 0.1%. It was also reported that of the total amount of Nitrogen in honey only 40–65% is in protein, the remaining part of total nitrogen resides in substances other than protein, such as amino acids. The honey proteins are mainly in the form of enzymes (Chukwuma and Eze, 2020). The honeybees add different enzymes during the process of honey ripening. The

enzymes added include diastase (amylase), which digest starch to maltose and is relatively stable to heat and storage, and invertase (saccharase or alpha glucosidase), which catalyses the conversion of sucrose to glucose and fructose. The invertase also catalyses many other sugar conversions and is mainly responsible for the sugar patterns of honey. Glucose oxidase and catalase are two other enzymes added by the honeybee, which regulate the production of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>); the H<sub>2</sub>O<sub>2</sub> serve as one of the anti-bacterial factors in honey.

Hives one, two and five honeybee samples showed high moisture contents (mean value 40.98, 43.84 and 49.70g/100 g respectively), and samples of hives three and four were low with 17.19 and 18.09g/100g. The result showed that the lower moisture percent contributed to the higher carbohydrate content in hives three and four and the values did not exceed the limit of 20.0 g/100g of honey as established by international norms. The hive three and four values also fell within the range of moisture contents reported by Buba *et al.* (2013). Moisture content is an important quality parameter, important above all for honey shelf-life (Udeh and Nwafor, 2021). It is the only composition criterion which as a part of honey standard has to be fulfilled in world honey trade (Zhang and Wang, 2021). The significance of moisture in honey derives from the fact that there is a relationship between honey water content and yeast count; at 17.0 g/100 g moisture (humidity) there is very minimal fermentation danger due to very low yeast count (Udeh and Nwafor, 2021). Thus, honey having high water content is more likely to ferment (Zhang and Wang 2021). A maximum

value of 20.0 g/100 g was established by the Codex Alimentarius Commission and EU Commission as the international standard for honey moisture contents. The total carbohydrate contents of the honeybee samples from all the hives were different from each other ranging from  $6.68 \pm 0.03$  to  $45.79 \pm 0.01$ . Therefore, the energy values for hive three and four samples of the honeybee are relatively higher than the honey samples of hive one, two and five. The energy value is mainly attributed by the high sugar content of honey samples. Osho and Osho (2020) reported that honey is primarily a high energy carbohydrate food and the honey sugars are easily digestible sugars similar to those found in many fruits. For this reason honey is regarded as a good food for both infants and adults. The fat contents of the honeybee samples investigated in this study fall within the range of 0.2 to 4.9 g/100 g. However, Khalil *et al.* (2020) reported total fat contents of honey in the range of 0.134 to 0.146 g/100 g; thus, indicating that honey contains very little amount of lipid and therefore not considered a good source of lipid.

## CONCLUSION AND RECOMMENDATIONS

Honeybee samples from five different hives were collected from Maitumbi, Minna and analysed for their biochemical and nutritional components. The results found that the honey samples exhibited a significant variation in the moisture, protein and carbohydrate content from proximate analysis. The moisture content indirectly affected the carbohydrates and energy value based on proximate analysis. It is suggested to collect more

honeybee samples for each hives of honeybee at different seasons in order to draw more convincing conclusion.

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