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Comparative Study of Physico-Chemical Properties of Pure Honey Harvested from *Jatropha curcas* Plantation and Honey Fortified with Moringa and Ginger

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ABSTRACT

This study was carried out to investigate the physicochemical properties of honey produced and harvested from *jatropha curcas* plantation and to compare them with the other two honey samples blended with ginger and moringa. The physicochemical parameters like: moisture content, pH, titratable acidity, total sugars, reducing sugar and non-reducing sugar were analyzed. Ash content of the honey is important because it represents its mineral content and forms part of proximate analysis for nutritional evaluations. The concentration of the selected metals (Cu, Ca, K, Fe, Zn, Cr) were also determined. The result showed that the pure honey fell within the permitted standards in all parameters determined, while the two honey samples blended with ginger and moringa respectively were not within the range of the standard values in some the parameters tested for, these includes moisture content, ash content and pH. The result of elemental analysis showed that the three samples of honey contain all these metals except chromium. Potassium was found to be in abundance compared with the level of other metals in each sample. The level of metal in both moringa and ginger fortified were higher which may be due to the presence of the addictives in the samples.Thus the the physicochemical properties recorded for honey for *Jatropha curcas* plantation horney showed its natural purity and good quality.

Key words: Honeybees, *jatropha curcas* plantation,physicochemical, parameters,Honey

INTRODUCTION

Honey is a natural sweet substance that is produced by some set of social bees. There are three families of social bees, which produce honey; these are: the, *Bomidae*, *Meliponidae* and *Apidae* (Smith *et al*., 2009). Honey is produced by honeybees from the nectar of blossoms or from the nectar secretion of living parts of plants or the excretion of plants sucking insects on the living parts of plants, which honeybees collect, transforms and combine with specific substances of their own, store and left in honey comb to ripen (Kebede *et al.*, 2012). Honey is composed primarily of sugars glucose and fructose with its third greatest component being water. It also contains other minor substances, such as organic acids, amino acids, proteins, minerals, vitamins and lipids. (Ferreira 2009;Terrab *et al.*, 2004).

The composition and flavour of honey varies, depending mainly on the source of nectar(s) from which it originates and to a lesser extent on certain external factors such as climatic conditions, humidity inside the hive and beekeeping practices in removing and extracting honey (Guler *et al.*, 2007).

Honey has numerous uses and functional applications worldwide such as in food systems, religious and magical ceremonies as well as in human and veterinary medicine, as in the treatment of wounds, ulcers, cough etc (Bogdanov *et al.*, 2014). The quality of honey is determined by its sensorial, chemical, physical, or biological properties. Internationally honey quality criteria are specified in Regulatory Standards, compiled in a Codex Alimentarius standard. (Bogdanov *et al.*, 2004).

Physicochemical parameters such as electrical conductivity, ash content, moisture content, free acid, mineral content, diastase activity, apparent sugar content have all been suggested as criteria for the characterization of honeys. These analyses helps food analyst to determine the chemical quality of the honeys analyzed (Cantarelli *et al*., 2008)

MATERIALS AND METHODS

Honey samples

Three honey samples were used for this research. The main honey, sample A was honey produced in and harvested from *jatropha curcas* plantation established in University of Ilorin, while two other samples B and C, were natural honey blended with ginger and moringa respectively.They were of commercial source. Confirmation of honey sample from *Jatropha curcas* Plantation was carried out from analysis of pollen presence in the honey (Ihtisham-ul-haq 1997;. Bogdanov *et al*, 2004).

Physicochemical analysis

Determination of moisture Content

The moisture content followed the AOAC (2000)
A certain weight (5g) of sample was weighed and placed into a pre weighed drying dish. The sample was then dried to constant weight in an oven at 105^{o}C for 4hrs.

Moisture content (%) = M_1-M_2 / M_1-M_0 x 100

Where:
M_o= Weight of Dish
M_1= Weight of the fresh Sample + dish
M_2 = Weight of the dried sample + dish

Determination of Ash Content

The determination of ash content according to AOAC (2000).Stainless steel crucibles were rinsed with distilled water and oven dried at 105^{o}C. 5g of honey will be weighed into the crucible and placed in a furnace at 110^{o}C for 30 minutes and then at 550^{o}C for 3hrs to constant weight. Care will be taken during heating so that no excess foaming takes place. This was repeated consequently till the weight became constant (ash became white or gray white).Weight of ash gave the ash content and was calculated by the following formula (Horwitz W. 2000)

$$\text{Ash (\%)} = \frac{\text{Weight of crucible + Ash - Weight of crucible}}{\text{Weight of sample}} \times 100$$

Determination of Total Solids

Percentage total solids for each sample were determined using the following formula (Horwitz, 2000).

