Comparative Study and Analysis of Proximate Compositions and Some Mineral Contents in Natural and Artificial Honey from Ogun State, Nigeria

Samuel Adeolu Olugbemi[1*], Oluwasegun Amos Amoniyan[1]
and Omolola Myriam Balogun[1]

[1]*Department of Science Laboratory Technology, School of Science and Technology, Gateway Polytechnic, Saapade-Remo, Ogun State, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. Author SAO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author OAA managed the analyses of the study. Author OMB managed the literature searches. All authors read and approved the final manuscript.

Article Information

Original Research Article

ABSTRACT

Use of honey is gaining ground worldwide as people are getting aware of the high nutritional values and beneficial health promoting effects of honey. This study was carried out in order to compare the proximate compositions and the mineral contents available in both natural honey and artificial honey obtained from some selected towns in Ijebu and Remo zones of Ogun State, Nigeria. The parameters were analyzed using standard methods of Association of Official Analytical Chemists (AOAC, 2005). From the results obtained, the proximate compositions (%) of the natural honey were - moisture content (17.85±0.74), carbohydrate (78.56±2.92), protein (2.43±0.23), fat (0.68±0.04), crude fiber (0.19±0.02), ash (0.29±0.05) while that of the artificial honey were moisture content (21.65±0.94), carbohydrate (76.25±2.67), protein (1.53±0.12), fat (0.30±0.07), crude fiber (0.12±0.01), ash (0.15±0.09). The natural honey compositions were generally better than those of the artificial honey compositions. This trend was also observed for the mineral contents – Na, K, Ca, Mg, Fe, Zn and other parameters such as pH, titratable acidity, electrical conductivity and total acidity analyzed.

Corresponding author: Email: samadeolugbemi@gmail.com;

1. INTRODUCTION

Honey is a sweet, viscous food substance produced by bees and some related insects. Bees produce honey from the sugary secretions of plants (floral nectar) or other insects (aphid honeydew) through regurgitation, enzymatic activity and water evaporation. Honey is stored in wax structures called honeycombs [1]. Natural honey is one of the most widely sought products due to its unique nutritional and medicinal properties as a result of the different groups of substances it contains. Honey is a worldwide recognized natural food which has high nutritional value and many beneficial health promoting effects. It consists mainly of carbohydrates (at least 60% mass ratio), particularly reducing sugars such as fructose and glucose as fast energy source upon consumption. The minor components in honey include amino acids, vitamins, organic acids, minerals and various phytochemicals [2].

Honey contains a variety of photochemical and other substances, such as organic acids, vitamins and enzymes, which may serve as a source for dietary antioxidants [3].

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 plant species on which the bees foraged [4,5].

The composition and the quality of honey depend on many factors such as, climatic condition during production, nectar composition, agricultural practices and handling of honey during extraction and storage. Physicochemical characteristic of honey may also depend on the bee species as well as geographical origin [6,7].

Honey has a long history of human consumption, and is used in various foods and beverages as a sweetener and flavoring agent. Flavors of honey vary based on the nectar source and various types and grades of honey that are considered [8,9].

Traditionally, honey has been used as a medicinal remedy for the treatment of wound, various ailments and diseases [10]. The healing capacity of honey is strongly influenced by the physical and chemical properties of honey [11,12].

Honey comes in a range of colors including white, amber, red, brown and almost black [13]. The flavor and texture also vary with the flower nectar from which they were made [8]. Due to the vast importance of natural honey and its commercial values but limited availability, people tend to produce honey from cane sugar or try to adulterate natural honey by addition of other sugars, syrups or compounds to change its flavor or viscosity. This process is sometimes used as a method of deception where buyers are led to believe that the honey is pure and natural, and also to increase the quantity available in the market in order to make more money. This study was aimed at assessing and evaluating the nutritive values and some mineral contents of both natural honey and artificial honey and to carry out comparative study on them in order to determine which is best for human consumption.

