Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.6, No.19, 2016

Physicochemical Analysis and Mineral Contents of Honey from Farmers in Western States of Nigeria

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Abstract
Honey samples were randomly collected from farmers in localities from Western States of Nigeria. Physicochemical and minerals were analyzed in the samples. Average mean pH was 3.34, moisture (16.6%), ash (0.46), ascorbic acid (2.61 mg/100g), citric acid (10.67%), lactic acid (121.71%), riboflavin (0.002 mg/100g), sugar content (65.78%), refractive index (1.49) and relative density (1.41 g/cm^3) were obtained from the proximate analysis. Average mean values of mineral contents in honey samples were Fe (3.80 mg/kg), Mn (1.47 mg/kg), Zn (1.82 mg/kg), P (29.64 mg/kg), Ca (55.93 mg/kg), Al (2.34 mg/kg), Cu (0.94 mg/kg), K (481.30 mg/kg), Mg (25.57 mg/kg) and Na (25.42 mg/kg).
Keywords: Chemical, honeys, minerals, farmers, Nigeria

1. Introduction

Honey is the substance made from the gathering of nectar, sugary deposits from plants and animals by honey bees (*Apis mellifera, Apis cerana indica* and *Apis mellipodae*) which is in their natural scientific models synthesized, purified and stored in combs in jelly liquid. The mechanism of honey synthesis by bees is same all over the world but the differences in honey observed in their physical and chemical properties are basically on geographical and botanical origins. The variation in taste, flavor, aroma and colour determines that honey is produced from many different flora substances majorly from plants (Omoya and Akharaiyi, 2010). This substantial variation is ably observed in the composition and nutritional values of honey. Commercial samples of honey available in various parts of the world are of highly different quality, on the basis of factors like geographical conditions, production season, processing, and source of nectar, packaging and storage period. Given the importance of honey as a nutrient full of energy and prebiotic compounds and its usage in disease treatment, the necessity of identifying the physicochemical and qualitative properties of this valuable nutrient is obvious (Mahmoudi *et al*., 2012). Quality of honey is mostly related to organoleptic, physicochemical and microbiological characteristics; qualitative physicochemical features of honey are indicated in European directive and codex food commission (EU, 2001). This feature includes content of moisture, ash, reducing and non-reducing sugars, acidity, starch, commercial and sugar content (Gomes *et al*., 2010). Honey consists of vast amount of different compounds that can be of nutritional and health benefits. Its therapeutic potential has been credited to its antimicrobial, anti-inflammatory, anti-oxidant properties, as well as boosting of the immune system and treatment of wounds (Manyi-Loh *et al*., 2011). Bashir (2009) reported that honey may inhibit the growth of a wide range of microbes. Honey has several important properties in addition to its composition and colour. It is composed mainly of carbohydrates, lesser amounts of water and a wide range of minor components (White, 1975).. This study is therefore aimed at investigating the physicochemical properties of honey samples four different pastures in Western Nigeria

2. Materials and Methods

Ten honey samples were randomly purchased directly from farmers in urban areas of Ondo, Oyo, Lagos and Osun States, Nigeria. The samples were filtered in the laboratory with sterile seitz filter with a pore size of 0.02 mm connected to an electronically operated vacuum pump. This was done to remove particles. The honey filtrates were stored in brown bottles at room temperature prior use.

2.1Ash content determination

Twenty milliliter of the each honey samples were weighed in crucible. The honey samples were charred on a Bunsen flame until samples turned black, dried and with no trace of foam. It was then ashed in a furnace at 600 ^oC and cooled in a desiccator until a constant weight was obtained (AOAC, 2000).

$$\% \text{ Ash} = \frac{(\text{Weight of crucible + ash}) - (\text{Weight of empty crucible}) \times 100}{\text{Sample weight}}$$

2.2 Moisture content determination

The moisture content was done by the use of Abbe refractometer. The refractometer was plugged, adjusted to 25

°C and allowed to stabilize for 30 min. The illuminating mirror was moved aside, the prism box was opened and the surface was wiped with cotton wool damped with acetone solution. The honey samples were introduced between the prism and the illuminating mirror was moved back to its position under the prism box. The mirror was adjusted to reflect sufficient rays of light into the box. The compensation prism was adjusted with the milled ring until the colour fringe became monochromatic and the boundary with sharp focus. The boundary was adjusted to coincide with the centre of the cross wires by using the prism control knob. The refractive indexes of the honey samples were read in the meter.

