**Microbiological and Physicochemical Quality of Honey in Minna Metropolis, Nigeria**

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**ABSTRACT**

Honey is a naturally sweet dark golden liquid produced by honey bees. Ten (10) honey samples from different locations in Minna, Nigeria were evaluated for their microbiological and physicochemical quality. The samples were subjected to microbiological assessment using standard pour plate method. Results revealed that the physicochemical properties of the honey samples had an average pH of 3.94, total titratable acidity of 34.45meq/kg, electrical conductivity of 40.85µS/cm, moisture content of 17.07%, total solid of 82.93%, ash content of

0.25% and water activity of 0.56 which were within the acceptable standard limit for international and Nigerian honey. The results of the microbiological assessment showed that the total bacterial counts ranged from 0-2.0x105cfu/mL and total fungal counts ranged from 0-

7.0x104cfu/mL. The data obtained from physicochemical assessment were subjected to one-way

analysis of variance (ANOVA) (P≤0.05) which showed that there was no significant difference in the levels of pH, ash content and water activity, while there was significant difference in the levels of moisture content, conductivity, total titratable acidity and total solid. Microbiological assessment revealed significant difference in both the total bacterial counts and total fungal counts. The bacteria were identified as *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa,* while the fungus was *Aspergillus fumigatus.* This study shows that some honeys are contaminated with microorganisms that could be hazardous to human health. Hence, the need for routine hygienic practices necessary to avoid microbial contaminants.

**Key words: Assessment; Honey; Microorganisms; Physicochemical; Quality. INTRODUCTION**

Honey is a mixture of sugars, water and other compounds (organic acid, formic acid, citric, succinic, latic, malic, gluconic acid and a number of aromatic acids). The specific composition of any batch of honey depends on the flowers available to the bees that produce the honey [1]. Honey has a density of about.36 kilograms per liter and 36% denser than water [2].

In view of the carbohydrates contents, honey is mainly fructose (about 38.5%) and glucose (about 31.0%) making it similar to the synthetically produced inverted sugar syrup which is approximately 48% fructose, 47% glucose, 5% sucrose and other complex carbohydrates [3]. Honey contains trace amounts of several vitamins and minerals [4]. As with all nutritive sweeteners, honey is mostly sugars containing vitamins or minerals in low amounts [5]. Honey also contains tiny amount of several compounds thought to function as antioxidants, including chrysin, pinobanksin,vitamins C, catalase, and pinocembrin [6]. Honey is produced by bees as a food source in cold weather. Bees use their stored honey as their source of energy [7].However, due to human benefit of honey; people have been able to semi-domesticate bee in artificial hives thus harvesting excess honey.

The average pH of honey is 3.9, but can range from 3.4 to 6.1. Honey contains many kinds of acids mainly amino acids and other organic acid. However the different types and their amounts vary considerably depending on the type of honey. These acids may be aromatic or aliphatic (non-aromatic). The aliphatic acids contribute greatly to the flavor of honey by interacting with the flavors of other ingredients. Gluconic acid, for instance is a flavor enhancer. The aromatic acids, such as malic acid, come mostly from the flowers, adding to the aroma and taste of the honey. Honey can contain up to 18 of the 20 amino acids. However amino acid content is almost negligible in honey accounting for only 0.05–0.1% of the composition. The main amino acid is proline [8].Organic acids comprise most of the acids in honey, accounting for

0.17–1.17% of the mixture. Gluconic acid which is the predominant organic acidis formed by the action of an enzyme called glucose oxidase. Other organic acids like formic, acetic, butyric, citric, lactic, malic, pyroglutamic, propionic, valeric, capronic, palmitic, and succinic are found in lesser amounts[9].

Honey is a natural product gotten from honey bees that contains some important nutritive and medicinal properties. However, as a result of its high demand, there is often adulteration of honey leading to its contamination by microorganisms.

