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# Selected Antinutrients and Proximate Compositions of Honey (*Apis mellifera*) Sample Collected from Biase, Southern Senatorial District of Cross River State, Nigeria

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## Abstract

Honey, is a natural product of the genus *Apis* from plant nectars, secretions and excretions of plant-sucking insects. Usually, it contains a variety of nutritional and anti-nutrient substances which varies depending on the plant species on which the bees forages. It had been noted that over the years, there have been a greater increase in the demand to know the best source of honey base on geographical location; this has led to increase in research of this honey type. This study was then undertaken to compare the proximate compositions and anti-nutrient compositions of honey sample harvested from Biase, southern senatorial district of Cross River State, Nigeria. The honey sample was analyzed following Standards Association of Official Analytical Chemists protocol for proximate compositions and anti-nutrient compositions using standard calibrated machines. Analysis of the results obtained showed that the honey sample was significantly rich in it nutritional compositions and less in anti-nutrient compositions ($P<0.05$). However, they are slightly high value of fats (6.7 ± 0.54%), and ash (17 ± 0%) contents from this sample, not only that, but only selected parameters were studied. Therefore, further study is recommended to be undertaken to ascertain other parameters not included in this study.

**Keywords:** Honey; Proximate; Antinutrients

## Introduction

Honey is a natural food, mainly composed of a complex mixture of carbohydrates and other minor substances, such as organic acids, amino acids, proteins, minerals, and vitamins. Honey is a gift of nature to mankind. Since ancient time, it has being in use in the household preparation. Honey is the sweet, viscous fluid produced by honeybees (*Apis melliflera*) using water, pollen, nectar of flowers or secretions from living parts of plants [1]. Honey is a sweet natural food made by bees using water, pollen and nectar from flowers [2]. The variety produced by honey bees (the Genus *Apis*) is the one most commonly referred to, as it is the type of honey collected by most beekeepers and consumed by people. Folayan and Bifarin reported that honey is produced by honey bee workers mainly from nectar of flower or honey dew on leaves. Nectar is reduced to honey containing predominantly carbohydrates with very little proteins, vitamins, minerals, enzymes, amino acids and as well as other several compounds like phenolic compounds thought to function as antioxidants.

Honey gets its sweetness from the monosaccharides: Fructose and glucose, and has about the same relative sweetness as granulated sugar. It has attractive chemical properties for baking and a distinctive flavor that leads some people to prefer it to sugar and other sweeteners.

Bees produce honey to act as a food store for the colony for periods when there are no flowers, or when the climate is adverse. For human, honey is a useful source of high carbohydrate foods; it consists of approximately 70-80% sugar, mainly from fructose and glucose. In addition to water (usually 17-20 percent), honey also contains small amounts of other substances, including minerals, vitamins, proteins and amino acids adding nutritional variety to human diets. The ash content of honey is mainly mineral trace elements. Minerals present are calcium, copper, iron, magnesium, manganese, potassium, sodium, and chlorides, phosphates, silicates and sulphates. These trace amounts of minerals may be important for human nutrition. Honey is widely used as a source of sugars for making honey wines and beers, and in the manufacture of many secondary products: breakfast cereals, bakery goods, and a multitude of other value-added products. Honey also contains a blend of flavonoids and phenolic acids which are antioxidants that eliminate potentially destructive free radicals in the human body.

These chemical components are of great importance as they influence the keeping quality, granulation, texture, as well as the nutritional and medicinal efficacy of honey. The major constituents of honey are nearly the same in all honey samples, however, the biochemical composition and physical properties of natural honeys varies greatly according to the floral source/variety of the honey bee species, geographic area, season, mode of storage, and even harvest technology and conditions [2-4]. Furthermore, honey sweetness depends on high fructose content and acidity with pH of ranges from 3.2 to 4.5. The predominant acid found in honey is gluconic acid. Its presence in all honey originated largely from the activity of glucose oxidase which the bees add at ripening and to a lesser extent from the bacterial action which occurs. A few plants give bitter honey: Agave sp. (sisal), Datura sp., Euphorbia sp., and Senecio sp. – in some societies (for example, in East Africa) these honeys are very popular. Buba reported that natural honey is one of the most widely sought products due to its unique nutritional

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and medicinal properties, which are attributed to the influence of the different groups of substances it contains.