2.3 Ascorbic acid estimation

Ten milliliter of honey sample was measured in a conical flask, mixed with 100 ml of oxalic acid solution (acid extraction solution) and filtered with a seitz filter with a pore size of 0.02 mm. 2 ml of the filtrate was pipetted and mixed with 5 ml of acid extracting solution (oxalic acid) in a conical flask and titrated with (dichlorophenol-indophenol (DPIP) solution until a light rose pink colour was persisted. The amount of ascorbic acid is calculated thus:

$$\frac{\text{Titre value of sample} \times \text{dilute factor}}{\text{Titre volume of standard} \times \text{volume used}} \times 100$$

2.4 Citric acid estimation

0.5 ml of honey sample was mixed in 50 ml distilled water and titrated with 1M NaOH using 0.5 ml of dilute phenolphthalein as indicator.

$$\text{Factor} = \frac{\text{Titre value} \times \text{equivalent length}}{\text{Wight taken}} \times 100$$

2.5 Lactic acid estimation

One milliliter of honey sample was mixed in 10 ml distilled water and 20 ml of 1M NaOH in a volumetric flask. This was stocked with a stopper and allowed to stand for 30min. 0.5 ml dilute phenolphthalein solution was added and titrated with 1M HCl.

$$\text{Factor} = \frac{\text{Titre values} \times \text{equivalent factor}}{\text{Weight taken}} \times \text{factor} \times 100$$

2.6 Determination of riboflavin (Vitamin B_2)

The assay solution was diluted in buffer solution of pH 4 in order to obtain between 10 and 20 µg/ml B_2, The sample was read in a spectrophotometer at 445 nm at 445 nm A^1 % 1cm = 308

$$\text{Riboflavin concentration (mg/ml)} = \frac{(\text{Absorbance} \times 100)}{(A^1\ \%\ 1\text{cm})}$$

2.7 Sugar content estimation

The sugar content of honey samples was determined by means of spectrophotometer according to AOAC (2000) but with little modifications. 0.5 ml honey sample was weighed in a beaker and 1ml of ethanol in 2 ml of sterile distilled water was added. The mixture was shaken and 10 ml of ethanol boiled to 100 °C was added and shaken for even mix up. 10 ml of this solution was centrifuged for 10 min to obtain a clear supernatant of free sugar for the analysis. The supernatant was decanted in a volumetric flask and made up to 100 ml with sterile distilled water. 1ml of solution was obtained in a test tube where 0.5 ml of 5% phenol and 2.5 ml of concentrated H_2SO_4 was added. Blank was prepared with a mixture of 1ml of sterile distilled water, 0.5 ml phenol and 2.5 ml concentrated H_2SO_4 for colour development. The spectrophotometer was calibrated with sterile distilled water. Also, at wavelength of 490 nm, standard of different concentration of glucose from 0-50 mg/ml was prepared. A concentration curve was plotted against absorbance to determine slope.

$$\text{Concentration of sugar} = \frac{\text{Absorbance} \times 100}{\text{Slop}\ \ 0.5} \times 10$$

2.8 Trace elements analysis

Five grams of honey were accurately weighed in a 120 ml glass beaker. 25 ml of nitric, 10 ml of perchloric acid were added and heated with a hot plate to mineralize organic material. The mixtures were heated to almost dry. The acid clear solutions were transferred to 50 ml volumetric flasks and diluted with deionized water. Three replicates were analyzed per sample. Elements P, Al, Fe, Mn, Zn, Cu, Ca, Mg, Na and K were analyzed by emission measurements obtained by direct nebulization in an inductively coupled plasma optical emission spectrometer (Unico – S1100-RS).