Total Solids (%) = 100 – Moisture Content

Determination of pH

The pH of honey was determined according to the method described by the International Honey Commission 2011. Five grams of each honey sample was diluted with 50ml distilled water to make a 10% solution. The pH was measured using a digital pH meter Model HI 8519 (Hannah Instrument) which was calibrated at room temperature using buffer solutions at pH 4 and 7. To ensure accurate pH measurement, the instrument was calibrated every time before use and recalibrated every two or three hours to compensate for any possible loss of sensitivity (Kebede *et al*, 2012).

Determination of Sugars

Five grams of sample was measured into a beaker and 100ml of warm water was added. The solution was then stirred until all soluble matters are dissolved and filtered through a filter paper into a 250ml volumetric flask. 100ml of the solution was pipetted and prepared into a conical flask, after which 10ml of diluted hydrogen chloride (HCl) was added and boiled for 5mins. On cooling, the solution was then neutralized to phenolphthalein with 10% NaOH and kept in a 250 volumetric flask. The solution was used for titration against Fehling's solution and the reading was calculated as follows: (Shahnawaz, 2013)

$$\textbf{Total Sugar (\%)} = \frac{\textbf{Factor (4.95) x dilution (250) x 2.5}}{\textbf{Titre x wt of Sample x 10}}$$

$$\textbf{Reducing Sugar (\%)} = \frac{\textbf{Factor (4.95) x dilution (250)}}{\textbf{Titre x wt of Sample x 10}}$$

Non reducing Sugar was estimated as the difference between the total sugar and the reducing sugar

Non-reducing Sugar = Total sugar – Reducing sugar

Determination of Titratable Acidity

A certain volume (25ml) of each sample (diluted) was titrated against 0.1N NaOH using phenolphthalein as an indicator (Agbagwa *et al* 2011). The relative amount of lactic acid was determined using the mathematical formulae:

Lactic acid (%) = Titre Value x Normality x 9
Volume of Sample

Elemental Analysis: Cu, Zn, Cr, Ca, K & Fe were determined using atomic spectrophotometer.

Elemental analysis was carried out using the solution of the ash after ash determination. This was carried out by measuring 5mls of 10% HCl solution; this was added to ash and warm in a water bath to dissolve. Where the ash did not dissolve, it was treated with 5mls of 10% nitric acid and warmed in a waterbath to dissolve. A stirring rod was used to stir quantitatively through a funnel into a clean dry 50ml standard volumetric flask. This solution of ash was used to check for the determination of elements: Cu, Zn, Cr, Ca, K, and Fe by direct aspiration via atomic spectrophotometer (Popek 2002;Agbagwa et al 2011)

Statistical Analysis

Analysis of results were carried using SPSS statistical program version sixteen. The means value were compared by using the least descriptive significant difference (LSD) at 0.05 probabilities Statistical descriptive statistics were applied for each of the quantitave parameters. (Shahnawaz *et al*, 2013;Matouskova, 1992)

RESULTS AND DISCUSSION

Confirmation of honey samples from *Jatropha* Plantation

Sixteen plant species, belonging to 13 families were observed in the honey samples collected from *Jatropha* Plantation with 9 pollen types . In the honey samples from *Jatropha* Plantation, pollen grains from *Guio gracilis* was more dominant (15.1%), followed closely by pollen grains from *Jatropha curcas* (14.1%), which is the dominant plant species in the area where beehives are located, and pollen grains from *Alchoneacordifolia*being the least with 0.6% frequency (Table 1). (Ibrahim 2012).