Efforts have been towards studying the physicochemical properties of honey. There seem to be dearth of information on the microbiota of honey been documented proving that a well- developed understanding of these is needed. Therefore, this study represents one of the few studies in this area. This will provide an insight to the microbiological characteristics of honey so as to lay a foundation of combining microbiological and physiochemical aspects of honey in order to enhance better understanding of the microorganisms associated with honey particularly some pathogenic microbes that could cause health hazard and human diseases. Hence the study was designed particularly to determine the quality and microorganisms associated with honey in Minna, Niger state, Nigeria.

**MATERIALS AND METHODS**

**Sample collection**

Ten (10) samples of honey were purchased from different locations in Minna, Niger state. These included; Bosso, Mobile, Chanchanga, Kpakungu, Mekunkele, Tundufulani, Saukakahuta, Tunga, Maitumbi and Central market. They were transported immediately to the laboratory of Federal University of Technology, Minna, Niger state, Nigeria for microbiological and physic- chemical analysis. The honey samples were stored in the refrigerator until further use.

**PHYSICOCHEMICAL ANALYSIS**

**pH**

The pH of the honey samples were determined by carefully measuring out 10 mL of each sample into a clean beaker. The pH meter was then immersed into the honey sample, after which the honey sample was stirred with pH meter gently and waited until the display on the pH meter was stabilized. The pH value was recorded accordingly after the reading had been stabilized.

**Titratable acidity (TTA)**

The total titratable acidity was determined using the method of [8]. One milliliter (1mL) of each honey sample was measured into 5mL distilled water and titrated against 0.02M NaOH using phenol red as indicator.

Total titratable acid was calculated as follows:

Total titratable acid = !.!" Mmol/L Equation (1)

!

Where X= Titre (mL) of alkali used

Y= Volume of samples

0.02 NaOH was determined to contain 20m Mol total titratable acid.

**Electrical conductivity**

Electrical conductivity of the honey samples were measured at 22℃ using a conductivity meter.

**Moisture content**

The moisture content of each sample was determined as follows:

Five gram (5g) of the sample was weighed and placed into a pre-weighed aluminum drying dish. The sample was dried to constant weight in an oven at 105℃ for 4 hours under vacuum [10].

Moisture content = !"!!"

!"!!"

Equation (2)

Where:

Mo=Weight of the aluminum dish M1=Weight of the fresh sample + dish M2=Weight of the dried sample+ dish

**Total solid**

The percentage total solid of each sample was determined using the equation:

Total solids (%) = 100 −Moisture content Equation (3)

**Ash content**

Ash content was determined by weighing 5g of each honey sample separately into a porcelain crucible previously ignited and weighed organic matter was charred by igniting the sample on a hot plate in the fume cupboard. The crucible was then placed in the muffle furnace and maintained at 600℃ for 6 hours. They were then cooled in desiccators and weighed immediately

[10].

The percentage ash was calculated as:

Ash%=(!"#$%& !" !"#!$%&'!!"#)!(!"#$%& !" !"#$% !"#!$%&')×!""

!"#$%& !"#$%&

Equation (4)

**Water activity (Aw)**

Water activity was determined using the equation below: Water activity (Aw) can be calculated as;

Aw = (!.!"#×!"#$ !"#$%) + 0.13. Equation (5)

(!""# !"#$%)

**MICROBIOLOGICAL ANALYSIS**

**Bacterial isolates**

One milliliter (1mL) of the honey sample was aseptically introduced into 9mL of sterile distilled water in a test tube. This was shaken and serially diluted. One milliliter (1mL) from an appropriately serially diluted sample was introduced into a sterile Petri dish and molten nutrient agar was poured on it using the standard pour plate method described by [9] and mixed properly. It was then allowed to solidify and then incubated for 24 hours at 37℃ for bacterial growth.

**Fungi isolates**

Appropriate serially diluted honey sample were inoculated onto Sabouraud dextrose agar (SDA)

to identify the fungal isolates. The plates were incubated at 28°C for 48-72 hours.

