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EXTRACTABLE SUGARS AND MICROBIAL QUALITY OF 5 HONEY SAMPLES FROM UMUDIKE FARMS AND DIFFERENT LOCATIONS IN SOUTH EASTERN NIGERIA

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ABSTRACT

The extractable sugars, moisture contents and microbial quality of 5 honey samples from Umudike farms (Eastern and Western) and different locations in the South Eastern parts of Nigeria (Ahiaeke, Amoaba and Nsukka) were investigated. All the honey samples investigated were observed to contain moderate amounts of reducing sugars ($P > 0.05$) and non reducing sugars but higher quantities of total soluble carbohydrates. The microbial assay carried out, revealed the presence of *Staphylococcus*, *Streptococcus* and Yeast in most of the samples. However, their loads fell within the range given by the Food and Agricultural Organization for microbial loads of foods. In addition, the honey from Eastern farm was observed to be sterile. The percentage moisture contents of all the samples ranged from 20.04 to 30.08 with the honey from Nsukka having the highest moisture content (29.45±0.10) while that from Western farm was the least (21.25±0.06). Results indicate that these honey samples could serve as useful adjuncts or dietary supplements in the management of diabetes. Finally, the honey from Eastern farm has high anti-microbial actions which confers very high biological functions on it such as treatment of ulcers, gastrointestinal diseases and enhancement of wound healing.

Keywords: Honey, Sugars, Locations, Microbial Loads

INTRODUCTION

Honey is a natural food product that is obtained when the nectar and sweet deposits from plants are gathered, modified and stored in the honeycomb by honey bees. From ancient times honey has been used in healing purposes **[1]**.

Honey has been credited for many biological and therapeutic purposes such as: treatment of colds, skin wounds and various gastrointestinal diseases **[2]**. This beneficial role is attributed to both antibacterial and anti-inflammatory properties of honey that arise from the high sugar which are the major components **[3, 4]** in addition to high osmolarity, acidity and content of hydrogen peroxide.

The composition and quality of honey depend on several environmental factors during production such as weather and humidity inside the hive, nectar conditions, treatment of honey during extraction and storage. Bacterial spores and less often *Clostridium* spp. may be present but honey possesses antibacterial activities that do not support bacterial growth or production of toxins **[5-8]**. However, there are reports that fungi grows and ferments or spoils unprocessed honeys **[7, 9]** although proper processing and handling can control spoilage.

Therefore, the microbiological quality of honey may give an idea of the hygienic conditions under which the product was processed, handled and stored.

Honey is composed mainly of carbohydrates, lesser amounts of water and a great number of minor components. Sugars are the main constituents of honey, constituting of about 95% of honey on dry weight basis **[3, 4]** and these have been credited for various anti-bacterial activities of honey. This thus confers on honey, high anti-microbial properties. The major sugars are the monosaccharide hexoses: fructose and glucose, which are products of the hydrolysis of the disaccharide-sucrose.

The microorganisms of interest in honey are those that can withstand the concentrated sugar, acidity and antimicrobial action of honey and they include certain yeasts and spore - forming bacteria (*coliforms*), indicative of the sanitary or the quality of the honey **[10]**.

In Nigeria, it is possible to adulterate honey and present it for sale as a natural genuine product. It is thus important that the dealers and consumers ascertain the quality of the honey that they are purchasing. In addition, there are several reports on the sugar composition and microbial quality of honey from other countries **[11, 12]**, however such

reports on Nigerian honeys especially those from the South Eastern parts of Nigeria are scarce in literature and this led to the study above.

MATERIALS AND METHODS

Phenol and glucose standards used were obtained from Sigma and Aldrich Chemical Company, Germany and were of analytical grade. All other chemicals that were used were purchased from Horse Lab, Umuahia, Abia State, Nigeria and were of analytical grade.

