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Proximate Analysis of Honey Samples: NIFOR Apiary and Open Market

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Abstract

Two (2) different honey samples were obtained, one from colonized hives in NIFOR apiary and the other randomly purchased from the open market. Proximate composition of honey analysis includes moisture content; ash content; crude fibre; crude fat (or lipid); crude protein; and carbohydrate. The results of the proximate analysis carried out showed that moisture content for NIFOR honey was higher than that of the Market honey with values of 18.505 g/100g and 12.890 g/100g respectively. Ash content for NIFOR honey was in agreement with the known standard having recorded values of 0.2g/100g meanwhile, the Market honey recorded lower values 0f 0.002g/100g. Crude fat content for NIFOR honey was 0.013g/100g and that of the Market honey was 0.025g/100g, implying that NIFOR honey had much lower fat content when compared with the market honey sample. NIFOR honey had Crude protein content value of 1.410g/100g doubling the content in the Market honey sample which valued at 0.781g/100g, although both samples were in agreement with the known standard, they were compared with carbohydrate content for NIFOR honey was slightly lower in values than that of the Market honey but was closer to the value from the known standard. Also results of the mineral analysis carried out on both NIFOR and Market honey samples showed that, Potassium (K), Sodium (Na), Calcium (Ca) and Magnesium (Mg) had higher values of 8.207mg/100g, 0.70mg/100g, 5.07mg/100g and 0.32mg/100g respectively in NIFOR honey which were obviously higher than the values of 2.163mg/100g, 0.50mg/100g, 3.95mg/100g and 0.22mg/100g respectively for Market honey. Manganese (Mn) content in NIFOR honey was 0.16mg/100g, although lower than value of 0.18mg/100g of Market honey but closer than the known standard when compared. Iron (Fe) content in the both samples were 1.10mg/100mg. Zinc (Zn) content in NIFOR honey was higher than that of the Market honey with values of 1.76mg/100mg and 0.16mg/100mg respectively. Copper (Cu) in NIFOR honey recorded values of 0.07mg/100mg which was closer to the known standard of 0.036mg/100mg when compared to that of Market honey which had a value of 0.12mg/100mg.Empirical results of the proximate and mineral analysis of both honey samples revealed that the tabulated t-values (2.571, 2.365) were greater than the calculated t-values (0.00658, 1.354) at an alpha level of 0.05 indicating a significant difference between the two samples. The proximate and mineral composition of both honey samples under investigation suggests that NIFOR honey is perceived to be higher in quality than the market honey.

Keywords

Apiary, Proximate analysis, Honey, Bees, Hives

1. Introduction

Natural honey is a viscous sweetener prepared by honey bees from the nectar or secretion of flowering plants [1]. Its major constituents include 80% carbohydrate (35% glucose, 40% fructose, and 5% sucrose), small amount of water (20%), while its minor components include minerals, proteins, vitamins, organic acids, flavonoids, phenolic acids, enzymes and other phytochemicals [2] [3]. Proximate analysis involves investigation of the nutritional contents of food and derivatives [4]. Proximate composition of honey includes moisture content, ash content, crude protein, crude fat (or lipid), crude fibre, nitrogen-free extracts [5]. The proximate composition of honey is vital in its quality assessment [6]. Honey contains mineral substances which are beneficial to human in small quantities [7]. These include potassium, chlorine, sulphur, calcium, sodium, phosphorus, magnesium, silicon, iron, manganese. The African honeybee, Apis mellifera adansonii is most commonly reared honey bee and the major producer of honey in south-western Nigeria [8]. Honey bees and their products are vulnerable to various diseases, parasites and pests [9]. These biotic stressors have been reported to be one of the major causes of colony losses in many African countries including Nigeria [5]. Some effective ways of controlling pests and diseases of honey bees is by practicing integrated hive management; an approach which makes use of different practices in a compatible manner towards maximizing hive production and maintenance of the health of the colony. The first step towards this is locating the apiary properly, then hive placement which is followed by maintenance. Hive inspection should be regular so as to monitor pests and diseases as well as ascertain the level of performance. There are obvious gaps in the available knowledge on modern bee hive management and the quality of honey and related products in this part of Nigeria and the world presently. Given the importance of honey as a nutrient full of energy and prebiotic compounds and its usage in disease treatment [10], this study seeks to evaluate the proximate and mineral analysis of two honey samples; NIFOR honey produced by bees at the Nigerian Institute for Oil Palm Research (NIFOR) apiary and honey purchased from the open market for quality assessment purpose.

