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Chemical Study of Natural and Farmed Samples of Honey from Riyom, Plateau State

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Abstract

Raw and processed samples of honey from natural and bee - hives (farmed honey) sources were collected and analysed for some physicochemical properties and heavy metals. The results indicate moisture content to range between 18.81% to 20.45%. Ash content was found to range between 1.26% - 1.86%. pH of the honey samples ranged between 5.15 - 5.83. Electrical conductivity values vary between 0.86 mS/cm to 0.91mS/cm. The acid and Hydromethylfurfural (HMF) contents of the sample were found to be (5.28 - 6.53) meq/kg and (3.87-4.64) mg/kg respectively. Diastase activity values were observed to be between 7.61 DN to 10.18 DN. Lead content of raw and processed natural honey was 0.0432 mg/L and 0.0846 mg/L respectively, while that of processed from farmed sample was 0.0156 mg/L. Copper levels were found to range between 0.0187 mg/L to 0.1062 mg/L, while nickel levels ranged between (0.0234-0.0896) mg/L for natural honey. The values for farmed honey were between (0.0354 - 0.1867) mg/L. Cobalt was only detected (0.1563 mg/L) in the processed sample from trees. Cobalt was observed to be 0.2481 mg/L and 0.4352 mg/L for raw and processed honey from bee-hives respectively. Chromium levels were in the range 0.0172 mg/L to 0.2842 mg/L. Cadmium was not detected in all the honey samples. Zn levels were found to range between 0.2136 mg/L to 0.2963 mg/L. The results revealed that raw samples from trees were relatively higher in some physicochemical parameters compared to the raw farmed samples. In all cases heavy metal contents of the processed samples were higher than those of the raw samples. Therefore, care should be taken during processing since honey could easily be contaminated during processing.

Keywords

Honey, Bee-hive, Hydromethylfurfural, Diastase, Riyom

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1. Introduction

Honey is a sweet dark golden viscous liquid substance made by honey bees from the nectar and sweet deposits from plants, modified and stored in the honeycomb to ripe and mature [1]. Buba *et al* [2] reported that honey is a sticky and viscous solution and it is known to contain about 80–85% carbohydrate (mainly glucose and fructose), 15–17% water, 0.1–0.4% protein, 0.2% ash and minor quantities of amino acids, enzymes and vitamins as well as other substances like phenolic antioxidants. Lachman *et al*, [3] reported that the most abundant elements found in honey are potassium, calcium, magnesium and sodium.

There are basically two types of honey; Comb honey which is honey in its original comb or portions thereof and extracted honey: that is when it is removed from the comb and presented in several forms, as liquid, crystallized or granulated and partially crystallized. According to Adams *et al,* [4], the aroma, flavour, colour and composition of honey is dependent on its source.

Apart from its nutritional value, honey is known to contain flavonoids, antioxidants which help reduce the risk of some cancers and heart diseases. Honey had been reported to be used in the treatments of ulcers and bacterial gastroenteritis.

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It has antibacterial properties; it impedes the growth of food-borne pathogens such as *E. coli* and *salmonella*. It is known to fight certain bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. It is also known for the treatment of coughs and throat irritation. Antibiotic activities of honey are based on its ability to dehydrate bacteria and hence render them inactive [5]. Honey had been found to contain some enzymes which produce hydrogen peroxide that kills bacteria.

Considering the importance of honey for both nutritional and medicinal value, honey farming has become a profession for most ruler dwellers. They usually keep bees hives which later produces honey for commercial purposes. Although bees as agents of pollination suggests that they may also be a good source of heavy metal contamination. Bees contribute immeasurably in the contamination of honey through the transfer of these metals from nectar, pollens, flowers and calyx of plants that might have been contaminated by heavy metals to the honey [5, 6, 7]. Also, since production and selling of honey has become a lucrative business in Nigeria, many of the honey dealers sometime adulterate the honey by addition of some chemical substances that could endanger the health of the consumers or introduce heavy metals through the process of addition of these chemicals. Occasionally, honey may be contaminated by heavy metals during processing, since most of them employ crude methods of processing.

This study therefore considers the assessment of both raw and process honey from trees (natural honey) and from bee-hive (farmed honey) obtained from Hawan Kibo hills of Riyom L.G.A Plateau State, Nigeria with the aim of checking the purity and levels of heavy metal contamination.