Harvesting of Honey

Five honey samples, collected from the beehives of Umudike farms (Eastern farm-Light brown) and Western farm (Extremely light brown) and different locations in the Eastern parts of Nigeria (Nsukka-Dark brown), Ahiaeke (Moderately dark brown) and Amoaba (Brown) were used for the study. The matured combs, laden with honey, were harvested into well covered containers.

Extraction Procedure

The Crushing method was employed for extraction of the various honey samples varieties from their different combs and this method is ideal for processing the bee wax. Each of the honey combs, laden with honey, was broken into trunks and tied up in a cheese cloth which was positioned into a press that was screwed with the aid of a jack to release the honey into a receptacle. The released honey was stored under room temperature (27-30^{o}C) using glass jars.

A measured amount (0.1ml) each of five different honey samples was dissolved with 1ml of methanol, made up to 100mls with distilled water and left overnight. The mixture was centrifuged at 3000 x g for 10 minutes and filtered. The filtrate was used for the analysis of total soluble sugars. Another portion (5ml) each of the five samples was oven dried at 65^{o}C for 24 hrs for determination of moisture content while the rest were stored in glass flasks at 4^{o}C and analysis was performed within two months of sampling.

Determination of Total Carbodydrates / Sugars

The phenol-sulfuric acid method **[3]** was used with modifications. Five microliters of the aqueous extract + 500ul (4% phenol in water) + 2.5ml (96% sulphuric acid in water) were mixed thoroughly. The resulting solution was further re-diluted with 4mls of water and the absorbance was read at 490nm against a reagent blank that contained 500ul (4%) phenol and 2.5ml (96%) sulphuric acid were added to 500µl (4%) phenol + 2.5ml (96%) sulfuric acid. The resulting solution was further re-diluted in 2mls of water and the absorbance was read spectrophotometrically

at 490nm against a reagent blank which contained 500ul (4%) of phenol and 2.5ml (96%) of sulfuric acid. The same procedure was followed for the glucose standard (0.1g in 100ml water) which was serially diluted to varying concentrations of 0.005, 0.01, 0.02, 0.03, 0.04 and 0.05mg/ml and results were expressed in mg/ml. The concentration of total soluble sugars in the samples were obtained from the equation: Y = 15.97x - 0.042* 2 (R2 = 0.879) where 15.97 = slope of the graph; x = absorbance of the sample; 0.042 = intercept; 2= dilution factor and R2 = correlation coefficient; Y = Unknown concentration of the sample.

Analysis of Reducing Sugars

The reducing sugar contents of the honey samples was determined using Fehling's method as described by the International Honey Commission **[13]**.

Analysis of Non-Reducing Sugar

The non-reducing sugar contents of the five honey samples was determined by the difference between the total soluble carbohydrates and reducing sugar contents of the five honey samples **[14]**.

Microbial Assay

The microbial load of the samples was determined using the pour plate techniques **[15]** with modifications. One ml each of the samples was dispersed into 9ml of sterile water in different test tubes which was shaken thoroughly for proper mixing. The mixture was diluted serially. From the 2nd dilution (10^{-2}), the whole setup was aseptically transferred into a sterile petridish. The petridish was gently stirred for the contents to mix evenly. The plates were incubated at 37°C for 18-24hrs. Counts of the number of colonies in each plates were done using a Gallenkamp Electronic Colony Counter. The mean of the counts was obtained and multiplied with the appropriate dilution factor and the counts were calculated using the formula:

$$\textbf{Mean count} = \frac{\textbf{Total Viable Counts}}{\textbf{Number of plates}}$$

The total viable counts (indicative of microbial load) of micro-organisms in each sample was expressed in colony forming units/ml (cfu/ml).

Characterization of Isolates

The macroscopic examination for physical morphology (colour, texture, odour, etc) and microscopic examination through gram staining and biochemical tests (Coagulase, oxidase, Indole, Urease, Methyl red, Citrate utilization and Sugar fermentation tests) were used in identifying all isolates and results were matched with the Bergy's manual for confirmation **[16]**.