In many countries, honey is regarded more as a medicine or special tonic, rather than as an every-day food. Honey does have medicinal properties that are acknowledged increasingly by modern medicine. Ancient Egyptians, Assyrians, Chinese, Romans and Greeks have traditionally used honey as a medicinal remedy, for the management of wound healing, skin ailments and various gastrointestinal diseases. Modern research has shown that honey may possess anti-inflammatory activity and stimulate immune responses within a wound. The therapeutic importance of certain types of honey has been attributed to its secondary metabolites of antibacterial potency. In this present study, the anti-nutrients and proximate composition was determined in honey sample collected from southern senatorial district, of Cross River, Nigeria.

## Materials and Methods

### Source of materials

The honey sample used for this study was obtained from Biase local government, southern senatorial district of Cross River State, Nigeria. It was collected in an aseptic container and transfer to the Department of Chemical science Cross River University of Technology, Calabar, where it was kept at room temperature for further analysis. All chemicals used were of analytical grade and were products of Sigma Chemical Co., USA.

### Experimental design

This experiment was designed to carry out quantitative analysis of both the proximate and anti-nutrient compositions of honey sample obtained from Biase local government.

**Materials:**

***Reagents:*** Sulphuric acid, sodium suphate, cupric sulphate, ethanol, boric acid, sodium hydroxide, double indicator, fomaline, isopropanol ether, acetic acid, indol-3-acetic acid, 2,6-dichlorophenolindophenols, methyl red, hydrochloric acid, antibomber, distilled water, potassium iodide, tannic acid, silver nitrate, 6 N $NH_4OH$, ferric chloride, phenolphthalein, diethyl tether, ferric hydroxide, NaOH, $HNO_3$, $Na_2CO_3$, $KMnO_4$, $CaCl_2$, IAA, TCA, Somogyi reagent, $CuCO_3$, and $ZnSO_4$.

***Apparatus/Equipment:*** Conical flask, measuring cylinder, burette, clamp, retort stand, hot plate, water bath, stirrer, beaker, funnel, fume cupboard, heating mantle, round bottom flask, soxhlet extractor, condenser, centrifuge, refrigerator, electrical weigh balance, electric shaker, crucible, and desiccator.

### Protein determination

This method involves three steps:

I. **Protein digestion:** To 1 g of honey in Kjeldahl flask, add 3 g of cupric sulphate, 10 gram of sodium sulphate, 20 ml of sulphuric acid, the colour changes to brown, and few grains of anti-bombing granules also added to the flask. The sample was placed on heating mantle in a fume cub board (safety cabinet) and concentrated at low temperature and gradually increasing the temperature until it changes from brown to pale yellow. It is then switch off and filter the mixture with Whitman filter paper.

II. **Distillation:** Measure 10 ml of the digested sample into around bottom flask which has been connected to a set up apparatus for distillation process and a receiving beaker containing 10 ml of 2% boric acid.

Pipette 30 ml of 10% $Na_2OH$ into the round bottom flask containing the digested sample after heating it for 2-3 minutes.

The distillation was continued until the volume of the distillate double the initial volume in the receiving beaker.

III. **Titration:** 0.1 $MHCl_2$ was prepared in 250 ml and turned into 50 ml burette. Then titrated against the distillate in the beaker. The titre value.

### Determination of fats content

**Apparatus/reagent:** Round bottom flask, soxhlet extractor, condenser, heating mantle and diethyl ether.

**Procedure:** It involves extraction and distillation.

**Extraction:** Set up the apparatus for extraction and add few grains of anti-bombing granules were added to the round bottom flask and 100 ml of diethyl ether was also added to the round bottom flask.