2. Literature Review

[11] carried out a study on the analysis of Biochemical Composition of Honey Samples from North-East Nigeria. They 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 characteristics of honey is influenced by multiple factors such as purity and sources of nectar, pollen composition, climatic conditions, method of harvest and extraction, storage conditions as well as geographical origin which influences the floral sources of nectar and pollen foraged by the honeybees [11]. These conditions significantly determine the relative composition of the physicochemical components such as simple-complex sugar ratio, moisture, acidity, hydroxymethylfurfural (HMF), viscosity, aroma, vitamins, ash, organic acids, amino acids and electrical conductivity. Studies on the physicochemical properties of honey have been conducted in Nigeria and some other parts of the world [12-18]. Although the foremost 22 constituents of honey are nearly alike in honey samples, the accurate chemical assemblage and physical characteristics of natural honey is similar depending upon the plant kinds wherever the bees feed [19-21]. There are facts showing that few types of honey comprise kynurenic acid that is a tryptophan metabolite with neuroactive action that can impart to its antimicrobic qualities [22]. In south eastern Nigeria, where honey is produced in commercial quantity, studies on the physicochemical properties of honey are vast and represent most of the honey producing areas within the region [14, 16]. There is need for continuous study of physicochemical and melissopalynological properties of honey in this region arising from changes in vegetation types due to climate change and anthropogenic factors. It has also been argued that the use of either physicochemical or pollen analysis alone in assessing the quality of honey has some limitations [23].

3. Materials and Methods

3.1 Collection of honey samples

Two (2) different honey samples were obtained, one from colonized hives in NIFOR apiary and the other was randomly purchased from the open market. The cut comb method was used to harvest honeycombs from hives in NIFOR apiary while honey extraction from harvested honeycomb was by the use of sieve clothe. Extracted honey samples were stored in clean airtight transparent buckets at an ambient temperature to avoid moisture absorption. The both honey samples were taken to the laboratory for proximate and mineral composition analyses to evaluate their nutritive value in human foods and that was conducted at the Department of Chemistry, Faculty of Physical Sciences, University of Benin.

3.2 Proximate analysis

After bringing the samples to uniform size, they were analyzed for proximate content including; moisture content, ash content, crude fiber, crude fat, crude protein, and carbohydrate and these were determined based on the official analysis methods from Association of Official Analytical Chemists [24].

3.2.1 Determination of moisture content

Moisture was determined by oven drying method. 1.5g of sample was accurately weighed in clean, dried crucible (W_1). The crucible was allowed in an oven at 100-105^{0}C for 6-12 hrs until a constant weight was obtained. Then the crucible was placed in the desiccator for 30 min to cool. After cooling it was weighed again (W_2). The percentage moisture was calculated by following the formula:

$$\%\text{Moisture} = \frac{W_1 - W_2 \times 100}{\text{Wt of sample}}$$

Where:
W1 = Initial weight of crucible + Sample
W2 = Final weight of crucible + Sample
Note: Moisture free samples were used for further analysis.

3.2.2 Determination of ash content

For the determination of ash, a clean empty crucible was placed in a muffle furnace at 600^{0}C for an hour, cooled in a desiccator and then weight of empty crucible was noted (W_1). One gram of each of the sample was taken in crucible (W_2). The sample was ignited over a burner with the help of blowpipe, until it is charred. Then the crucible was placed in muffle furnace at 550^{0}C for 2-4 hrs. The appearances of grey white ash indicate complete oxidation of all organic matter in the sample. After ashing, furnace was switched off. The crucible was cooled and weighed (W_3). Percentage ash was calculated by following the formula:

$$\%\text{Ash} = \frac{\text{Difference in Wt of Ash} \times 100}{\text{Wt of Sample}}$$

Difference in Wt of Ash = $W_3 - W_1$

3.2.3 Determination of crude fiber

A moisture free and ether extract sample of crude fiber made of cellulose was first digested with dilute H_2SO_4 and then with dilute KOH solution. The undigested residue collected after digestion was ignited and loss in weight after ignition was registered as crude fiber.

Reagents:

- Solution of sulphuric acid (0.128M) 7.1 ml, 98% per 1000 ml of distilled water.
- Solution of Potassium hydroxide (0.223M) 12.5 g per 1000 ml of distilled water.