2.9 Sensory evaluation

Equal volume of each of honey samples was dispensed on a serving dish for sensory quality assessment at breakfast time. With a 10 member panel of regular honey users either for healing or food supplement, appearance, colour, flowing properties, flavor, taste, texture, thickness, mouth fell and overall acceptability was evaluated. The parameters were rated on a 9 point hedonic scale. The ratings were described as dislike extremely (1), dislike very much (2), no preference (3), like extremely (4), like moderately (5), like very much (6) and like extremely (7).

However, the experiment was replicated three times and data obtained were analyzed using analysis of variance to determine difference and Ducan's multiple range tests to separate mean.

3. Results

3.1 Results of physicochemical analysis

pH of honey samples ranged from 2.40-4.06, with lowest pH mean value of 2.40 in samples from Lagos State and highest mean value of 4.06 in samples from Oyo State. Lowest ash content .with mean value of 0.16% was recorded in samples collected from Ondo State and highest of 1.00% from Oyo State, meanwhile samples from Lagos State were void of ash contents. Moisture content of honey samples ranged from 14.4–17.8%. Lowest evaluated mean value of 14.4% was recorded in samples collected from Lagos State and highest moisture of 17.8% each from Osun and Oyo States respectively. Ascorbic acid evaluated in the honey samples was between 2.41-2.68 mg/100g. While Lagos State samples had mean value of 2.41 mg/100g, Ondo, Osun and Oyo States samples had 2.68 mg/100g each of ascorbic acid content. Citric acid content was extremely low in samples collected from Lagos (5.46%) compared with highest value (12.77%) recorded from Ondo and Oyo States samples. Lactic acid content of honey samples was generally high in all samples. Highest mean value of 63.3% was recorded from Lagos State samples, followed by Oyo State with mean value of 61.0%, Ondo with 59.48% and least evaluated average mean of 58.5% from Osun State samples. Riboflavin content from honey samples was in the range of 0.001-0.002 mg/100g, with Lagos State samples evaluated for 0.001 mg/100g, while Ondo, Osun and Oyo States honeys had mean values of 0.002 mg/100g each. Reducing sugar content was highest in Lagos State samples with mean value of 78.8%, followed by samples from Osun States with 66.5% while least mean value of 56.7% was recorded in Oyo State samples. Refractive index evaluated in samples from Ondo and Osun States was 1.48% each respectively, while Lagos and Oyo States samples were evaluated for 1.50% each. Relative density of samples was similar and values were between 1.40-1.44. Osun and Oyo States honey samples had same mean values of 1.40 each, Ondo State honeys had mean value of 1.41 and Lagos State honeys with mean value of 1.44 (Table 1).

3.2 Results of Trace elements contents

Table 2 illustrates trace elements present in honey from studied areas. However, significant differences ($p<0.05$) were not observed among the honey samples from Western States of Nigeria.

Table 3 illustrates the analyzed trace elements in comparison with that obtained from other countries. In most cases, similarities were observed in some of the trace elements mainly for Fe, Mn, P, and Cu as reported by researchers in other countries of Italy, Argentina, Turkey, Cameroun, Egypt and Spanish.

3.3 Results of Sensory evaluation

The result obtained from the sensory evaluation showed differences in parameters considered to actualize acceptability and non acceptability of samples. Means followed by the same letter within each column are not significantly different at ($p\leq0.05$). Though significant differences occurred in parameters evaluated, the honey samples were endorsed for acceptability hence none of the samples' rating fell below average for partial acceptability according to international standard rating. Osun State honey samples were rated highest on the overall acceptability with a score of 9.8, followed by Ondo State having a score of 9.3, Lagos State 8.8 and Oyo State with least score of 8.0 (Table 4).