Statistical Analysis

Results are reported as the means ± standard deviations of triplicate experiments. Data was subjected to one way analysis of variance using the Statistical Package for Social Sciences (Version 15.0) and differences between means were separated using the Duncan Multiple Range Test and considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Reducing sugar refers to any sugar that has an aldehyde group or is capable of forming one in solution through isomerisation. The hyperglycemia that results from diabetes mellitus has been associated with the production of reducing sugars via polyol and glycolytic pathways. These reducing sugars can easily interact with lipids and proteins (non-enzymatic glycation reaction), increasing the production of reactive oxygen species, leading to increased oxidative stress and diabetic complications. The percentage reducing sugar contents of all the varieties of honey investigated, which did not differ significantly from each other ($P > 0.05$) was found to be low.

Codex Alimentarius **[17]** gave the standard for a good honey as one in which the moisture content is not more than 21% (because of rapid aerobic growth of honey fermenting yeast), sucrose of not more than 5% and reducing sugar content of not less than 65%. This statement with respect to reducing sugar content could be considered to be relative because people take honey for different purposes such as: biological functions in the treatment of ulcers, healing of wounds and antimicrobial purposes, all of which arise majorly from its reportedly high sugar contents. Its also recommended as a dietary supplement for diabetic diets because its believed that its consumption at least in moderate quantities, could lead to insulin secretion, reducing the levels of glycosylated haemoglobin (HbA1c), thereby leading to its anti-diabetic actions as reported by several authors **[18, 19, 20]**. The actual mechanism of action of this second therapeutic action of honey remains unclear but is believed to be due to the presence of minerals, flavonoids, phenolic compounds (that function to protect the pancreas from oxidative stress and damage) and fructose. The presence of fructose in honey is also implicated to lower blood glucose levels through increased expression or activation of some enzymes such as glucose6-phosphate dehydrogenase, aldolase B, phosphofructokinase-1 and glycogen synthase and inhibition of glucose 6-phosphatase and phosphorylases. Moreover, adulterated honeys tend to have hyperglycemic actions because of higher glucose/fructose (reducing sugars) ratios, the

glucose moieties tending to increase the blood glucose levels. In this regard, the lower reducing sugar contents of all the varieties of honey investigated as obtained in **Table 1**, compared with the standard reported by Codex Alimentarius **[21]** would be of comparative advantage for diabetics. Moreover, different honey from different sources have varying glycemic index ranging from low to intermediate **[22]**, which variation could be attributed to the floral source, processing, handling, storage and environmental conditions **[2, 23]**. However, the lower amounts of the reducing sugars in all the five honey samples investigated compared with the standard given by Codex Alimentarius **[17]**, implies higher prevalence to microbial attack.

Oligosaccharides are subgroup of Low Molecular Weight Carbohydrates (LMWC) in foods, which consist of digestible mono- and disaccharides and non-digestible oligosaccharides such as glucose, fructose, sucrose, raffinose and stachyose. Oligosaccharides are hydrolyzed by the concentrated sulfuric acid during the phenol-sulfuric assay and form monomers such as glucose, fructose and galactose (all which of are reducing sugars and have the same absorbance at 490nm) as well as sucrose. All the honey ssamples were studied as shown in **Table 1**, were observed to contain considerable amounts of total soluble carbohydrates (%) with the honey from eastern farm, having the highest amount of total carbohydrates (79.94±5.42) while that from Nsukka farm had the least (56.40±0.00). The Shikimic acid pathway that participates in the production of most of the phenolic compounds in plants require soluble carbohydrates as their basic compounds to carry out such task **[24]**. The significant quantities of soluble carbohydrates (sugars) in all the honey samples investigated as shown in **Table 1** indicate that they could serve as substrates for the production of aromatic amino acids and phenolic compounds through the Shikimic acid pathway which confers high phenolic and antioxidant potentials on these varieties of honey.

