# Evaluation of some biochemical, microbiological and organoleptic characteristics of some honey samples in Nigeria.

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**Abstract:** This work evaluated honey samples for their nutritive value, wholesomeness and effect of sucrose and honey on food functional properties. Honey samples obtained from Saki (A), Minna (B) and Maiduguri (C) exhibited the following characteristic features: 18 - 24% moisture content, 1.43 - 2.72% protein content, 0.49 - 0.86% ash, 73.7 - 78.6% carbohydrate, mainly sugars. No fibre or fat was detected. The pH values were between 3.2 - 3.6 which signify the honeys to be classified as acidic food. The most predominant minerals are Sodium (Na) (6.30 - 7.02), Potassium (K) (5.6 - 7.6), Calcium (Ca) (2.14 -3.40), Magnesium (Mg) (0.21 - 1.90) and Phosphorus (P) (2.40 -3.60) ppm. The mean viable microbial population counts are 0.5 x $10^7$ - 1.15 x $10^7$ cfu / ml at $10^{-6}$ dilution and 0.2 x $10^8$ - 0.8 x $10^8$ cfu/ml at $10^{-7}$ dilution. At $10^{-6}$ and $10^{-7}$ dilution, no mould growth was found except in Minna (B) honey. Honey samples from different parts of Nigeria are shown to be rich in nutrients and endowed with organoleptic properties. Some spoilage organisms were also isolated from the honey samples: *Xanthomonas campestris, Micrococcus roseus, Staphylococcus saprophyticus, Lactobacilllus fructivorans, Serratia marcescens and Aerococcus viridans.*

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## Introduction

Honey is a natural substance produced by bees , consisting basically of a complex mixture of carbohydrates, especially glucose and fructose, organic acids, amino acids, minerals, vitamins, enzymes, pollens, and pigments (Crane 1987, Fallico et al. 2004) Its nutritional quality, medicinal, and sensory properties have attracted thousands of consumers( Carlos et al, 2009**)**.It is a mixture of concentrated aqueous solution of inverted sugars and complex mixture of other saccharides, amino acids, proteins, organic acids, vitamins, minerals, Maillard reaction products and both enzymatic and non-enzymatic antioxidants, including glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids and carotenoid derivatives. (Smith 1960,Almamary ,2002,Gheldof 2002)

Bees obtain all their nutritional components from nectar, pollen and water. Nectar is reduced to honey containing predominantly carbohydrates with a very little protein, vitamins and minerals. Fully ripened honey consists of levulose / fructose (41%) and dextrose / glucose 35% and 22 others which are more complex than the monosaccharides present in quite minute quantities.( White *et al,*). Of the 22 complex sugars, the oligosaccharides identified are maltose, isomaltose, maltulose, nigerose, turanose, kojibiose, laminarihiose, α,β- trehalose and gsentibiose. Ten trisaccharides are present: melezitose, maltotriose, 3 - α - isomaltosylglucose, 1-kestose, panose, isomaltotriose and isomaltopentaose. Most of these sugars do not occur in nectar but may arise from enzymes added by honey bee during honey ripening or by chemical action in the concentrated acid sugar mixture of honey. (Gheldof *et al, 2002* )

The presence of phytochemicals such as flavonoids and phenolic acids that suggests the role of honey, along with fruits and vegetables, as a nutritional source of natural antioxidants responsible for protecting human health has been reported. *Gheldof et al 2003,,* McKibben 2002 , Schramm *et al 2003,* Tonks 2001,Tonks 2003) Its antibacterial, anti-inflammatory, antioxidant and anticancer properties have been extensively discussed.(Orsolic 2005,Swellam 2003,Blasa, 2006,Board 1972). Vitamin C and most of the Vitamin B complex are present in variable amounts. (Oszmianski ,1990).

Apart from being a high energy substance, honey has high digestibility, high acidity as well as a high taste appeal. By this characteristic antioxidant property, honey when applied at 10% has been found to inhibit enzymatic browning in apple slices and grape juice.(Khan 1985) It has been demonstrated that it is the proteins, peptides and amino acids in honey that exert an inhibitory effect on polyphenol

oxidase activity by chelating the essential Copper (Cu) at the active site of polyphenol oxidase thus forming stable complexes with $Cu^{2+}$.( Chen, 1998) It has also been revealed that the antioxidant content and the efficacy of honeys in inhibiting polyphenol oxidase activity vary in accordance with the type of honey. Agunbiade 1996

Honey has been used in the treatment of necrotic pressure sores, ulcers, burns and wounds, thereby eliminating characteristic odours of wounds. It is used also in treating external eye infections and diabetic foot( Aljadi , 2004)

**Materials and Methods**

The honey samples used in this study were obtained from Saki, Oyo state, South West of Nigeria, Minna, Niger state, North central of Nigeria and Maiduguri, Borno state, North east of Nigeria. Collection was by the conventional method of a beekeeper and each was kept in sterile bottle and covered with a lid.