Results of physicochemical analysis

Results obtained on physicochemical analysis were summarized in Table 2. The **moisture content** of the samples A, B, C are 18.20%, 22.13% and 22.60% respectively. Sample A fell within the maximum allowable content for honey as determined by the International Honey Commission (<20%) while samples B and C were higher. This was in agreement with the findings of Cantarelli *et al.*, 2008) who reported that the moisture content in honey was recorded in the range of 14% to 19%. Furthermore, it was in agreement with the standard of the Codex Alimentarius (Baroni *et al.*, 2009) which has a limit of less than 19% but samples B and C fell above the limits, which may be due to the presence of ginger and moringa respectively in the samples respectively (Martin *et al*, 1999; Shahnawaz, 2013).

The moisture content is an important criterion for evaluating the grade ripeness of honey and its shelf life. In general, the amount of water present causes honey to ferment, spoil and

loose flavor, with ensuing honey quality loss (Saxena *et al..*, 2010; Fredes and Montenegro 2006) reported that honey containing lower moisture content has a longer shelf life, which means that sample A possess the quality for this longer shelf life and resistant to microbial attack than samples B and C.

The maximum **Ash Content** was found in sample B (0.8%) followed by sample C (0.6%) whereas sample A had the least ash content (0.2%). The ash content of sample A was in agreement with that of Ihtisham-ui-haq (1997), who analyzed different varieties of honey for determination of ash content and draw a range of (0.008 to 0.49) % ash.

The Ash content of the all the samples are in accordance with those of White (1975) who worked on different varieties of honey and obtained ash content in the range of 0.020 to 1.028%. Honeys normally have low ash content but the high content of ash in samples B and C could be due to the presence of ginger and moringa respectively. The variation may also be due to many environmental factors, such as soil conditions, atmospheric conditions, beekeeping techniques and physiology of each plant. The ash content is normally used to determine botanical origin (Fredes and Montenegro 2006).

Honey is naturally acidic, irrespective of its geographical origin, which may be due to the presence of organic acids, that contribute to its flavor and stability against microbial spoilage. **The pH** of sample A (4.02) was in accordance with those made by the Codex Alimentarious Commission (2001) where acceptable ranges of pH of honey was predetermined between 3.2 and 4.5. Similarly the pH of sample A was also similar to those of Ibrahim *et al* (2012) who worked on Algerian Honey samples having a range of 3.70 to 4.00. The result was also in agreement with the findings of Hussain (1989), who reported the pH of 3.0 to 5.0 in pure honey. Sample B (6.09) and C (6.15) are not in accordance with these findings, which showed that the blending of those honey samples with ginger and moringa affected the pH of the honey.

The pH of honey samples is important during extraction process because it affects the texture of honey as well as its stability and shelf life [Terrab *et al.*, 2002] hence sample A of lowest pH have a better stability and longer shelf life than sample B and C.

The result of this study also include the concentration of **sugars** (Table 2)of which **Total sugar** was 76.10%, 68.20% and 68.40% in samples A, B and C respectively, whereas **reducing sugars** was determined to be 74.00%, 65.00% and 65.45% respectively. These results can be compared to the results of other researchers such as Shahnawaz (2013) and Joshi (1997), who also reported closely related findings of total sugar ranging from 53.30% to 80.7% in different varieties of honey. None of the sample exceeded the limit set for total sugar content by the European Community Directive (2001). The result of the reducing sugars was also in agreement with the findings of Latif *et al*. (1956), who reported 65 to 76% in different varieties. Likewise, non reducing sugar was determined to be 2.10%, 3.20% and 2.95% in samples A, B and C respectively. This result also authenticated agreed to Codex Alimentarius Commission [2001] given the fact that the range of non-reducing sugar in honey is 1.15 to 12%.

Electrical Conductivity represents a parameter used in routine honey quality control, and can be considered as a valid criterion for the determination of honey's botanical origin or more specifically, for the differentiation between nectar honey and honeydew honey (Ibrahim *et al*., 2012). None of the analyzed sample (Table 2) showed electrical conductivity values higher than 0.8mS/cm (variation between 0.22 and 0.58 mS/cm), suggesting that all samples are from nectar honey. Sample A has the least conductivity while sample B and C are higher, which showed the presence of more minerals and lower acid content, which must have been due to blending it with ginger and moringa.

Result of the Elemental Analysis

The result of the **metal characterization** of the three samples is detailed in Table 3. A total of six element, were determined. Similar to previous studies, the predominant metals observed in honey samples investigated in this study were K, Ca, Fe, Cu and Zn. Also the result of this study was similar to the work of (Agbagwa *et al*., 2011) where chromium was not detected at all in all samples.