4. Discussion

The mean pH value of samples from the studied areas was resolved at 3.34 which is low enough to suspect adulteration of the samples. It is of a reputable index that microbial contaminations could cause deterioration thereby making samples to lose their freshness. From this obtained value, it is evident that the honey samples were acidic in nature which is a general model of assessing honey quality. This acid nature of honey quality according to Mahmoudi *et al*, (2012), is extremely important during harvest of honey and storage, its effect on preservation growth of microorganisms improving the stability and durability of honey. Similar results in pH were reported in honey samples from Portugal, Argentina, Cameroun, Iran and Turkey respectively by Gomes *et al*, (2010); Contarelli *et al*., (2008); Tchoumboue *et al*, (2007); Mahmoudi *et al*, (2012); Kahraman *et al*, (2010). The pH mean value acceptable in honey is in the range of 3.4 - 6.1 (Annon 2001-2004) and in the results we

reported "pH of honey samples ranged from 3.16-4.0" which are within the minimum acceptable value.

Average moisture of 16.4% was recorded from honey samples in this study. Quality honey should have a low moisture content to avoid fermentation by associated microorganisms and enzymatic factors. The international standard of moisture in quality honey is ($\leq$ 20%) and the results obtained are within this range and therefore ascertained the honey samples of low moisture content that will not support proliferation of microorganisms for spoilage of the honey samples. Also evidenced the low moisture content of samples, was the absence of sugar granules during the various analyses carried out on samples. Though honey samples from studied areas were varied in values, all were within the range of international acceptable values. The variation in obtained values could be depended on degree of honey maturity in hives before harvest as reported by Finola *et al*., (2007) and could also be the season of harvest and storage conditions operated by different farmers in the different localities. Similar moisture contents in honey were reported by Kahraman *et al*., (2010); Tysset *et al*., (1980) and Duman *et al*., (2008). However, the percentage moisture content reached in this study reflected in the thickness and flowing properties of the samples thus even meeting international standards for acceptability in virtually all parameters.

A relatively low ash content mean value of 0.46% was evaluated. Ash contents in honey could be affected by nectar ingredients for honey production (Al-Khalifa and Al-Arify, 1999; Annon, 2001-2004). Similar ash contents as recorded in this study have been reported by Adenekan *et al*, (2010); Sahinler and Gul, (2004); Kahraman *et al*., (2010). Ash content was however not recorded from samples for Lagos State during this study. The absence of ash content in honey from this area of study among others does not implied that from time to time honey produced in this area could be void of ash content. Ash contents in honey represent the direct measure of inorganic residues after honey carbonization. The variability in ash of honey samples from the studied areas can be explained by the floral sources of honey (Vit *et al*., 1998).

Acid in honey accounts for less than 0.5% of the solids, but this level contributes not only to the flavour, but is in part responsible for excellent stability against microorganisms. Ascorbic mean value in the honey samples was 2.16 mg/100g. This value is moderately high to assist in combating free radicals hence ascorbic acid is a naturally occurring organic compounds with antioxidant property. Hence floral sources for the production of honey by bees are majorly from plant products, hydrophilic compounds such as vitamin C is expected to be available as regards the ascorbic value recorded. Hydrophilic compounds have potentials in reducing hazards of reactive oxygen nitrogen species (Omoruyi *et al*., 2012). This could be one applauds in the healing ability of honey. Mean value of citric acid was 10.66%. This may be as a result of resident microorganisms fermenting the sugar components in the honey which of course will add to the taste and enhance its preservative ability against microbial spoilage. The acidity of honey is due to the presence of organic acids, particularly the gluconic acid, in equilibrium with their lactones or esters and inorganic ions such as phosphate and chloride (Al-Khalifa and Al-Arify, 1999).