**Analytical Procedures**

***Proximate Analysis***

The Proximate concentrations of the honey samples was determined by the Standard AOAC (1990) methods for estimating moisture. Ash, crude fibre, crude oil, crude protein calculated as Nitrogen (N x 6.25) by the Kjeidahl method.

***pH Determination***

pH of each honey sample was determined by the method of Ojeleye 1991.

***Viscosity Determination***

Samples were run through Standard burettes and allowed to train 1ml at a time. The time taken for 1ml sample to flow was recorded with the aid of a stopwatch.

***Refractive Index***

Abbey Refractometer was used to determine the refractive index of each honey sample. From a reference sugar table, the refractive index and brix of the honeys could be convertibly estimated.

**Mineral concentrations of Honey samples**

Samples were wet washed, using AOAC method and analysed for Calcium, Magnesium, Potassium and Sodium, using Atomic Absorption Spectrophotometer.(AOAC,1990)

Phosphorus was estimated by a reaction between Phosphorus and Molybdovanate forming a Phosphomolybdovanate complex measurable colorimetrically at 420nm.

**Microbial Assay of Honey Samples**

Viable Quantitative and Fungal Population counts The methods of Miles and Misra, described by Collins and Lyne were used.[23] Serial dilutions of $10^{-6}$ and $10^{-7}$ were made and 1ml each was pipetted into Petri dishes and Nutrient Agar and Potato Dextrose Agar were separately aseptically dispensed and carefully mixed. The organisms in Nutrient agar mixture were incubated at $37^0$C for 24 hours while that of organism in Potato dextrose agar mixture was incubated at $22^0$C for 5 days. The colonies formed were counted using the colony forming units/ml sample. Organisms found in honey samples were characterized.

**Results and Discussion**

**Table 1** shows the proximate composition of the three honey samples. Ash content was between 0.49 - 0.86%. Moisture contents of the three honeys ranges from 18-24%. Honey Regulation 197651 No 180 Council Directive 74/4009/EEC stipulated honey moisture content should not be more than 21%. (Egan,1978) it is apparent that the water content varies greatly and may range widely. The amount of moisture is a function of factors involved in ripening, including, among others, the original moisture of the nectar. According to the United States Standards, extracted honey may not contain more than 18.6% moisture. Moisture level of about 17% has been found to be optimum. When honey is not hermetically sealed, because of its hygroscopic nature, it absorbs moisture. Honey with less than 17.1% water will not ferment in a year, irrespective of the yeast count. Between 17.1 and 18% moisture, honey with 1000 yeast spores or less per gram will be safe for a year. However when the moisture is above 19% honey can ferment even with only one spore per gram. This study shows that honey is a high energy carbohydrate food. Honey is a supersaturated sugar solution, with more than 95% of its dry mass consisting of sugar and water, although different valuable nutrients such as vitamins, minerals, enzymes, flavoring organic compounds, free amino acids and numerous volatile compounds constitute minor components (Baroni et al) .The carbohydrate in honey of about 98% obtained in this study is similar to the figure range of 95-99.9% of White and Doner.(25) The crude protein content of 1.43 -2.72% obtained in this study shows that honey is not an adequate source of dietary protein

**Table 1. Proximate Composition of Honey Samples in %**

| Honey sample | Dry matter | Crude protein | Ash | Fat | Sugars |
|---|---|---|---|---|---|
| **A. Saki** | 76.00±2.4 | 1.43±0.1 | 0.86±0.1 | Nil | 97.71±2.5 |
| **B. Minna** | 81.80±2.0 | 2.72±0.1 | 0.49±0.1 | Nil | 97.80±2.2 |
| **C.Maiduguri** | 77.80±2.2 | 1.83±0.1 | 0.64±0.1 | Nil | 97.53±2.4 |

Values are expressed as means of duplicate determinations ± standard deviation.

Table 1 shows the Proximate composition of Honey samples in %

**Table 2. Physicochemical characteristics of Honey samples**

| Sample | pH | Flow rate | Refractive Index |
|---|---|---|---|
| A Saki | 3.6 | 1.65 | 1.4765 |
| B Minna | 3.5 | 0.30 | 1.4910 |
| C Maiduguri | 3.2 | 0.45 | 1.4810 |