Potassium accounted for the most abundant (29.25 to 283.5mg/L), which may be due to the level of potassium in plant tissues. Nutritionally, the presence of these metals in honey makes it an excellent food supplement for humans. From previous, it appears that the elemental composition of honey depends on the soil composition, plant type, season and environmental conditions.

CONCLUSION

Physicochemical analyses have been carried out on three honey samples and results were compared to various results of other studies and regulatory standards. From the result of these analyses, it was found that sample A, which is from University of Ilorin *jatropha curcas* plantation met the standard in all the tested parameter while sample B and C blended with ginger and moringa respectively were not in some parameters.

The study allowed the qualitative analysis of the honey samples and it showed that Unilorin honey (sample A) is a pure honey as a result of the fact that the physicochemical parameter were within the standard. Finally physicochemical parameters enable a researchers to determine the quality of honey and the extent to which honey has might have been adulterated.

Table 1: Frequency of the plant species in *Jatropha* Plantation honey sample

Plant species	Family	Pollen type	Frequency (%)
Alchoneacordifolia	Euphorbiaceae	Tricolpate	0.6
Anacardiumoccidentale	Anacardiaceae	Triporate	5.9
Apoisamericana	Fabaceae	Tricolporate	2.5
Astromytus spp.	Myrtaceae	Syncolprate	2.8
Canomyricamonticola	Myricaceae	Triporate	4.7
Capsicum annuum	Solanaceae	Tricolporate	13.5
Cephatalusoccidentale	Rubiaceae	Tricolporate	5.2
Elaeisguineensis	Arecaceae	Tricolpate	4.5
Guiogracilis	Sapindaceae	Parasyncolporate	15.1
Jatropha curcas	Euphorbiaceae	Panporate	14.1
Pendiculoussibthorpii	Schnophulariaceae	Tricolporate	1.5
Phyllanthuscasearoides	Euphorbiaceae	Triporate	4.0
Sabal palmetto	Araceae	Monosulcate	3.7
Syzigiumguineense	Myrtaceae	Tricolpate	7.5
Taxa bacata	Taxaceae	Ulcerate	9.5
Zea mays	Poaceae	Monoporate	3.7

Source: AbdulRahaman et al. (2013)

Table 2. Physicochemical analysis

SAMPLE	Moisture content (%)	AC (%)	Total Sugar (%)	R. Sugar (%)	N.R Sugar (%)	pH	E.C (mS/cm)	Acidity (%)	Total solids (%)
A	18.20*	0.20*	76.10*	74.00*	2.10*	4.02*	0.35*	1.08*	81.80*
B	22.13*	0.80*	68.20*	65.00*	3.20*	6.09*	0.55*	4.32*	77.87*
C	22.60*	0.60*	68.40*	65.25*	2.95*	6.15*	0.58*	4.68*	77.40*
Lit value & range	13.4-19.5	0.02-1.028	53.30 – 80.78	65.0 – 76.0		3.2 -4.5	0.22 - 0.58		

Values were obtained after triplicate analysis *Significance at $P < 0.05$

KEYS

MC = Moisture content E.C = Electrical conductivity

AC = Ash content

R. Sugar = Reducing Sugars

N.R Sugar = Non reducing Sugar

Table 3. Elemental Analysis

Ca (mg/L)	K (mg/L)	Fe (mg/L)	Cu (mg/L)	Zn (mg/L)	Cr (mg/L)
4.13	29.25	0.91	0.64	2.94	0.00
5.24	283.57	2.23	0.66	48.03	0.00
5.36	276.35	2.75	0.79	3.76	0.00

Values were obtained after triplicate analysis

References

AbdulRahaman A. A, Solomon, O. R., Adeyemi S. B., Liadi M. T., Ahmed R. N., Belewu, M. A. and Oladele F. A. (2013). Melisopalynological Analysis of Honey Samples from Jatropha Plantation and Unilorin Apiary Farm. International Journal of Phytofuel and Allied Sciences Vol. 2(1) 81 -92.

Agbagwa O.E, Otokumefor T.V, Frank-Peterside and Nnnenna.(2011). Quality assessment of Nigerian honey and Manuka honey. *Journal of Microbiology and Biotechnology Research*.1 (3).20-31.