**Characterization and identification of microbial isolates**

**Bacterial isolates**

The bacterial isolates were characterized based on colonial morphology, cultural characteristics, Gram’s reaction and biochemical tests as described by[11,12]. The bacterial isolates were identified by comparing their characteristics with those of identified species using the schemes of [13-15].The biochemical tests carried out on the bacterial isolate were catalase test, carbohydrate

fermentation test, citrate utilization test, coagulase test, indole test, methyl red test (MR), starch hydrolysis and Voges-proskauer test (VP).

**Fungal isolates**

Fungal identification was carried out using mycological atlas [16]. The fungi isolated were stained using lactophenol cotton blue solution and characterized based on the colour of the aerial and substrate hyphae, type of hyphae, shape and kind of asexual spore, sporangiophore and conidiophore and the characteristic of spore head. The isolates were identified by comparing their characteristics with those of known taxa using the schemes of [17].

**RESULTS**

The results from the research showed that the pH and total titratable acidity from all the locations ranged from 3.7-4.2 and 26.0-40.59 respectively (Table 1). There was no significant difference in the pH value for all the locations sampled, while there was significant difference in the values of the total titratable acidity for all the locations sampled.

The results from the investigations shows that the ash content and water activity from all the locations ranged from 0.16-0.36 and 0.53-0.59 respectively (Table 1).There was no significant differences in the ash content and water activity from all the locations sampled.

The results from the study shows that the conductivity, moisture content and total solid from all the locations ranged from 16.70-60.14, 11.60-21.35 and 78.65-88.40 respectively (Table

1).There was significant difference in the conductivity, moisture content and total solid from all the locations sampled.

**Table 1: Physico-chemical parameters of honey samples from different locations**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Locations** | **pH** | **Total titratable**  **Acidity(meq/kg)** | **Conductivity**  **(**𝝁𝑺/𝒄𝒎**)** | **Moisture Content (%)** | **Total solid (%)** | **Ash Content (%)** | **Water**  **Activity** |
| Mobile | 4.1a | 34.74d | 24.55h | 21.35a | 78.65g | 0.18a | 0.53a |
| Saukakahuta | 4.2 a | 35.0d | 16.70j | 11.60g | 88.40a | 0.16a | 0.55a |
| Kpankungu | 4.1a | 30.0f | 22.40i | 20.32b | 79.68f | 0.27a | 0.57a |
| Tunga | 3.8 a | 26 .0g | 33.40g | 15.86e | 84.14c | 0.28a | 0.54a |
| Maitumbi | 4.0 a | 37.5c | 40.13f | 17.03d | 82.97d | 0.19a | 0.56a |
| Central  Market | 3.7 a | 29.45g | 55.26c | 16.53e | 83.47c | 0.36a | 0.59 a |
| Tundun  Fulani | 3.8 a | 38.0c | 47.48e | 18.71c | 81.29e | 0.25a | 0.55a |
| Chanchanga | 4.0 a | 39.50b | 49.20d | 16.53e | 84.53b | 0.33a | 0.55a |
| Mekunkele | 4.0a | 40.59 a | 60.14a | 18.71c | 83.24d | 0.32a | 0.56a |
| Bosso  Market | 3.7a | 33.67e | 59.20b | 15.47f | 82.93d | 0.19a | 0.54 a |

Values (a, b, c, d, e, f, g, h, i, j) on the same column with different superscript are significantly different (p<0.05)

while those with the same superscript are not significantly different (p>0.05).

It was revealed that Mobile, Kpankungu, Tundun-fulani, Mekunkele and Bosso market had total viable bacterial count of 2.0x10!, 1.5x10!, 1.8x10!,1.0x10! and 5.0x10!respectively, while Saukakahuta, Tunga, Maitumbi, Central market and Chanchanga had zero counts of bacteria growth (Table 2). There was significant difference in the total viable bacterial counts from all the locations sampled.