**Table 2** reports the physico-chemical characteristics of the three honey samples. The pH values of 3.2-3.6 recorded in this work is lower than 4.3 - 6.0 pH range reported by Adebiyi et al, 2004. Low honey pH shows the three samples to be acidic. . Glucose oxidase in honey has been implicated in the conversion of dextrose to gluconolactone which in turn forms gluconic acid, the principal acid in honey. Glucose oxidase also forms hydrogen peroxide during its action on dextrose. This end product (hydrogen peroxide) is well known for its antiseptic property even in a diluted honey.(Wahdan,, 1998) In addition to gluconic acid, other acids, including lactic acid are said to be present in honey. High acidity of honey in combination with high sugar content therefore confers on honey high antimicrobial property. The refractive indices of the three honeys ranging from 1.4765 – 1.4910 are similar to 1.460 – 1.488 values of Adebiyi et al, 2004. When refractive index is extrapolated on a reference standard table, it may serve as a rapid and simple measure of the % of total soluble sugar solid in honey at $20^0$C

**Table 3. Mineral constituents of honey in mg/kg**

| Sample | Na | K | P | Ca | Mg |
|---|---|---|---|---|---|
| A Saki | 6.84±0.2 | 6.08±0.15 | 3.60±0.16 | 3.39±0.2 | 1.90±0.15 |
| B Minna | 7.02±0.2 | 7.60±0.14 | 2.40±0.17 | 2.44±0.12 | 0.26±0.01 |
| C Maiduguri | 6.30±0.2 | 5.61±0.20 | 3.45±0.12 | 2.14±0.18 | 0.21±0.17 |

Values are expressed as means of duplicate determinations ± Standard Deviation.

**Table 3** shows the mineral composition in mg/kg of the three honey samples. The major minerals are Na, K, P and Ca while Mg constitutes a very minute proportion especially in Minna and Maiduguri honey samples. All these minerals had their origin from the soil .The mineral levels obtained in the present work are quite lower than those that have been reported.(23)This wide disparity may be due to variation in the vegetations and soil composition of minerals at the different locations from which the honeys were produced.

**Table 4. Fungi and Coliform counts in Honey**

| Sample | Dilution factor | Mean mould count (cfu/ml) | Coliform count (cfu/ml ) |
|---|---|---|---|
| A Saki | $10^{-6}$ | $0.5 \times 10^7$ | Nil |
| | $10^{-7}$ | $0.2 \times 10^8$ | Nil |
| B Minna | $10^{-6}$ | $0.8 \times 10^7$ | $0.1 \times 10^7$ |
| | $10^{-7}$ | $0.35 \times 10^8$ | Nil |
| C Maiduguri | $10^{-6}$ | $1.15 \times 10^7$ | Nil |
| | $10^{-7}$ | $0.8 \times 10^8$ | Nil |

cfu/ml = Colony forming unit per ml sample

**Table 4** reports the total bacterial and fungal counts in the three honey samples.

Maiduguri sample (C) with the highest microbial counts at dilution $10^{-6}$ and $10^{-7}$ produced 1.15 x $10^{7}$ cfu/ml and 0.8 x $10^{8}$ cfu/ml respectively. Sample A (Minna honey) on the other hand, with the least microbial counts at dilutions $10^{-6}$ and $10^{-7}$ produced 0.5 and 0.2 cfu/ml respectively. It was only in Minna Sample B that fungi were detected at $10^{-6}$ dilution. No coliform was however isolated in any of the honey samples. The presence of fungi, presumably yeast, in B may explain the possibility of fermentation of honey under extreme acidity and high osmolarity.

**Table 5. Microbial Isolates in Honey Samples**

| Sample | Isolate | Organisms |
|---|---|---|
| A & B | a | *Lactobacillus fructivorans* |
| A & C | b | *Staphylococcus saprophyticus* |
| B & C | c | *Xanthomonas campestris* |
| ABC | d | *Micrococcus roseus* |
| A | e | *Serratis marcescens* |
| B | f | Unidentified |
| C | g | *Acrococcus viridans* |

**Table 5** shows the isolates from the three honey samples (A, B & C). All the organisms were either osmophilic or they survived only in the resting spore form ( Wahdan H ,1998). They were also acid fermenters. The spoilage organisms apparently attacked honey sugars fermentatively at low pH of 3.2 – 3.6 under which the proteolytic and lipophilic organisms may be incapacitated. It should be noted, however, that lactic acid bacteria are only weakly proteolytic and lipolytic.

**Conclusion**

This study has confirmed honey to be a high acid and high sugar food. These two characteristics also show that it can harbour spoilage organisms (fermenters) only sparingly especially if bee-keepers can maintain a high degree of cleanliness in terms of personal hygiene, equipment cleaning, careful process control and good packaging, distribution and good storage etc. Its usefulness as an antimicrobial, anti-oxidant agent has been highlighted by some other workers. By its osmotic effect due to high sugar content and its acidity, honey may be used as a dressing for wounds, inflammations and diabetic sores. This application makes honey unsuitable for pathogens and it thus hastens healing. As an anti-oxidant it counteracts free radicals, destructive chemical agents which have been linked to many diseases. In addition to the above, honey is an energy giver and therefore it is recommended for all and sundry consumption and especially for the diabetics as an alternative to sucrose.

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