High amount of potassium, calcium, phosphorus, magnesium and sodium were recorded in honey samples than other minerals analyzed in this study. From the studied areas, Lagos State had higher values in minerals such as copper, iron, manganese, zinc and magnesium. The abundance of these minerals in Lagos State samples than others may be from wastes generated by the many industries located in this area which bees regularly visits in collection of materials for honey production. Similar results were reported by Tuzen *et al*., (2007) in honey from Turkey and other countries such as Spain, Italy. Higher values of these elements were reported from honey samples from Egypt by Rahed and Soltan, (2004) and lower values than what was obtained in this study were reported in honey samples from Argentina by Cantarelli *et al*, (2008). These minerals among others are essential for humans, and they may play an important role in a number of biochemical processes (Garcia *et al*., 2005). The variation of mineral elements in honey samples from one country to another is not controversial hence reports from many researchers have emphasized that minerals can be highly indicative of the geographical origin of honey and can be used as environmental indicators (Przybylowski & Wilczynska, 2001). Honeybees may continuously be exposed to contaminants present in the surrounding area for the duration of their foraging activity (Conti & Botre, 2001). Therefore honeybees and their products can be considered representative bio-indicators of the environmental pollution (Conti & Botre, 2001). Bogdanov *et al*., (2007) emphasized that minerals detected from honey are originated from both natural sources (soil and plants) and anthropogenic sources. Minerals such as Zn and Cu are well known as potential air or soil contaminants of anthropogenic origin, and are of course also found as natural ingredients of soil minerals, as are Fe and Mn. Apart from direct excretion via nectar, such elements might find other ways to honey: by deposition as dusts or aerosols onto flower and nectars, onto leaf surfaces and honeydew or on the bees themselves (Bogdanov *et al*., 2007).

The higher rating of honey samples from Osun and Ondo States than Lagos and Oyo States could be as a result of less industries generating wastes adopted by bees for the production of honey in these areas. Oyo and Lagos States are larger population and with many industries of various products where pollutants to reduce honey quality could be adopted by honey bees from effluents generated from industries and homes; and

environments such as markets, eateries, and other commercial areas where indiscriminate human wastes disposal are common routine by individuals.

Conclusions

The honey samples evaluated were from the Western States of Nigeria. The physicochemical parameters analyzed were within the range of international standard. The acid level of samples discouraged microbial proliferation for spoilage of samples. Quality honey samples were obtained from less industrial areas than highly industrial areas in the studied location.

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Table 1: Proximate composition of honey Sample

Sample collection area	pH	Moisture (%)	Ash (%)	Arscorbic acid (mg/100g)	Citric acid (%)	Lactic (mg/100g) (%)	Riboflavin content	Sugar (%)	Refractive index	Relative density (g/cm^3)
Ondo	3.16^{b}	15.6^{b}	0.16^{c}	2.68^{a}	12.77^{a}	59.5^{c}	0.002^{a}	61.1^{bc}	1.48^{a}	1.41^{a}
Osun	3.75^{ab}	17.8^{a}	0.66^{ab}	2.68^{a}	11.61^{ab}	58.5^{c}	0.002^{a}	66.5^{b}	1.48^{a}	1.40^{a}
Lagos	2.40^{c}	14.4^{c}	0.00^{c}	2.41^{b}	5.46^{c}	63.3^{a}	0.001^{a}	78.8^{a}	1.50^{a}	1.44^{a}
Oyo	4.06^{a}	17.8^{a}	1.00^{a}	2.68^{a}	12.77^{a}	61.0^{b}	0.002^{a}	56.7^{c}	1.50^{a}	1.40^{a}
Average Mean value	3.34	16.4	0.46	2.61	10.67	60.60	0.002	65.78	1.49	1.41

^{abc} Means with different letters in a same parameter are significantly different from each other (p<0.05)
Each value is a mean standard deviation of triplicate determinations per sample