2. Materials and Methods

2.1. Samples Collection

Samples of raw and process honey from natural source (from tree) and from bee-hives were collected from Hawan Kibo hills in Riyom L.G.A Plateau State, Nigeria in April, 2014. Unwanted materials such as wax sticks, dead bees and particles of combs were removed by straining using cheese cloth before analysis.

Moisture Content and ash contents were determined using the method described by AOAC [8].

2.2. Determination of Total Solids

Percentage total solids of each sample were determined using the following formula:

$$\text{Total solids (\%)} = 100 - \text{Moisture content}$$

2.3. Determination of pH

The pH values of the honey samples were measured using pH meter (Equiptronics digital pH meter model EQ-610). 10% aqueous solution of the honey was prepared by weighing 10 g of honey and diluting with 90 mL of distilled water. The mixture was stirred thoroughly using glass rod. The pH electrode was inserted into the solution and the reading was recorded as it stabilizes. Before use, the pH meter was calibrated at pH 4.0 and 7.0 using standard buffer solutions [9].

2.4. Determination of Conductivity

Electrical conductivities of the honey samples were measured using digital conductivity/pH meter model EQ-610. 10% aqueous solution of the honey samples was prepared by weighing 10 g of honey and diluted with 90 mL of distilled water. The mixture was stirred thoroughly using glass rod. The glass rod electrode was dipped into the solution. The reading was recorded as it stabilizes. Three replicates determination were carried out and average was taken [10].

2.5. Determination of Titratable Acidities [12]

10 % aqueous solution of the honey was prepared by weighing 10 g of honey and diluting with 90 mL of distilled water. 25 mL of the sample was titrated against 0.1M NaOH solution using phenolphthalein as indicator until the solution turned colourless and persisted for at least 10 seconds. The relative amount of titratable acid was determined using the mathematical formulae: Titratable acid (%) = [Titre value x morality x 9] / [Volume of sample].

2.6. Determination of Hydroxymethylfurfural (HMF)

HMF was determined spectrophotometrically according to the method described by White [12]. 5.0 g of undiluted honey was dissolved in 25 mL of distilled water, transferred quantitatively into a 50 mL volumetric flask, followed by the addition of 0.5 mL of Carrez solution I and 0.5 mL of Carrez solution II and made up to 50 mL mark with distilled water. The solution was filtered through filter paper, rejecting the first 10 mL of the filtrate. Aliquots of 5.0 mL were placed in two test tubes; to one tube was added 5.0 mL of distilled water (sample solution); to the second was added 5.0 mL 0.2% sodium bisulphate (reference solution). The absorbance of the solutions at 284 and 336 nm was determined using a UV–visible spectrophotometer. The quantitative value of HMF was calculated using the formula:

$$\text{HMF (mg/kg)} = (A284 - A336) \times 149.7 \times 5 \times D/W$$

2.7. Determination of Diastase Activity

The diastase activity was determined in accordance with the method of described by White [12]. 5 g of honey was dissolved in 15 mL water; then 2.5 mL of acetate buffer (pH 5.3) was added and transferred to a 25 mL volumetric flask. 10 mL of this solution was then mixed with 5 mL of 2% starch solution in a test tube and incubated at 40°C for 15 minutes. After 5 minutes, 1mL of the solution was taken and 7.0×10^{-4} meq/l of iodine solution was added. The absorbance was then read in a spectrophotometer at 660 nm until readings obtained were less than 0.235 absorbance units. The unit of diastase activity, the Schade unit, is defined as that amount of enzyme which will convert 0.01 gram of starch to the prescribed end-point in one hour at 40°C under the conditions of test. Results are expressed in Schade units per gram of honey. The diastase activity was calculated and expressed as Diastase Number (DN).

2.8. Digestion of Honey Samples for Metal Analysis

2 g of samples were placed in a dry and clean digestion tube. 10 ml concentrated HNO_3 was added and the mixture thoroughly mixed by stirring and placed on the digestion block whose temperature was gradually increased up to 100°C and maintained for 1.5 hours for complete oxidation. The temperature was then raised to 200°C and the sample heated to near dryness to complete the destruction of organic matter. 2 ml of HNO_3 was then added and the reaction mixture heated to a final volume of 5ml and filtered and transferred into a 100 ml volumetric flask. The flask was filled to mark with de-ionized water. The solution was then transferred into clean, dry plastic bottles for analysis. A blank solution was prepared by subjecting 2 ml of de-ionized water through the same process. All the samples were digested in triplicates. The concentrations of Pb, Cu, Ni, Co, Cr, Cd and Zn in the final solution were determined by atomic absorption spectrometer (AAS) (iCE 3000 series).