Procedure: Weighed 0.153 g sample (W_0) weighed and transferred to porous crucible. Then placed the crucible into Dosi-fiber unit and kept the valve in OFF position. After that, added 150 ml of preheated H_2SO_4 solution and some drops of foam-suppresser to each column. Then opened the cooling circuit and turned on the heating elements (power at 90%). When it started boiling, power was reduced at 30% and left for 30 min. Valves were opened for drainage of acid and rinsed with distilled water thrice to completely ensure the removal of acid sample. The same procedure was used for alkali digestion by using KOH instead of H_2SO_4. Sample was dried in an oven at 150^{0}C for 1 hour, then allowed to cool in a desiccator and weighed (W_1). Sample crucibles were kept in muffle furnace at 55^{0}C for 3-4 hours. Samples were cooled in desiccator and weighed again (W_2). Calculations were done by using the formula:

$$\%\ \text{CrudeFiber} = \frac{W_1 - W_2 \times 100}{W_0}$$

3.2.4 Determination of crude fat

Extraction method for fat determination was implied. It consisted of extracting samples with some organic solvent, since all the fat materials e.g. fats, phospholipids, sterols, fatty acids, carotenoids, pigments, chlorophyll etc. are extracted together therefore, the result are frequently referred to as crude fat. Fats were determined by intermittent soxhlet extraction apparatus. Crude fat was determined by either extract method using Soxhlet apparatus. Approximately 1g of moisture free sample was wrapped in filter paper, placed in fat free thimble and then introduced in the extraction tube. Weighed, cleaned and dried the receiving beaker was filled with petroleum ether and fitted into apparatus. Turned on water and heater to start extraction. After 4-6 siphoning, allow ether to evaporate and disconnect beaker before last siphoning. Transferred extract into clean glass dish with ether washing and evaporated ether on water bath. Then placed the dish in an oven at 105^{0}C for 2 hrs and cooled it in a desiccator.

The percent crude fat was determined by using the following formula:

$$\%\ \text{Crude Fat} = \frac{\text{Wt. of ether extract} \times 100}{\text{Wt. of sample}}$$

3.2.5 Determination of crude protein

Principle: Protein in the samples were determined by Kjeldahl method. The samples were digested by heating with concentrated sulphuric acid (H_2SO_4) in the presence of digestion mixture. The mixture was then made alkaline. Ammonium sulphate thus formed, released ammonia which was collected in 2% boric acid solution and titrated against standard HCl. Total protein was calculated by multiplying the amount of nitrogen with appropriate factor (6.25) and the amount of protein was calculated.

Reagents:

- 0.1N HCl (standard)
- Concentrated sulphuric acid
- Sodium hydroxide solution 40% w/w
- Digestion mixture: Potassium sulphate (K_2SO_4) and copper sulphate ($CuSO_4$).
- Boric acid: Dissolved 40g of boric acid in sufficient distilled water and made the volume up to 100 ml.
- Indicator: Methyl red.

Procedure: Protein in the sample was determined by Kjeldahl method. 0.5-1.0 g of samples was taken in digestion flask. Add 10-15 ml of concentrated H_2SO_4 and 8 g of digestion mixture i.e. K_2SO_4 CuSO4 (8:1). The flask was swirled in order to mix the contents thoroughly then placed on heater to start digestion till the mixture becomes clear (blue green in color). It needed 2 hours to be completed. The digest was cooled and transferred to 100 ml volumetric flask and volume was made up to mark by the addition of distilled water. Distillation of the digest was performed in Markam Still Distillation Apparatus [25]. Ten milliliters of digest was introduced in the distillation tube then 10 ml of 0.5 N NaOH was gradually added through the same way. Distillation was continued for at least 10 min and NH_3produced was collected as NH_4OH. The distillate was then titrated against standard 0.1 N HCl solution till the appearance of pink color. A blank was also run through all steps as above.

Percent crude protein content of the same sample was calculated using the following formula:

$$\% \text{ Crude Protein} = 6.25^{*} \times \%N \text{ (*. Correction factor)}$$

$$\%N = \frac{(S - B) \times N \times 0.014 \times D \times 100}{\text{Wt of the sample} \times V}$$

Where:
S = Sample titration reading
B = Blank titration reading
N = Normality of sample after digestion
D = Dilution of sample after digestion
V = Volume taken for distillation
0.014 = Milli equivalent weight of Nitrogen

3.2.6 Determination of Carbohydrate

This is calculated by difference as follows:
100 – (%Moisture Content + %Ash Content + %Crude Protein + %Crude Fat + %Crude Fiber).

3.3 Mineral determination

Mineral contents were determined by atomic absorption spectrometry, flame photometry and spectrophotometry according to the methods of [24].