5 g of the sample is carefully put into the extractor, to prevent it from touching the wall of the extractor. Also the various points of attachment are grease to prevent evaporation in the set up during the extraction process.

The sample is heated until the colour of the extract passing through the evaporating arm turn colorless.

**Distillation of the extract:** The extract was distilled until it turns gel-like and the extract place in a desiccator to completely dry. After drying, measure the weight of the round bottom flask containing the extract. Wash the flask and then measure the weight of the flask after washing.

### Determination of carbohydrates (glucose/ fructose) concentration in honey sample

**Principle:** Fructose, glucose, and other hexoses produce hydroxymethyl fufural on heating with a strong acid. The hydroxymethyl fufural formed can react with some reagent forming colored complexes with the color with the color intensity proportional to the concentration of the hexose in the test sample.

**Procedure:** Add 1 ml of the fructose/glucose standard solution (5 mg/100 ml) and then test sample into two different test tubes. Pipette 0.2 ml IAA reagent and 8.0 ml HCl into each of the two test tubes and mix well. Maintain the test tubes at a temperature of 37°C in a water bath. Stop the reaction after standing for 80-90 minutes. Cool and measure the absorbance of each tube at 520 nm against a reagent blank made up of 1 ml of distilled water. Indol-3-acetic acid is 0.5 g of recrystallized IAA in ethanol.

### Determination of ash content

**Procedure:** Take the weight of the empty crucible and the lid and measure 2 g of the honey sample into the crucible and weight again to obtain initial weight.

Then place it on a hot plate at a temperature of 150°C for 3 hours.

Remove and transfer the crucible with the sample into a desiccator and allowed to cool.

Weigh it again to obtain the final weight.

### Determination of moisture content

**Procedure:** Take the weight of the empty crucible and lid.

Measure 2 g of honey sample into the crucible and weigh to obtain initial weight.

Heat the sample in an oven at a temperature 100$^0$C for 3 hours.

Remove it and put in a desiccator to cool. Weigh it again to obtain the final weight.

### Determination of cyanide

**Principle:** Cyanide glycosides are anti-nutrients that contain the cyanide (-NC) group [5].

**Method:** 1.0 g of honey, at room temperature and standing respectively is weigh into 250 ml flat bottom flask. 200 ml of distilled water was introduced into the flask, and allow for two hours (for hydrolysis to occur). Full distillation was carried out and 150 ml conical flask containing 20 ml of 2.5% NaOH solution. Before distillation 0.1 g of tannic acid was introduced to the heated flask as anti-foaming agent. After the distillation 100 ml of the aliquot (distillate) containing cyanogic compound was measured into another conical flask plus 8 ml of 6N $NH_4OH$ solution, 2 ml of 5% KI was also added to the flask and mixed well before titrated with 0.02 N silver nitrate ($AgNO_3$). The black background with permanent turbidity indicate end point.

### Determination of phytates

2.0 g of honey at room temperature and standing which was added into reagent bottle with 50 ml of 0.18 M trichloricacetic acid (TCA) at room temperature. The bottle was place on an electric shaker for 2 hours to extract the phytate.

The extracts were filtered and neutralized by adding 2 drops of phenolthalein in 10 ml of the extract sample plus 0.5 M NaOH, the aliquots were treated with 4.0 ml of 0.034 Ferric chloride solution and place on boiling water. After 45 minutes, the precipitated ferric phytate where collected by centrifugation, washed twice with 30 ml of TCA and once with 50 ml of distilled water. The precipitates obtain were suspended in 3.0 ml of 1.5 M NaOH solution plus 2 drops phenalphtallein indicator for the neutralization and diluted to 30 ml distilled water, the resulting ferric hydroxide was then centrifuge, washed with water and dissolved in 40 ml of 3.2 N $HNO_3$ and made up to 100 ml with distilled water. The iron content was then determined by the UV/V spectrophotometer using the wavelength of 248.3 nm.