2. MATERIALS AND METHODS

2.1 Sample Collection

The honey samples used for this study were bought from twelve different local markets in some selected cities and towns within Ijebu and Remo areas of Ogun State, Nigeria, namely: - Ijebu-Ode, Ago-Iwoye, Ijebu-Igbo, Atan, Sagamu, Isara, Ipara, Ode-Remo, Iperu, Ikenne, Ilishan and Ogere. Twelve of natural and artificial honey samples each were bought from each local markets within the selected towns in the month of May, 2018 where local honey merchants rarely (if at all) subject honey to quality analysis by regulatory agencies.

The following physical tests were carried out on the samples in order to ascertain the purity of the honey samples and to distinguish the natural honey from the adulterated or artificial ones.

(i) Thumb Test – Natural honey stay intact on a thumb when a small drop of the honey is placed on the thumb while artificial honey spills and spreads around.

(ii) Water Test – Natural honey settles right at the bottom of the water in glass cup while artificial honey dissolves in the water.

Table 1. Sampling areas within Ijebu and Remo Areas of Ogun State, Nigeria

Natural Honey Sample	Artificial Honey Sample	Sampling Area
NH1	AH1	Ijebu-Ode
NH2	AH2	Ago-Iwoye
NH3	AH3	Ijebu-Igbo
NH4	AH4	Atan
NH5	AH5	Sagamu
NH6	AH6	Ishara
NH7	AH7	Ipara
NH8	AH8	Ode-Remo
NH9	AH9	Iperu
NH10	AH10	Ikenne
NH11	AH11	Ilishan
NH12	AH12	Ogere

NH – Natural Honey, AH – Artificial Honey

(iii) Flame Test – A lighted wooden splint in a natural honey continues to burn while it is extinguished by an artificial honey.

(iv) Water-Vinegar Mix – A well-mixed mixture of natural honey with some water and 2-3 drops of vinegar will not be foamy unlike the artificial one.

2.2 Proximate Compositions and Mineral Contents

The Proximate compositions of the samples were analyzed chemically according to the official methods of analysis described by the Association of Official Analytical Chemists [14] and Charrondiere, et al. [15]. All analyses were carried out in replicate.

2.2.1 Determination of moisture content: by oven drying method

2.2.1.1 Procedure

a) Crucible was dried in an oven and cooled in a desiccators and then weighed, W_1.
b) 2g of sample was weighed into the crucible. Weight of crucible + sample = W_2
c) The crucible and the weighed sample material were heated in an oven at a temperature of 105^0C for 5 hours until the weight remained constant.
d) The crucible with the dried sample material was transferred into a desiccators with the aid of a pair of tongs and allowed to cool and reweighed, W_3.

$$The \ \% \ Moisture \ Content = \frac{W_2 - W_3}{W_2 - W_1} \times 100\%$$

2.2.2 Determination of total solids

The total solid content determination was calculated by subtracting the percentage moisture content from the total percentage i.e. 100% of the sample before drying.

$$The \ \% \ Total \ Solid \ Content = \frac{W_3 - W_1}{W_2 - W_1} \times 100\%$$

2.2.3 Determination of the ash content

The inorganic residues which are in form of their oxides give an idea of the mineral content in the original food. The inorganic residues are the remnant after the removal of the moisture content and burning off of the organic matters with muffle furnace at 550^0C.

2.2.3.1 Dry ashing procedure

a) Weight of a dried crucible, W_1.
b) 2 g of the sample was transferred into the crucible. Weight of crucible + weight of the sample = W_2
c) The crucible with the sample was transferred into the muffle furnace regulated at 550^0C for 4 hours until the sample was fully ashed.
d) The crucible with the ashed material was transferred into the desiccators with the aid of a pair of tongs and allowed to cool.
e) Weight of the cooled crucible with the ashed material = W_3.