3.3.1 Wet digestion of samples

For wet digestion of samples, exactly (1.0000 g) of the sample was taken in digesting glass tube. Twelve milliliters (12ml) of HNO_3 was added to the food sample and mixture was kept for overnight at room temperature. Then 4.0 ml perchloric acid ($HClO_4$) was added to the mixture and was kept in the fume block for digestion. The temperature was increased gradually, starting from 50^0C and increased up to 250-300^0C. The digestion was completed in about 70-80 min as indicated by the appearance of white fumes. The mixture was left to cool down and the content of the tubes were transferred to 100 ml volumetric flasks and the volumes of the contents were made to 100 ml with distilled water. The wet digestion solution was transferred to plastic bottles labeled accurately. Stored the digest and used for mineral determination.

3.3.2 Determination of Iron (Fe), Zinc (Zn), Calcium (Ca), Manganese (Mn), Copper (Cu) and Magnesium (Mg) by Atomic Absorption Spectrometry.

Principle: In this technique the atoms of an element are vaporized and atomized in the flame. The atoms then absorb the light at a characteristic wavelength. The source of the light is a hollow cathode lamp, which is made up of the same element, which has to be determined. The lamp produces radiation of an appropriate wavelength, which while passing

through the flame is absorbed by the free atoms of the sample. The absorbed energy is measured by a photo-detector read-out system. The amount of energy absorbed is proportional to the concentration of the element in the sample.

Procedure: The digested sample was analyzed for mineral contents by Atomic Absorption Spectrophotometer – Buck Scientific (Model 210 VGP) Made in USA, in Chemistry Department Laboratory, Faculty of Life Sciences, University of Benin, Benin City. Different electrode lamps were used for each mineral. The equipment was run for standard solutions of each mineral before and during determination to check that it is working properly. The dilution factor for all minerals except Mg was 100. For determination of Mg, further dilution of the original solution was done by using 0.5 ml original solution and enough distilled water was added to it to make the volume up to 100 ml. Also for the determination of Ca, 1.0 ml lithium oxide solution was added to the original solution to unmask Ca from Mg. The concentrations of minerals recorded in terms of "ppm" were converted to milligrams (mg) of the minerals by multiplying the ppm with dilution factor and dividing by 1000, as follows:

$$\mathrm{MW} = \frac{\text{absorbency } (\text{ppm}) \times \text{dry wt.} \times \mathrm{D}}{\text{Wt. of samples} \times 1000}$$

Note: Dilution factor for magnesium is 1000, and for other minerals including calcium, iron, potassium, sodium, manganese and copper is 100.

3.3.3 Determination of Sodium (Na) and Potassium (K) by flame photometer

Principle: The flame photometer measures the emission of radiant energy when atoms of an element return to their ground state after their excitation by the high temperature of the flame. The degree of emission is related to the concentration of the element in the solution.

Procedure: Na and K analysis of the sample were done by the method of flame photometry. The same wet digested food sample solutions as used in AAS were used for the determination of Na and K. Standard solutions of 20, 40, 60, 80 and 100 milli equivalent/L were used both for Na and K. The calculations for the total mineral intake involved the same procedure as given in AAS.

3.4 Data analysis

All data from both NIFOR honey and market honey samples were subjected to T-test.

4. Results

4.1 Hive management

There were observations of healthy colonies of Apis mellifera adansonii in the hives, as all stages of the honey bees had no signs of noticeable disease. However, some species of both insects and associated predators such as ants, small hive beetle (*Aethina tumida*), lesser wax moth (*Achroia grisella*), Termites (*Macrotermes spp*), praying mantis (*Sphodromantis viridis*) and monitor lizards (*Varanus niloticus*) were seen around the hives. There was no form of honey theft in the apiary as honey combs were intact in the different hives. As at the time of harvest, most of the colonies had about 75% of heavy combs filled with sealed and capped honey.

4.2 Proximate analysis.

The result of proximate analysis obtained from the honey samples from NIFOR apiary and the open market is presented in Table 1 below:

Table 1

S/N	PARAMETERS	NIFOR HONEY (NH)	MARKET HONEY (MH)	STANDARDS	UNITS
1	Moisture Content	18.505	12.890	17.1	g/100g
2	Ash Content	0.296	0.107	0.2	g/100g
3	Crude Fiber	0.002	0.002	0.2	g/100g
4	Crude Fat	0.013	0.025	0	g/100g
5	Crude Protein	1.410	0.781	0.3	g/100g
6	Carbohydrate	79.774	86.195	82.4	g/100g

*[26] Standard.
The results of the T-test of this table goes thus;
Calculated t-value = 0.00658
Tabulated t-value = 2.571

The tabulated t-value is greater than the calculated t-value at an alpha level of 0.05. Therefore, we can accept the null hypothesis which means there is significant difference between the two samples.