### Determination of tannins

Method: 1.0 g of honey sample was weighed into a separating funnel agitated for few minutes. The flask was left to stand for 30 minutes at room temperature, being shaken every 5 minutes. At the end of 30 minutes, it was centrifuge and extract gotten. 2.5 ml of the supernatant (extract) was dispensed into 50 ml volumetric flask. Similarly 2.5 ml of 0.1 g of standard tannic acid solution was added.

1.0 ml of Folin–Denis was measured into each of the flask, followed by 2.5 ml of saturated $Na_2CO_3$ were diluted to the mark of 50 ml flask, and incubated for 90 minutes at room temperature.

The absorbance was measure at 700 nm in a Genway model 6405 UV/ V spectrophotometer. Readings were taken with the reagent blank at zero.

### Determination of oxalates by titration method

This determination involves three major steps:

I. **Digestion:** 2 g of the honey at room temperature was added to 190 ml of distilled water in 250 ml digestion flask. 10 ml of 6 M HCl was added into the flask and connected to Kjeidahl digestion apparatus and digested at 100$^0$C for 1 hour in the fume cub board and after the digestion, the flask was cool with lab water and make up to 250 ml and filtered respectively [6].

II. **Oxalate precipitation:** 125 ml of filtrate was measured into four beakers and four drops of methyl red indictor was added to the beakers respectively. This was followed by the addition of 25% of $NH_4OH$ solution (drop wise) until the test solution changes from salmon pink color to faint yellow color and this was also measure by PH indicator paper to PH of 4.5. The test samples were then heated to 90$^0$C, cool and filtered to removed precipitate containing ferrous ion. The filtrates were again heated to 90$^0$C and 10 ml of 5% $CaCl_2$ solution was added while stirring constantly. After heating and cooling, the samples were left over night at 5$^0$C in terresstitic refrigerator. At the end of the test the sample, the solution were centrifuged at 2500 rpm for 5 minutes, then the supernatant was decanted and the precipitate was completely dissolved in 10 ml of 20% V/V $H_2SO_4$ solution.

III. **Permanganate titration:** At this point the total resultant filtrate was made up to 300 ml with distilled water. Aliquot of 125 ml of this filtrate was heated until near-boiling and then titrated against standardized $KMnO_4$ solution to faint pink color which was persisted for 30 second. At the end the calcium oxalate content was calculated using the formula in the appendix.

### Statistical analysis

The results were statistically analyzed after triplicate sampling and mean values and standard deviation presented and multiple range test with significant at $p<0.05$.

## Results

The results of proximate compositions of honey sample obtained from Bias.

| Parameters % | Mean plus standard values |
|---|---|
| Moisture | 7.66 ± 0.05 |
| Protein | 3.06 ± 0.54 |
| Fats | 6.7 ± 0.54 |
| Ash | 17 ± 0 |
| Fructose | 11.86 ± 0 |
| Glucose | 17.63 ± 0.07 |
| *Data are mean values of triplicate determinations ± standard deviation | |

**Table 1:** Proximate analysis of honey sample.

| Parameters (%) | Mean plus standard values |
|---|---|
| Phytate | 0.38 ±0.05 mg/100 g |
| Tannin | 0.59 ±0.05 mg/100 g |
| Cyanide | 0.005 ±0.01 mg/100 g |
| Oxalate | Nil |
| *Data are mean values of triplicate determinations ± standard deviation | |

**Table 2:** Results of Selected anti-nutrient compositions of honey sample.

## Discussion

Proximate analysis is usually carried out to determine the nutritional values of food and food based products. The nutrient contents are essential not only for health promotion, but also for metabolic energy. The proximate property of honey sample used for this study is presented in Table 1 above.