3. Results and Discussion

The moisture content of the raw and processed honey from trees and bee-hives was found to range between 18.81% to 20.45% (Table 1 and 2). There was significant difference in the moisture content of the honey at ($p < 0.05$). The results also indicate the moisture content of processed honey from the bee-hives to be higher. This could be traceable to adulteration during processing. Although, all the values are below the maximum limits (21%) accepted by the WHO and Codex Alimentarius. The lower value of moisture obtained in this study could probably be attributed to the time of harvest, besides; it implies that the honey from this area could be store for long time with minimal attack by micro-organisms.

The ash content of the raw and processed honey from the natural source was found to be 1.26% and 1.66% respectively (Table 1), while that from the bee-hives (Table 2) was 1.36% and 1.86% for raw and processed honey respectively. There was no significant difference in the ash content of the samples. The differences in soil texture and atmospheric conditions as well as the type and physiology of each plant could be responsible in the variation in ash content among honey samples [13]. Higher ash content is an indication of higher micro and macro nutrients composition of honey [1].

The pH value of the raw and processed honey from the natural source (Table 1) was found to be 5.63 and 5.83 respectively, while that from the bee-hives (Table 2) was 5.15 and 5.63 for raw and processed respectively. There was no significant difference in pH values of raw and processed honey from the natural source. The pH of honey samples analysed compared favourably with samples from other geographical locations. According to Buba [2], the acidic pH of honey is desirable because acidification promotes wound healing by causing oxygen release from haemoglobin. In addition, the pH of honey is low enough to prevent growth of many species of bacteria on wounds. Honey pH can provide a good indication of its botanical origin and it can also be used for the prediction of honey degradation during storage. Honeys with pH ranging from 3.5 to 4.5 are said to originate from nectar of plants.

The conductivities of the raw and processed honey from natural source (Table 1) was observed to varied between 0.91 mS/cm to 0.86 mS/cm , while that from bee-hives (Table 2) was 0.90 mS/cm and 0.86 mS/cm respectively. There was no significant difference in the electrical conductivities of the samples at $p<0.05$. Conductivity of honey depend on ash and acidic contents, i.e. the higher the ash and acid contents, the higher the conductivity. The conductivity data in this study shows that all the samples fall within the range required by the international standards. Conductivity is a good criterion for determining botanical origin of honey and recently, it is determined in routine honey quality control instead of the ash content. On comparative basis the conductivity data obtained in this work are higher than those reported for some commercial Nigerian honeys; 0.02 to 0.06 mS/cm with an average of 0.03 mS/cm [14].

Acid values were found to range between 5.28 meq/kg to 6.53 meq/kg (Tables 1 and 2). These values are far less than the 50meq/kg acceptable. All the values observed were below the acceptable limits, this is an indication of rawness of the honey samples. High acidity can be indicative of fermentation of sugars into organic acids [15].

The Hydromethylfurfural (HMF) content of raw and processed honey from natural source (Table 1) was observed to be 4.51 mg/kg and 4.64 mg/kg respectively, while that from bee-hives was 3.87 mg/kg for raw and 4.15 mg/kg (Table 2) for processed respectively. The results also shows that all the honey samples analysed had HMF contents within the specifications of international standards; i.e., not more than 40mg/kg. Hydromethylfurfural is a decomposition product of fructose and it is found only in trace amount in raw honey. However, its concentration increases with storage and prolonged heating of honey [2]. Other factors that can increase the concentration of HMF in honey are; pH, storage temperature, moisture, acidity, metals, amino acid and simple sugars (glucose and fructose).

Table 1. Physicochemical properties and heavy metals of Natural honey (from Trees).

Parameters	Raw	Processed	[39]WHO limits	[39]CODEX limits
Moisture (%)	18.81±0.39	19.23±0.72	21.0	21.0
Ash (%)	1.260±0.013	1.66±0.018	0.04	0.06
Total solids (%)	81.19±0.47	80.77±1.31		
pH	5.63±0.022	5.83±0.034	Less than 7.00	Less than 7.00
Conductivity(ms/cm)	0.91±0.0045	0.86±0.020	1.20	0.80
Titratable acidity (meq/kg)	5.29±0.054	6.53±0.48	50.0	80.0
Hydromethylfurfural (mg/kg)	4.51±0.11	4.64±0.81	40.0	40.0
Diastase activity (DN)	7.78±0.14	7.61±0.18	Greater than 3.00	Greater than 8.00
Pb (mg/L)	0.0432±0.893	0.0846±0.71	0.50	
Cu (mg/L)	0.0187±0.033	0.1062±0.035	0.05-0.50	
Ni (mg/L)	0.0234±0.082	0.0896±0.041		
Co (mg/L)	ND	0.1563±0.547		
Cr (mg/L)	0.0196±0.229	0.2846±0.238	1.00	
Cd (mg/L)	ND	ND	0.05-0.20	0.05-0.20
Zn (mg/L)	0.2267±0.0016	0.2893±0.043	5.00	5.00

ND=Not Detected

Table 2. Physicochemical properties and heavy metals of honey from hives (farmed honey).