4.3 Mineral analysis.

The result of mineral analysis obtained from the honey samples from NIFOR apiary and the open market is presented in Table 2 below:

Table 2

S/N	MINERALS	NIFOR HONEY (NH)	MARKET HONEY (MH)	STANDARDS	UNITS
1	Potassium (K)	8.207	2.163	52	mg/100g
2	Sodium (Na)	0.70	0.50	4	mg/100g
3	Calcium (Ca)	5.07	3.95	6	mg/100g
4	Magnesium (Mg)	0.32	0.22	2	mg/100g
5	Manganese (Mn)	0.16	0.18	0.08	mg/100g
6	Iron (Fe)	1.10	1.10	0.42	mg/100g
7	Zinc (Zn)	1.76	0.16	0.22	mg/100g
8	Copper (Cu)	0.07	0.12	0.036	mg/100g

* [26] Standard.
The result of the T-test of this table goes thus;
Calculated t-value = 1.354
Tabulated t-value = 2.365

The tabulated t-value (2.365) is greater than the calculated t-value (1.354) at an alpha level of 0.05. Therefore, we can accept the null hypothesis which means there is significant difference between the two samples.

5. Discussion

The result of the proximate analysis carried out shows that, moisture content for NIFOR honey was 18.505 g/100g while the Market honey recorded a value of 12.890 g/100g. Ash content for NIFOR honey was 0.296 g/100g while that of Market honey was 0.107 g/100g. Clearly, it can be seen that NIFOR honey had higher moisture and ash content values than the market honey and these values were closer to the known standard of 17.1g/100g and 0.2g/100g respectively when compared. The values of Crude fiber content for both NIFOR honey and Market honey was the same 0.002 g/100g. The Crude fat content for NIFOR honey was 0.013g/100g and that of the Market honey was 0.025g/100g. This implies that NIFOR honey had lower fat content when compared with the known standard. NIFOR honey had Crude protein content value of 1.410g/100g which was double the value of the Crude protein content in the Market honey sample. The Carbohydrate content value for NIFOR honey was 79.774g/100g, and was seen to be closer to the known standard as compared to that of the Market honey which was 86.195g/100g. Also results of the mineral analysis carried out on both NIFOR and Market honey samples show that, Potassium (K) had a value of 8.207mg/100mg in NIFOR honey which was obviously higher than the 2.163mg/100mg value recorded in the Market honey. NIFOR honey had Sodium (Na) content of 0.70mg/100mg which was also higher than the 0.50mg/100mg values recorded in the Market honey sample. In the same light, Calcium (Ca) in NIFOR honey had higher values of 5.07mg/100mg, over that of Market honey which was 3.95mg/100mg values when compared with a known standard of 6mg/100g. Magnesium (Mg) in NIFOR honey was also higher with values of 0.32mg/100mg as against 0.22mg/100mg values of the Market honey when compared with a known standard of 2mg/100g. Manganese (Mn) content in NIFOR honey was 0.16mg/100g, although lower than value of 0.18mg/100g of Market honey but closer than the known standard when compared. Iron (Fe) content in the both samples were 1.10mg/100mg. Zinc (Zn) content in NIFOR honey was higher than that of the Market honey with values of 1.76mg/100mg and 0.16mg/100mg respectively. Copper (Cu) in NIFOR honey recorded values of 0.07mg/100mg which was closer to the known standard of 0.036mg/100mg when compared to that of Market honey which had a value of 0.12mg/100mg. The T-test result on the proximate analysis of both NIFOR and market honey samples revealed that the tabulated t-value (2.571) is greater than the calculated t-value (0.00658) at an alpha level of 0.05 meaning there is significant difference between the two samples. Also, T-test results of the mineral analysis revealed a tabulated t-value (2.365) which is greater than the calculated t-value (1.354) at an alpha level of 0.05 meaning that there is a significant difference between the two samples.

6. Conclusion

The two (2) honey samples under investigation were found to have various quantities of the analyzed minerals present in them. Results from two major parameters moisture content and ash content which are used in determining honey quality however indicated that NIFOR honey recorded values that were closer to or in agreement with the known standards thereby meeting the requirements of a quality honey as recommended by the International Honey Commission and the U.S. Department of Agriculture, Agricultural Research Service. There is the need to conserve the existing flora around the study location, most especially those that encourages *Apis mellifera adansonii*. Also, more research in beekeeping around this region should be conducted so as to further strengthen the management of best practices for high honey quality.

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