This honey sample from Biase southern senatorial district of Cross River State has moisture content of 7.66 ± 0.05%. The moisture content obtained conforms to the range reported for floral honeys by Badawy. It also indicate that the sample comply with USDA, (2007) standard of honey grading. The moisture content of the sample was below 21% which is regarded as good according to the Codex Alimentarius specifications [1]. The moisture content of honey is one of the criteria that determine the shelf stability of honey. Thus the higher the moisture, the higher the probability that honey will ferment upon storage by osmotolerant yeasts. A high moisture content of honey is also an indicator of adulteration [7].

Low value was recorded for the protein from the honey samples, which was 3.06 ± 0.54%. The result obtained was in agreement with that reported by other authors. The value for fat was 6.7 ± 0.54%. High fat content makes foods to be susceptible to rancid spoilage during storage. The value for fat was 6.7 ± 0.54%. High fat content makes foods to be susceptible to rancid spoilage during storage.

The values obtained for the ash content was 17 ± 0%. Ash content is a reflection of the total inorganic minerals that are present in a sample after incineration.

The carbohydrate content of the honey sample was 65.58%, with fructose and glucose having 11.86 ± 0 and 17.63 ± 0.07 respectively.

The anti-nutrient contents of the honey sample as shown in Table 2 contains phytate at 0.38 ± 0.05, tannin at 0.59 ±0.05, cyanide at 0.005 ± 0.01 and 0% of oxalate content.

Tannin is non-toxic and can generate physiological responses in animals that consume them. Tannin can be toxic to filamentous fungi, yeast and bacterial. The higher content of tannins in the honey will be more active as antifungal, antibacterial, antidiarrheal, antioxidant and antihemorrhoidal agent [8].

The knowledge of the phytate level in foods is necessary because high concentration can cause adverse effects on the digestibility. Phytate diet of 1-6% over a long period of time decreases the bioavailability of mineral elements in monogastric animals [9-11]. Phytic acid is a strong chelator, forming protein and mineral-phytic acid complexes thereby decreasing protein and mineral bioavailability. Phytate is associated with nutritional diseases such as rickets in children and osteomalacia in adult humans respectively. However, the phytate are quantitatively the second abundant antinutritive contents of honey from Biase, Southern Senatorial District of Cross River State. It is established that only high content of this antinutrient elecite deleterious effect in the body metabolism [12-15].

Next to phytate, cyanide is the second most abundant antinutrient in the sample. It has been established that excess cyanate in the body inhibits the cytochrome oxidase [16]. This may stop ATP formation and the release of inorganic phosphate to body tissues. Consequently, the body suffers energy deprivation and subsequent death. High level of HCN has been implicated in cerebral damage and lethargy in man and animal.

Oxalate was not detected in honey sample from Biase, Southern Senatorial District of Cross River State. Oxalates had being known to cause irreversible oxalate nephritis when ingested in large doses. It is an antinutrient and prevents the absorption of some vital nutrients in food, especially divalent metals ($Ca^{2+}$, $Mg^{2+}$ etc.) and fatty acids by forming salts [17,18]. Oxalate intoxification (high ingestion of oxalate) causes malasorption syndromes leading to steatorrhoea, in which fatty acids are not absorbed, causing formation of insoluble calcium salt of fatty acid. Oxalates also cause gastrointestinal tract irritation, blockage of the renal tubules by calcium oxalate crystals, development of urinary calculi and hypocalcaemia [19].

They are significantly low levels of antinutrients in this honey sample and as such generally regarded as safe [1]. The antinutrients presents in honey sample, irrespective of their concentration are natural components of honey. This is indicating the importance and necessity in taking moderate amount of honey in our daily diet. The absence of the antinutrients especially may be attributed to their flora source [20].

## Conclusion

This study reveals that honey possesses some nutritional quality that can be used as supplement for the need of human. The results of this study show the variability of some quality characteristics of the honey sample from Baise, Southern Senatorial District of Cross River State. The honey was mostly of good quality when compared with Codex Alimentarius honey specifications.