$$The \ \% \ of \ Total \ Ash \ Content \ =$$

$$\frac{Weight \ of \ Ash}{Weight \ of \ sample} \times 100\%$$

3

2.2.4 Determination of crude fiber

a) 2.0 g of the sample, (W_1) was weighed into the fiber flask, and 100 mL of 0.25 M of H_2SO_4 was added and the mixture was heated under reflux for an hour with the heating mantle.
b) The hot mixture was filtered through a fiber sieve cloth. The filtrate was thrown off and the residue was returned to the fiber flask to which 100 mL of 0.31 M NaOH was added and heated under reflux for another 1 hour.
c) The mixture was filtered using a fiber sieve cloth and 10 mL of acetone was added to dissolve any organic constituent.
d) Residue was rinsed with about 50 mL of hot water on the sieve cloth before it was finally transferred into the crucible. The crucible and the residue were oven dried at 105^0C overnight to drive off moisture.
e) The oven dried crucible containing the residue was cooled in a desiccator and later weighed to obtain the weight W_2.
f) The crucible with weight W_2 was transferred to the muffle furnace at 550^0C for 4 hours.
g) Sample was cooled in a desiccators and weighed after cooling to obtain W_3. The difference $W_2 - W_3$ gives the weight of the fiber.

$$The\ \%\ Crude\ Fiber\ = \frac{W_2 - W_3}{W_1} \times 100\%$$

2.2.5 Determination of protein

Protein content in food samples is determined mainly or usually by the conventional Kjedahl method. However, Various modifications of Kjedahl method are available and but the one of them employed in this work is Nessler's Reagent method.

a) 2 g of the sample was weighed and transferred into a digestion flask.
b) 10 mL of concentrated H_2SO_4 was added.
c) The sample and the acid were heated gently until digestion was completed.
d) This was determined when the solution became clear.
e) The digest was finally clarified by adding another strong oxidizing agent, hydrogen peroxide (H_2O_2).
f) The solution became very clear signifying total digestion.
g) Digest was then transferred into a 100 mL volumetric flask and made to volume with ammonia free water to form the stock solution.
h) 5 mL of the aliquot was transferred into another 25 mL volumetric flask and 2 mL of Nessler's reagent was added.
i) The mixture was made up to volume with ammonia free water.
j) Change in color from yellow to brown was observed, which shows the presence of Nitrogen.
k) The intensity of the color is directly proportional to the amount of Nitrogen in the sample which is also related to the quality of the protein in the sample.
l) Color developed was measured using spectrophotometer at about 460-462 nanometer, for the amount of protein.

The % Protein Content = %N x 6.25

2.2.6 Determination of fat content

a) 1.0 g of each sample was put into cleaned, dried conical flask.
b) Samples were heated with 10 mL alcohol for 10 minutes on a water bath at a temperature of 62^0C.
c) Samples were allowed to cool using in desiccators.
d) 12 mL diethyl ether was added into the sample in each flask and the reagent was shaken properly.
e) 0.5 mL dilute ammonia was added to the samples in each flask.
f) 4.5 mL water and 12.5 mL light petroleum were added and mixed together gently.
g) Upper layer of each sample was siphoned off into a cleaned weighed beaker.
h) Extracts were then heated to remove solvent by evaporation and were then weighed and values were recorded.

$$The\ \%\ Fat\ Content$$
$$= \frac{Weight\ of\ extract}{Weight\ of\ sample} \times 100\%$$

2.2.7 Determination of carbohydrate

Total carbohydrate content of each samples were determined by difference. This was done by subtracting the percentage of moisture, ash, protein, fiber and fat obtained from 100%.

The % Carbohydrate = 100% - (% moisture + % ash + % protein + % crude fiber + % fat) [15]

2.2.8 Determination of pH measurement

Standardized pH meter using buffer solutions 4.0, 7.0 and 9.0 was dipped into the honey sample to determine the pH.

2.2.9 Determination of titratable acidity

a) 1 mL of the sample was diluted to 100 mL. 10 mL of the aliquot of the diluent was pipetted into a 100 mL conical flask.
b) 1 drop of 1% of phenolphthalein indicator was added and shaken properly to give a pink color.
c) The mixture was titrated against 0.1M NaOH solution until the pink color was discharged to a clear colorless solution at the equivalence point.

d) $The\ \%\ of\ Titratable\ Acid\ =$
$$\frac{Titre\ value\ \times Mol.(NaOH)\times F \times Df \times 10}{Volume\ of\ aliquot\ taken}$$

F = Equivalence of a particular acid, Df = Dilution factor

2.2.10 Determination of the mineral compositions

The mineral compositions in the honey samples evaluated include: Sodium and Potassium determined using flame photometer (Model: Corning 410), Magnesium, Calcium, Zinc and Iron were determined using atomic absorption spectrophotometer (Model: Buck VGP 210) after digestion [16].