**Table 2*:* Total viable bacterial counts from different locations**

**Locations Total bacterial count (cfu/mL)**

Mobile 2.0x10!a Saukakahuta 0e Kpankungu 1.5x10!b Tunga 0e Maitumbi 0e

Central market 0e Tundun-fulani 1.8x10!a Chanchanga 0e Mekunkele 1.0x10!c

Bosso market 5.0x10!d

Values (a, b, c, d, e) on the same column with different superscript are significantly different (p<0.05) while those with the same superscript are not significantly different (p>0.05).

Results from Table 3 revealed that Chanchanga and Bosso market had total fungi count of 7.0 x10!and 3.0 x10!respectively, while Mobile, Saukakahuta, Kpankungu, Tunga, Maitumbi, Central market, Tundun-fulani and Mekunkele had zero counts of fungi growth. There was significant difference in the total fungi counts from all the locations sampled.

**Table 3: Total fungi counts from different locations**

**Locations Fungi count (cfu/mL)**

Mobile 0c Saukakahuta 0c Kpankungu 0c Tunga 0c Maitumbi 0c Central market 0c Tundun-fulani 0c

Chanchanga 7.0x10!a

Mekunkele 0c

Bosso market 3.0x10!b

Values (a, b, c) on the same column with different superscript are significantly different (p<0.05) while those with the same superscript are not significantly different (p>0.05).

The results from the microbial analysis revealed that the honey samples harbour different microorganisms which include *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Aspergillus fumigatus* (Table 4).Table 5 shows the biochemical tests carried out for bacteria isolates.

**Table 4: Frequency of occurrence of bacteria and fungi isolates from honey**

**Microorganisms No of organisms isolated Frequency of occurrence (%)**

*Bacillus subtilis* 2 28.57

*Staphylococcus aureus* 2 28.57

*Pseudomonas aeruginosa* 1 14.3

*Aspergillus fumigatus* 2 28.57

Total 7 100

**DISCUSSION**

The physicochemical properties of the different samples of honey are given in Table 1 above. The pH values of the honey samples ranged from 3.7-4.2. The pH values correlate and corroborate with the pH range of 3.2 - 4.5 as reported by [16] for international honey. The pH range obtained in the present study was however lower than the range of 4.31-6.0 reported by [18] for Nigerian honey from other locations. This may be due to adulteration of the honey with glucose solutions, dextrose, molasses, sugar syrup, invert sugar, flour, corn syrup, starch, or any other similar product, other than floral nectar or low-quality honeys with a high water content, because the honey has been taken from cells that are not properly covered with wax. The acidic pH of honey is desirable since acidification has been shown to promote healing by causing oxygen release from hemoglobin [19]. The pH of honey is low enough to prevent the growth of many species of bacteria.

**Table 5: Biochemical tests for bacteria isolates**

**Locations**

**Gram Reaction**

**Cell shape**

**Catalase**

**Coagulase**

**Oxidase**

**Indole**

**Citrate utilization**

**H2S production**

**Urease**

**MR**

**VP**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | | | | | | | | | | **Suspected Bacteria** |
| Mobile | + | Rod | + | − | − | − | + | − | − | − | + | *Bacillus subtilis* |
| Bosso | + | Rod | + | − | − | − | + | − | − | − | + | *Bacillus subtilis*  *Staphylococcus* |
| Tundunfulani | + | Cocci | + | + | − | − | + | − | − | + | + | *aureus*  *Staphylococcus* |
| Kpakungu | + | Cocci | + | + | − | − | + | − | − | + | + | *aureus*  *Pseudomonas* |
| Mekunkele | − | Rod | + | − | + | − | + | − | − | − | − | *aeruginosa* |

**+:** Positive; **−:** Negative; MR: Methyl red; VP: Voges-proskauer.

The moisture content of the honey samples ranged from 11.60-21.35%. The moisture content of the samples falls within the range of 11.47 - 22.20 reported by [19] for international honey and by [20] for Nigerian honey from other locations. The variations in the moisture content of honey have been ascribed to the composition and flora origins of honey [19]. Moisture content is practically the most important quality parameter, since it affects storage life and processing characteristics. The strong interaction of sugar in honey with water molecules may decrease the water available for microorganisms. The low moisture content of honey also forms an important part of the system which protects the honey from attack by microorganisms.