### References

1. Codex Alimentarius (2001) Draft revised standard for honey. Alinorm 01/25 19-26. Official Journal of the European Communities L10: 47-52.
2. Cantarelli MA, Pellerano RG, Marchevsky EJ, Camina JM (2008) Quality of honey from Argentina: study of chemical composition and trace elements. J Arg Chem Soc 96: 33-41.
3. Ebenezer IO, Olubenga MT (2010) Pollen characterization of honey samples from North Central Nigeria. J Biol Sci 10: 43-47.
4. Adeniyi KA, Daudu OAY, Abubakar A, Ismail AD, Busari MB, et al. (2014) Comparative Analysis of the Proximate Composition of Nigerian Bitter and Sweet honey from Apis mellifera. Int J Sci Res Pub 4: 1-4.
5. AOAC (2005) Official of Analysis The Association of Official Analytical Ahemist. 18th Marylad. AOAC International. William Harwitz (ed).
6. Onwuka GI (2005) Food analysis and instrumentation; theory and Practice. Nephthalic Prints, Surulere, Lagos, Nigeria, 219. Sharpe, A. G., (1976): The Chemistry of Cyano Complexes of the Transition Metals; Academic Press: London, 1976.
7. Nyau V, Mwanza EP, Moonga HB (20013) Physico-chemical qualities of honey harvested from different beehive types in Zambia. African J Food Agri Nutri Development 13.
8. Khaliqur R, Hussain A, Shafqat U, Zai IUM (2013) Phytochemical Analysis and Chemical Composition of Different Branded and Unbranded Honey Sample. Int J Microbiol Res 4: 132-137.
9. Sulieman AME, Abdelhmied BA, Salih ZA (2013) Quality Evaluation of Honey Obtained from Different Sources. Food and Public Health 3: 137-141.
10. Adebiyi FM, Akpan I, Obiajunwa EI, Olaniyi HB (2004) Chemical/physical characterization of Nigeria honey Pakistan. J Nutr 3: 278-281.
11. Andrussow L (1927) Über die schnell verlaufenden katalytischen Prozesse in strömenden Gasen und die Ammoniak-Oxydation (V). Berichte der deutschenchemischen Gesellschaft 60: 2005-2018.
12. African Journal of Food Agric Nutrition and Development 13: 7415-7427.
13. Baroni MV, Arrua C, Nores ML, Faye P, Diaz del MP, et al. (2009) Composition of honey from Cordoba (Argentina): Assessment of North/South provenance by chemometrics. Food Chem 114: 727-733.

14. Boralle N, Gottlieb HE, Gottlieb OR, Kubitzki K, Lopes LMX, et al. (1993) Oligostilbenoids from Gnetum venosum. Phytochemistry 34: 1403-1407.

15. Dinnebier RE, Vensky S, Panthöfer M, Jansen M (2003) Crystal and Molecular Structures of Alkali Oxalates: First Proof of a Staggered Oxalate Anion in the Solid State. Inorg Chem 42: 1499-1507.

16. FAO (1980) Compositional Analysis methods In: Manuals of food quality control. Food analysis, general techniques, additives, contaminants and composition of food and Agricultural organization of the United Nations. Pp: 203-232.

17. Kambai C, Popoola V, Ugbe CJ, Janfa N, Ukanyirioha C (2015) Proximate and Mineral Elements Composition of Honey from Selected Hives in Jos Metropolis, Nigeria. J Environ Sci Toxicol Food Technol 9: 94-98.

18. Chua LS, Adnan NA (2014) Biochemical and Nutritional Components of Selected Honey Samples. Acta Sci Pol Technol Aliment 13: 169-179.

19. Mullaney EJ, Ullah AHJ (2012) Phytases: attributes, catalytic mechanisms, and application. Inositol phosphates: linking agriculture and the environment. Pp: 97-110.

20. Ndife J, Abioye L, Dandago M (2014) Quality Assessment of Nigerian Honey Sourced from Different Floral Locations. Nigerian Food J 32: 48-55.