The percentage non-reducing sugar contents of the honey samples were determined by the difference between the total soluble sugar/carbohydrates and the reducing sugar since sucrose is the dominant non-reducing sugar in honey and thus the results obtained is seen as the apparent sucrose. The results of the non-reducing sugar contents of all the samples of honey investigated indicated that while the honey from Eastern farm had the highest amounts of non-reducing sugars (26.70±0.58%), the honey from Ahiaeke farm

had the least (4.56±3.34%). The lighter honeys were observed to contain higher amounts of sugars than the darker honeys which is a significant finding in this study. The reason behind this cannot really be explained but it worth's being noted. Honey contains non-reducing sugars such as maltose, isomaltose, turanose, maltulose and sucrose, the major non reducing sugar moiety being sucrose which is reported to have a high glycemic index, thereby raising the blood glucose levels. Thus the low amounts of the non-reducing sugar contents of some of these varieties of honey indicate that they could fall under the categories of honey with low glycemic index. In addition, the non-reducing sugar contents of some of the honey samples that were studied fell within the range of 6.8-12.25% given by the International Honey Commision **[13]** except those that were obtained from Eastern farms.

The microbial loads of all the five honey samples that were studied, as shown in **Table 2**, ranged from 2 x 10^2 to 9 x 10^2 with the honey from Ahiaeke farm having the highest loads while that from Nsukka farm had the least load. In addition, the honey from Eastern farm was observed to be sterile. The microbial loads of all the samples decreased in the following order: Ahiaeke farm honey > Amoaba farm honey> Western farm honey > Nsukka farm honey > Eastern farm honey. The higher amounts of microbial loads in the honey that was obtained from Ahiaeke farm could be attributed to its lower amounts of reducing, non-reducing and total soluble sugars compared with other varieties of honey investigated as these sugars have been credited for various anti-microbial actions of honey. However, it is curious to observe that the honey from Nsukka farm, which also had a low sugar content, in addition to possessing the highest amounts of moisture as obtained in **Figure 1**, contained lower amounts of microbial loads than the honey that was obtained from Amoaba, Ahiaeke and Western farms. It is possible that the honey that was obtained from this source could contain high quantities of polyphenols or glucose oxidase or both as these have been reported to possess antibacterial properties **[21]**. This statement is further strengthened with the observation that the only micro-organism that could grow in it was yeast, a fungi. Thus the species of bacteria that grew in some of the varieties of honey that were investigated could not have grown in it due to its antibacterial actions that could possibly have originated from possession of high amounts of polyphenols or glucose oxidase or both. This is another significant finding in this present study.

The sterility of the honey that was obtained from Eastern farm as shown in **Table 2**, could be attributed to its low moisture contents, high amounts of soluble sugars and possibly high amounts of phenolic compounds although this was not determined in this study. Their interactions could through synergistic interactions, possibly inhibit the growth of any microorganism. This highlights the high anti-microbial action of the honey from this source and the very high biological functions it confers on it such as treatment of ulcers, gastrointestinal diseases and enhancement of wound healing and this is also a significant finding in this present study.

Micro-organisms that were isolated from all the species of honey investigated include: Yeast, *Streptococcus* and *Staphylococcus* aureus. Similar kind of results was obtained by Snowdown and Cliver **[10]**. The identification of these micro-organisms indicate possible contamination of the environment under which the samples were processed or the processing and storage methods used as these could affect the microbial quality of honey.

Although these organisms are potentially pathogenic and could lead to the spoilage of honey, their counts (less than 10cfu/ml) recorded are quite low to cause any health hazards. Results obtained are consistent with recommendations of FAO **[23]** for microbial loads of food substances and this confirms that honey contains considerable antimicrobial properties that can delay the growth of many micro-organisms.

The percentage moisture contents of all the five varieties of honey investigated, ranged from the honey from 20.04 to 30.08 with the honey from Nsukka having the highest moisture content (29.45±0.10) while that from Western farm was the least (21.25±0.06). With the exception of the honey from Nsukka, the moisture content of others fell within the range given by ANNON **[8]** and Crane **[25]**.