3. RESULTS AND DISCUSSION

The results of the proximate analysis and the mineral contents of the honey samples bought from selected cities and towns in Ijebu and Remo areas of Ogun State, Nigeria are summarized in Table 2.

The proximate compositions and the mineral contents of both natural and artificial honeys were as shown in Table 2.

The moisture content in the analyzed honey samples of natural honey and artificial honey were 17.85±0.74% and 21.65±0.94% respectively. The natural honey was found to be within the limit prescribed by Codex Alimentarius Commission [17] of (21 g/100 g) while the artificial honey was slightly higher than the permissible limit but falls within the range of

20.62 to 37.31% reported by Chua and Adnan [2] and Oyeyemi et al. [6] that reported 20.50 to 21.78%. The high moisture content in the artificial honey may be attributed to the intentional adulteration by the sellers and the processes involved in the production. Thus, the shelf life of the artificial honey would be shorter than that of the natural honey.

The ash content of honey is a parameter that is used in determining the floral origin of a honey and this represent the mineral and trace element contents in the honey. The ash contents of the natural and the artificial honey were 0.29±0.05% and 0.15±0.09% respectively. These results fall within the limits of <0.6g/100g by Codex Alimentarius Commission, [17] and also agreed with the values of 0.44 to 0.50% reported by Oyeyemi et al. [6] and 0.12 to 0.50 by Adenekan et al. [11]. This study revealed that natural honey with higher ash content contains more minerals than the artificial honey.

The natural and artificial honey were analyzed for the crude fiber and the results obtained were 0.19±0.02% and 0.12±0.01% respectively. The results in this study agreed with some previous works reported in literatures that honey contains little or no fiber [2,8]. Natural honey will therefore contain higher amount of indigestible carbohydrates than the artificial honey and this which in turn aids easy digestion.

This study revealed that the protein content of the natural honey was 2.43±0.23% and that of the artificial honey was 1.53±0.12%. These results agreed with the values of 1.43 to 2.72% reported by Agunbiade et al. [18] but relatively lower than 5.65 to 6.25% reported by Oyeyemi et al. [6] while they were higher than those reported by Chua and Adnan [2]. Since protein is responsible for the thickness of honey and also for building human body, the higher protein content in the pure honey makes it thicker and more nutritious than the artificial honey.

From this study, natural honey showed higher fat content of 0.68±0.04% while that of the artificial honey was 0.30±0.07%. The results obtained here were similar to the results of 0.80 to 1.23% previously reported by Oyeyemi et al. [6]. Some studies reported also revealed that honey contains little or no fat [2,19]. This implies that the natural honey will be a better source of lipid than artificial honey.

Table 2. Results of proximate compositions and the mineral contents of the honey samples from selected Ijebu/Remo Towns

Parameter	Natural Honey	Artificial Honey	Parameter	Natural Honey	Artificial Honey
Moisture Content (%)	17.85±0.74	21.65±0.94	Na (mg/Kg)	0.03±0.01	0.02±0.00
CHO (%)	78.56±2.92	76.25±2.67	K (mg/Kg)	0.07±0.02	0.05±0.01
Protein (%)	2.43±0.23	1.53±0.12	Ca (mg/Kg)	0.06±0.01	0.02±0.00
Fat (%)	0.68±0.04	0.30±0.07	Mg (mg/Kg)	0.02±0.00	0.01±0.00
Fibre (%)	0.19±0.02	0.12±0.01	Fe (mg/Kg)	0.51±0.04	0.20±0.03
Ash (%)	0.29±0.05	0.15±0.09	Zn (mg/Kg)	0.15±0.02	0.07±0.01
			pH	3.85±0.11	4.28±0.21
			%TTA	2.70±0.13	1.28±0.09
			Electrical Conductivity (µS/cm)	4.53±0.31	2.71±0.47
			Total Acidity (meq/Kg)	35.80±1.31	28.95±1.83

CHO = Carbohydrates, TTA = Total Titratable Acidity

The carbohydrate contents in both samples were high. This implies that honey makes a good source of energy to the body. The carbohydrate contents of both natural and the artificial honey were 78.56±2.92% and 76.25±2.67% respectively. These results corroborated the previous work by Chua and Adnan [2] who reported 61.89 to 78.67%. This study also revealed that natural honey will be a better source of energy than artificial honey.