Parameters	Raw	Processed	[39]WHO limits	[39]CODEX limits
Moisture (%)	18.85±0.15	20.45±0.11	21.0	21.0
Ash (%)	1.36±0.017	1.86±0.019	0.04	0.06
Total solids (%)	81.15±0.32	79.55±1.73		
pH	5.15±0.080	5.63±0.034	Less than 7.00	Less than 7.00
Conductivity(ms/cm)	0.90±0.0022	0.86±0.018	1.20	0.80
Titratable acidity (meq/kg)	5.28±0.032	5.67±0.180	50.0	80.0
Hydromethylfurfural (mg/kg)	3.87±0.110	4.15±0.160	40.0	40.0
Diastase activity (DN)	9.06±0.086	10.18±0.045	Greater than 3.00	Greater than 8.00
Pb (mg/L)	ND	0.0156±0.84	0.50	
Cu (mg/L)	0.024±0.054	0.0867±0.035	0.05-0.50	
Ni (mg/L)	0.035±0.117	0.1867±0.41		
Co (mg/L)	0.2491±0.99	0.4352±0.16		
Cr (mg/L)	ND	0.0172±0.238	1.00	
Cd (mg/L)	ND	ND	0.05-0.20	0.05-0.20
Zn (mg/L)	0.2136±0.015	0.2963±0.063	5.00	5.00

ND=Not Detected

The diastase activity, calculated as diastase number (DN) for raw and processed honey from tree was 7.78 DN and 7.61 DN respectively (Table 1). The value of diastase activity for bee-hives for both raw and processed honey (Table 2) was 9.06 DN and 10.18 DN respectively. Diastase activity plays an important role in judging quality of honey. A maximum value of 8 diastase units has been set by the Codex Alimentarius Commission as the acceptable limit for international market [11].

Results of heavy metal content of the honey indicate Pb level to be 0.0432 mg/L and 0.0846 mg/L for the raw and processed natural honey respectively. The lead content of processed bee-hives honey was 0.0156 mg/L. Lead was not detected in raw bee-hives honey. Lead is present in uncontaminated soils at concentrations less than 200 mg/kg but higher levels can be obtained from areas subjected to anthropogenic emissions [6]. Copper levels in honey were found to range between 0.0187 mg/L to 0.1062 mg/L. The results revealed that copper content of the natural honey to be higher than that of the bee-hives. This can be linked to the pollination activities of the bees and assimilation of copper from the soil by these plants since Cu is found naturally in the earth's crust as sulphides, sulphates, sulphosalts, carbonates and other compounds [16].

Nickel was also observed in all the samples at levels of 0.0234 mg/L and 0.0896 mg/L for raw and processed honey from tree. The raw and processed honey from bee-hives was 0.0354 mg/L and 0.1867 mg/L respectively. Nickel content of the honey was within the permissible limit for consumption. Cobalt was not detected in raw honey from tree but a level of 0.1563mg/L was observed in the processed honey (Table 1). Cobalt was observed in both the raw and processed honey from bee-hives (0.2481 mg/L and 0.4352 mg/L respectively Table 2). Chromium levels in the honey were found to range between 0.0172 mg/L to 0.2842 mg/L. However Cr was not detected in raw honey from wood. Cadmium was not detected in all the honey samples. Zn levels in the honey samples were found to range between 0.2136 mg/L to 0.2963 mg/L. In all case it was observed that the processed honey had higher levels of heavy metals than the raw samples.

4. Conclusion

Results of the physico-chemical assessment of honey produced from Hawan Kibo hills in Riyom L.G.A Plateau State of Nigeria revealed that it had not been sternly adulterated hence values were found to be in conformity with permissible levels. Also, heavy metal levels were found to be in varying amounts, although, most of which were below the permissible levels. It is therefore recommended that the processing methods should be modify so as to avoid contamination from heavy metals.

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