Table 1: Sugar Contents of 5 Honey Samples (g/100g) from Different Locations

Location	Reducing sugar	Total soluble carbohydrates	Non-reducing sugar
Western	**55.56±4.77^{ab}**	**62.23±2.50^{a}**	**6.67±3.21^{a}**
Eastern	**51.56±6.10^{ab}**	**78.26±6.51^{b}**	**26.70±0.58^{b}**
Ahiaeke	**48.14±5.36^{a}**	**52.70±3.00^{a}**	**4.56±3.34^{a}**
Amoaba	**67.98±11.89^{b}**	**79.94±5.42^{b}**	**11.96±9.15^{a}**
Nsukka	**50.56±2.28^{ab}**	**56.40±0.00^{a}**	**5.84±3.22^{a}**

Means with the Same Superscript Along Each Vertical Column are Not Significantly Different From Each Other (P > 0.05). N = 5

Table 2: Microbial load of 5 Honey Samples from Different Locations

Location	Dilutions	Colonies	Load(Cfu/ml)	Isolated micro-organisms
Amoaba	10^{-2}	8	8×10^2	Yeast, *Streptococcus*
Ahiaeke	10^{-2}	9	9×10^2	*Staphylococcus aureus*, Yeast
Western	10^{-2}	3	3×10^2	Staphylococcus, Yeast
Nsukka	10^{-2}	2	2×10^2	Yeast
Eastern	10^{-2}	Nil	Nil	Nil

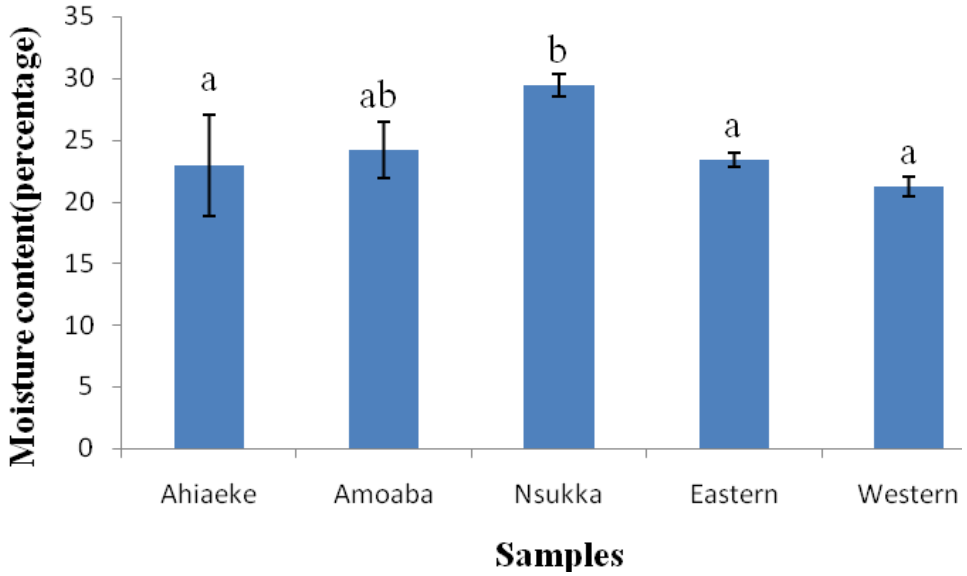

Figure 1: Percentage Moisture Contents of 5 Honey Samples from Different Locations

CONCLUSION

Most of the honey samples used for the study especially that from the Eastern Farm of the National Root Crops Research Institute, Umudike, Nigeria could compete favourably with the honey from other countries in terms of energy yield and biological functions because of its sterile quality. These varieties of honey could be recommended to diabetics as sources of energy during periods of energy need in the form of glucose. In addition, the study showed that lighter honeys may contain higher amounts of sugars than the darker honeys. The isolation of the following micro-organisms: *Streptococcus*, *Staphylococcus aureus* and Yeast, underscores the need for periodic sterilization of the processing environment or storage media of honey. Finally, the study shows that locations plays a crucial role in the composition of honey.

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