This study revealed the total acidity of the natural and artificial honey to be 35.80±1.31% and 28.95±1.83%. The total acidity of the samples studied falls within the acceptable limit of 50 meq/kg.

Honey generally has low pH which is responsible for its antiseptic and antimicrobial properties. The lower the acidity, the more potent for it to be used for curing and preventing infections. The pH of the natural and artificial honey was 3.85±0.11 and 4.28±0.21 respectively. The pH results of this study were higher than the values of 3.21 to 3.50 reported by Chua and Adnan [2] but fall within the acceptable range of 3.0 to 4.3 prescribed by Bogdanov [20]. The lower pH and the higher total acidity of the natural honey make it a better antiseptic and antimicrobial agent than the artificial honey.

The titratable acidity for both the natural and artificial honey was found to be 2.70±0.13% and 1.28±0.09% respectively. Comparatively, these values were higher than the values of 0.03 to 0.19% reported by Lawal et al. [21]. The value of

the natural honey falls within the values of 2.31 to 2.73 reported by Oyeyemi et al. [6] while that of the artificial honey was lower

The results of the concentrations of the mineral contents of the honey samples analyzed in mg/Kg as shown in Table 2 revealed that the concentrations of the minerals in the natural honey were generally higher than their corresponding minerals in the artificial honey. The concentrations of the minerals found in the natural honey samples were in the order of Iron (0.51±0.04) > Zinc (0.15±0.02) > Potassium (0.07±0.02) > Calcium (0.06±0.01) > Sodium (0.03±0.01) > Magnesium (0.02±0.00). Artificial honey samples results also followed the same trend with the natural honey except for calcium and sodium that had the same concentration: Iron (0.20±0.03) > Zinc (0.07±0.01) > Potassium (0.05±0.01) > Calcium (0.02±0.00) = Sodium (0.02±0.00) > Magnesium (0.01±0.00). The values obtained from this study were not in conformity with that of Agbagwa et al. [22] who reported dominance of potassium while Oyeyemi et al. [6] reported dominance of calcium in the honey samples analyzed. However, the mineral contents of honey may differ 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 [6].

Natural honey will be a better source of these minerals than the artificial honey as iron plays an important role in hemoglobin formation, normal functioning of the central nervous system and oxidation of carbohydrate, protein and fats [23]. Sodium and Potassium found in the intracellular

fluid help to maintain electrolyte balance and membrane fluidity [6] while magnesium protects and manages high blood pressure and cardiovascular diseases [24].