The ash content of the honey samples ranged from 0.16-0.36% and it falls within the acceptable range of 0.10-0.36 reported by [22] for Nigerian honey from other locations and by [20] for international honey. The floral origin of honey has been reported responsible for the variability in ash content [22].The ash content is a measure of the mineral elements in honey.

The electrical conductivity values of the honey samples ranged from 16.70 - 60.14 𝜇��/𝑐��. These values fall within the range of 9.4 - 172.9 reported by [19] for Nigerian honey from other locations. Electrical conductivity measures all ionisable organic and inorganic substances present in honey. It has been related to the botanical origin of honey and very often used in routine honey control instead of ash content [21].

The total titratable acidity values ranged from 26.0-40.59meq/kg. The total titratable acidity values falls within the range of 40meq/kg reported by [18] for international honey and by [20] for Nigerian honey from other locations. The acidity of honey contributes to its stability against microorganisms and to flavour.

The values of the water activity varied from 0.53-0.59. These values fall within the range of 0.53 and 0.59 reported by [20] for international honey. The water activity of honey varies slightly. It is obviously related to the floral source of nectar. Honey is a supersaturated sugar solution with a low water activity, which means that there is insufficient water available to support the growth of bacteria and yeast. Although some yeast can survive in high water content causing spoilage of the honey.

Total solids in the present study varied from 78.65-88.40. The total solid is a measure of dissolved solids in the honey samples. A reduction or absence of total solids in honey samples is an indicator that further processing has been done on the honey samples. The total solids of honey samples obtained from this study falls within the acceptable range of 78.60 - 88.45 reported by [19] for Nigerian honey from other locations. The data obtained from physicochemical quality assessment were subjected to One Way Analysis of Variance (ANOVA) (P≤ 0.05) which showed that there were no significant differences in the levels of pH, ash content and water activity, while there was significant difference in the levels of total titrable acidity, moisture content, total solid and conductivity.

The results in the present study revealed that some of the honey samples purchased from different locations in Minna subjected to microbiological quality assessment were contaminated with pathogenic bacteria which include *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa.* Previous study carried out on honey revealed the presence of these bacteria [7].

The high counts of bacteria detected in honey may be due to contaminations such as the activities of personnel, equipment, containers, wind, dust, and the digestive tract of the honey bees and nectar, while the zero counts of bacteria may indicate proper handling and storage of the honey products. There were moulds in the honey samples from Chanchanga and Bosso identified as *Aspergillus fumigatus* but zero counts were detected in the other locations. This was however not surprising since [19] reported counts of less than 10 cfu/mL in Moroccan honey while some French honeys had zero counts of moulds and yeasts as reported by [25].The low counts of moulds and yeast may be due to the inhibitory properties of honey such as osmotic effect, hydrogen peroxide, acidity, phenolic compound that discourage the growth of many microorganisms [23, 24]. The data obtained from the microbiological quality assessment were subjected to One-Way Analysis of Variance (ANOVA) (p≤0.05) which revealed that there were significant difference in both the total viable bacterial counts and the total fungi counts.

**CONCLUSION**

The microbiological quality of honey in the present study has revealed that microorganisms are present in some honey purchased from different locations in Minna metropolis, Nigeria. Physicochemical quality assessment shows that some of the honeys were within the acceptable limits. Some of the microorganisms isolated from these samples are pathogenic and could be hazardous to human health. Hence, the need for routine hygienic practices necessary to avoid microbial contaminants.

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