Baroni M.V, Arrua C, Nores M.L.; Fayer P, Diaz M.D, Chiabrando G.A and Wunderlin D.A. (2009). *Food Chem*. 2009, 114, 727.

Bogdanov S. Ruoff K. and Persano-oddo L. (2004). Physicochemical methods for the characterization of unifloral honeys: A review. *In Apidologie*, Vol. 35, no 1, p. S4-S17

Bogdanov Stefan (2014). Honey in Medicine. *Bee Product Science*. www.bee-hexagone.net.

Cantarelli M.A, Pellerano R.G, Marchevsky E.J and Camina J.M (2008). Quality of honey from Argentina: Study of chemical composition and trace elements. In the *Journal of the Agentine Chemical Society*, Vol. 96,2008, no. 1-2, pp. 33-41

Ferreira I.C.F.R., Aires E., Barreira J.C.M., Estevinho L.M., (2009), " Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. *Food chem.*. 114, 1438-1443.

Fredes C and Montenegro G (2006). Honey metals and other trace elements in honey bee in Chile. *Cien. Inv. Agric*., 33: 50-58

Guler A. Bakan A. Nisbet C and Yavuz O. (2007). "Physicochemical analysis of pure honey" *Food chem.* 105, 1119

Horwitz W. (ed) (2000) Official method of analysis of chemists, Sugar and sugar products 2(44) 20-33

Hussain M.H.(1989).Studies on the production and some properties of honeys from Dhoefer (Omar).Proc. Fourth Intl. Conf. Api. Tropical climates. Int. Bee Res. Ass., pp.256-264

Ibrahim Khalil, Mohammed Moniruzzaman, Laid Boukraa, MokhtarBenhanifia. Md. Asiful Islam, Md. Nazmul Islam, SitiAmrahsulaiman and SiewHuaGan.(2012). Physicochemical and Antioxidant properties of Algerian Honey. *Molecules*, 17.11199-11215. http://www.mdpi.com/journal/molecules.

Ihtisham-ul-haq (1997). Effects of floral type, geographical locations and honey bee species on the water activity and other physicochemical parameters of honey M.sc Thesis. Dept. food Sci. Technol., *Faculty of Nutrition Sciences*, NWFP

Agricultural University of Peshawer, Pakistan.

Joshi V.K (1997). Fruit Wine, Naumi Solar (HP). Directorate of Extension Education, Uni. of Hortic and Forestry Ch. 2 pp 78-86

Kebede N, Subramanian P.A and Grebrekidan M. (2012). Physicochemical analysis of Tigray Honey: An attempt to determine major quality markers of Honey. *Bull. Chem. Soc, Ethiop.* 26(1), 127-133.

Latif A. QuayyumH.A, and Haq M (1956). Research on the composition of native honey. *Pak. J. Sci. Res.*, 8: 163-165.

Martin P., Lullman C. and Bogdnov S. (1999) Honey quality and international regulatory standards,Review of the International Honey Commisssion. Mitt. Gebiete Lebenism Hyg.108 - 125.

Matouskova O., Chalupa J., Hruka K and Cigler M. (1992) Statistic programme STATplus version 1.01. pp 89 -124

Popek S. (2002) A procedure to identify a honey type *Food chem*. 79 402 - 406

Saxena S.; Gautam S. and Sharma A. (2010).Physical biochemical and antioxidant properties of some Indian honeys. *Food Chem*. 118, 391-397.

Shahnawaz M., Sheikh S.A., Hussain M., Abdulrasak and Khan S.S., (2013), "A study on the determination of physicochemical properties of honey from different valleys of Gilgit- Baltistan. *International Journal of Agricultural Science Research journals*. Vol. 2(2), pp 049-053.

Smith E.G, De Clerk-Floate R.A, Van Hezewik B.H, Moyer J.R and Pavelik E (2009). Cost of mass-producing the root weevil, Mongulosecruciger, a biological control agent for houndstongue (Cynoglossumofficianale L.). *Biol. Cont*. 48:281-286

Terrab A, Angeles F.R, Dolorez H and Francisco J.H. (2004). Characterization of Spanish thyme honeys by their physicochemical characteristics and mineral content. *Food Chem*. 88, 537-542.

Terrab A, Diez M.J and Heredia F.J. (2002). Characterization of Moroccan unifloral honeys by their physicochemical characteristics. *Food Chem*. 79, 373-379.