4. CONCLUSION

This study confirmed that honey possesses important nutritional properties and possible therapeutic ability. The study revealed that both natural and artificial honeys meet the Codex Alimentarius [17] specifications for honey. It was also revealed that, important proximate and mineral compositional differences exist between natural and artificial honey. Nonetheless, the study established that natural honey is better than the artificial counterpart nutritionally. Consumption of honey is also shown here to aid the achievement of recommended dietary allowance (RDA) of the minerals contained in honey thus preventing effects of the deficiency of such mineral. The reduced nutritional quality of the artificial honey could be attributed to the methods employed during processing and/or intentional adulteration by the sellers in order to make more money. The authors hereby conclude that more comparative work should be done on the natural and artificial honey to determine the level of adulteration, the type of sugar and their respective abundance in each. Furthermore, government should intensify on sensitization and enlightenment of the consumers on the need to patronize honey certified by the food regulatory agencies only.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Eva C. Bees and Beekeeping Science, Practice and World Resources. Heinemann Newnes, Oxford; 1990.
2. Chua LS, Adnan NA. Biochemical and nutritional components of selected :honey samples. Acta Sci. Pol., Technol. Aliment. 2014;13(2):169-179.
3. Rebiai A, Lanez T. Comparative study of honey collected from different flora of Algeria. Journal of Fundamental and Applied Sciences. 2014;6(1):48-55.
4. Adeniyi KA, Daudu OAY, Abubakar A, Ismail AD, Busari MB, Abdulsalami H, Usman OKA. Comparative analysis of the proximate and nutritional compositions of Nigerian bitter and sweet honey from *Apis mellifera*. International Journal of Scientific and Research Publications. 2014;4(11):2-4.
5. James OO, Mesubi MA, Usman LA, Yeye SO, Ajanaku KO. Physical characteristics of some honey samples from North Central Nigeria. International Journal of Physical Sciences. 2009;4:464 -470.
6. Oyeyemi SD, Kayode J, Owolabi MO. Comparative nutritional studies on honey samples in Ado Ekiti, Ekiti State, Nigeria. Donnish Journal of Medicinal Plant Research. 2015;2(2):016-020.
7. Amril A, Ladjama A. Physicochemical characterization of some multifloral honeys from honeybees *Apis mellifera* collected in the Algerian Northeast. African Journal of Food Science. 2013;7(7):168-173.
8. Kambai C, Popoola V, Ugbe CJ, Janfa N, Ukanyirioha C. Proximate and mineral elements composition of honey from selected hives in Jos Metropolis, Nigeria. IOSR Journal of Environmental Science, Toxicology and Food Technology. 2014; 9(9):94-98.
9. Vaughn MB. Pollen contents of honey. CAP Newsletter. 2001;24(1):10–24.
10. Basauldo C, Sgroy V, Finola MS, Marioli JM. Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. Veterinary Microbiology. 2007;124:375-381.
11. Adenekan MO, Amusa NA, Okpezel VE, Owosibo AO. Nutritional and microbiological composition of honey samples obtained from Ogun State, Southwestern Nigeria. European Journal of Sustainable Development. 2012;1(2): 271-286.
12. Nwankwo CM, Ezekoye CC, Igbokwe SO. Phytochemical screening and antimicrobial activity of apiary honey produced by honey bee (*Apis mellifera*) on clinical strains of *Staphyloccus aureus*, *Escherichia coli* and *Candida albicans*. African Journal of Biotechnology. 2014;13(23):2367-2372.
13. Eleazu CO, Iroaganachi M, Okoronkwo J. Determination of the physico-chemical composition, microbial quality and free radical scavenging activities of some commercially sold honey samples in Aba, Nigeria: The effect of varying colours. Journal of Nutritional Food Science. 2013;3(2):189.

14. AOAC, Official Methods of Analysis of AOAC International, 18th ed. AOAC. International, Maryland, USA; 2005.
15. Charrondiere UR, Chevassus-Agnes S, Marroni S, Burlingame B. Impart of different micronutrient definitions and energy conversion factors on energy supply estimations. J. Food Compos. Anal. 2004;17(3-4):339-360.
16. Escuredo O, Sojo M, Fernandez-Gonzalez M. Descriptive analysis of rubus honey from the North-West of Spain. Int. J. of Food Sci. and Tech. 2011;46:2329-2336.
17. Codex Alimentarius. Draft revisal standard for honey Codex Alimentarius Commission, FAO, Rome, Alinorm. 2001; 25:19-26.
18. Agunbiade SO, Arojojoye OA, Alao OO. Evaluation of some biochemical microbiological and physic-chemical properties of Moroccan honey. Int.J. Agric. Biol. 2012;7(5):773-776.
19. Singh N, Bath KP. Quality evaluation of different types of Indian honey. Food Chemistry. 1997;58:129-133.
20. Bogdanov S. Honey quality and International standards. Review by the International Honey Commission. Bee World. 1999;80(2):61-68.
21. Lawal RA, Lawal AK, Adekalu JB. Physico-chemical studies on adulteration of honey in Nigeria. Pak. J. of Biol. Sci. 2009;12: 1080–1084.
22. Agbagwa OE, Otokunefor TV, Peterside FN. Quality assessment of Nigeria honey and Manuka honey. J. Microbiol. Res. 2011;1(3):20-31.
23. Adeyeye EI, Okokiti MKO. Proximate composition and some nutritional valuable minerals of two varieties of *Capsicum annum*. (Bell and Cherry peppers). Discovery Innovation. 1999;11:75-81.
24. Vorman J. Magnesium; nutrition and metabolism. Molecular aspect of medicine. Washington D.C. 2003;23:27.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/60072