Table 2: Mineral content (mg/kg) in honey from studied area

Trace element	Ondo State	Osun State	Lagos State	Oyo State	Average mean
Fe	3.6^{ab}	3.7^{ab}	4.11^{a}	3.8^{ab}	3.80$_{ab}$
Mn	1.45^{a}	1.40^{a}	1.45^{a}	1.48^{a}	1.47^{a}
Zn	1.64^{ab}	1.81^{ab}	2.06^{a}	1.76^{ab}	1.82^{ab}
P	27.4^{a}	28.56^{ab}	30.1^{a}	32.5^{a}	29.64^{ab}
Ca	58.41^{a}	50.75^{c}	56.11^{ab}	58.45^{a}	55.93^{ab}
Al	2.44^{ab}	2.62^{a}	2.16^{c}	2.14^{c}	2.34^{ab}
Cu	1.16^{a}	0.76^{b}	1.18^{a}	0.66^{b}	0.94^{b}
K	475.46^{a}	480.11^{a}	483.40^{a}	486.18^{a}	481.30^{a}
Mg	26.14^{a}	25.11^{a}	28.03^{a}	25.18^{a}	25.37^{a}
Na	24.19^{a}	26.11^{a}	25.10^{a}	26.27^{a}	25.42^{a}

^{abc} Means with different letters in a same line are significantly different from each other (p<0.05)
Each value is a mean standard deviation of triplicate determinations per sample

Table 3: Comparison of minerals (mg/kg) in honey produced from other countries

	Nigeria	Argentina	Italy	Egypt	Turkey	Cameroun	Spanish
Fe	3.80	3.91	3.8	113.2	6.6	-	4.56
Mn	1.47	0.33	1.54	11.7	1.0	0.03	3.10
Zn	1.82	1.08	2.5	7.2	2.7	-	2.12
P	29.64	28.80	-	-	-	1.80	51
Ca	55.9	56.35	257	192	51	22.68	92.2
Al	2.576	-	-	-	-	-	21.48
Cu	0.94	0.19	0.91	1.7	1.9	0.02	0.60
K	481.30	482.76	1195	1500	296	20.02	1283.9
Mg	25.37	23.38	56.7	102	136	0.92	55.3
Na	25.42	25.56	96.6	378	117	0.62	99.7

Table 4: Sensory evaluation of honey samples

	Ondo State	Oyo State	Osun State	Lagos State
Appearance	7.2 0.± 6a	6.4 ± 0.5b	7.2 ± 0.4a	7.5 ± 0.5a
Colour	6.3 ± 0.4b	7.3 ± 0.7a	6.2 ± 0.4b	6.3 ± 0.5b
Flowing property	6.7 ± 0.4^{ab}	6.1 ± 0.3^{b}	6.6 ± 0.5^{ab}	7.1 ± 0.9^{a}
Flavour	6.2 ± 0.4^{a}	6.8 ± 0.4^{a}	6.4 ± 0.5^{a}	6.1 ± 0.2^{a}
Taste	8.8 ± 1.0^{ab}	7.2 ± 1.2^{a}	9.4 ± 0.6^{a}	9.0 ± 0.8^{a}
Texture	6.3 ± 0.5^{a}	6.1 ± 0.3^{a}	6.3 ± 0.5^{a}	6.5 ± 0.5^{a}
Thicknes	6.6 ± 0.5^{b}	7.7 ± 0.5^{a}	7.0 ± 0.2^{a}	6.4 ± 0.5^{b}
Mouth feel	1.7 ± 0.4^{c}	7.3 ± 2.8^{a}	6.7 ± 0.5^{b}	6.9 ± 0.3^{b}
Overall Acceptability	9.3 ± 1.3^{a}	8.0 ± 1.8^{b}	9.8 ± 1.0^{a}	8.8 ± 1.0^{b}

^{abc} Means with different letters in a same line are significantly different from each other ($p<0.05$)
Each value is a mean standard deviation of triplicate determinations per sample