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Physico-Chemical Studies on Adulteration of Honey in Nigeria

[1]R.A. Lawal, [2]A.K. Lawal and [3]J.B. Adekalu
[1]Department of Chemical Science, Fountain University, P.M.B. 4491, Osogbo, Osun State, Nigeria
[2]Federal Institute of Industrial Research Oshodi, Lagos, Nigeria
[3]Department of Biochemistry, Lagos State University, Ojo, Lagos, Nigeria

Abstract: The extent of adulteration of honey samples from various geographical locations in Nigeria was evaluated. In order to ascertain the quality and extent of adulteration of the honey samples, the total titrable acidity, brix content, pH, colour, viscosity, moisture content, total solids, ash content, hydroxymethyl furfural and microbiological analysis were carried out. Honey samples from Akwa-Ibom, Ondo and Ogun had a high hydroxymethyl furfural with coliforms and total bacteria counts being absent, while honey samples from Shaki, Yola and Ibadan had a low hydroxymethyl furfural and some total viable counts were present in them. These results indicate that honey samples from Akwa-Ibom, Ondo and Ogun were completely free of adulteration. However, honey samples obtained from Shaki, Yola and Ibadan were discovered to have undergone some form of adulteration.

Key words: Honey, hydroxymethyl furfural, adulteration, coliform counts, Winkler, toluidine

INTRODUCTION

Honey is the sugary substance produced from the nectar of flowers by the worker bees (Alfred, 2004). It is the oldest sweet known to man. Rock paintings show it being collected from bees nest at least 15,000 years ago. US honey production in 2007 was 148 million pounds and down by 4% from a year earlier (USDA, 2008). Canada had a annual honey production of 62 million pounds in 2008, which was one tenth less than its production in 2007 (Canada Honey Council, 2008). There has been a steady decline in World production. Honey is found in beehives in large quantities in Nigeria. Honey contains mainly sugars (National Honey Board, 2008). Honey contains more carbohydrates (82.3%) than does any other animal product. A unique property of honey is its predominant sugar, fructose. This sugar is primarily responsible for its sweet taste. Honey contains a large amount of glucose but is low in sucrose (<8%) (USDA, 2008). At normal temperatures, honey is frequently saturated with respect to glucose and exists as clear syrup mainly preferred by consumers. The chief sources of micro-organisms in honey are the nectar of the flowers and the honey bee. Yeasts have been shown to come from the nectar and from the intestinal content of the bee (Frazier and Westhoff, 1994). Honey has an acidic pH and low moisture content and hence well preserved. Honey is often spoiled by some osmophyllic yeasts and moulds. Due to its large nutritive and high medicinal value (Molan, 1999; Subrahmanyam, 1999; Dumfort *et al*., 2000), there is a high demand for it. This high demand for honey coupled with poverty results in its scarcity. Hence, adulteration of the product often takes place resulting in a reduction in its nutritive and medicinal value. Pure honey is not easy to come by. Majority of what has been sold locally in Nigeria is caramelized sucrose (Omode and Ademukola, 2008). Reports have indicated that this caramelized sucrose sold do not have any medicinal value. It is on the basis of this great adulteration of honey that this study was conducted to evaluate the extent of adulteration of honey samples from various geographical locations in Nigeria.

MATERIALS AND METHODS

Collection of samples: Commercial honey samples were obtained from six various geographical locations within Nigeria; Aqua Ibom, Ibadan, Ogun, Ondo, Shaki and Yola through August, 2007. The honey samples were stored in clean bottles and immediately transferred to the laboratory for analysis. The analysis were done at FIIRO in Lagos, Nigeria.

Determination of pH: The pH of the honey samples were determined by carefully measuring out 10 mL of each sample into a clean beaker and its pH determined using a pH meter (Unicam, 9450 model).

Corresponding Author: Lawal Ridwan A., Fountain University, P.M.B. 4491, Osogbo, Osun State, Nigeria
Tel: +234-08056036852

Determination of the total titrable acidity: Twenty five milliliter of each sample (diluted) was titrated against 0.1 N NaOH using 0.25 mL phenolphthalein as an indicator. The relative amount of lactic acid was determined using the mathematical formula:

$$\text{Lactic acid (\%)} = \frac{\text{Titre value} \times \text{Normality} \times 9}{\text{Volume of sample}}$$

Determination of the colour: The colour of the honey samples was determined using the spectrophotometer (Spectronic 20 D model) to read their absorbance at a wavelength of 660 nm against distilled water.

Determination of viscosity: This was carried out using the method of Akoh (1991). Five milliliter of each sample was carefully measured out into an Ostwald U-tube viscometer (model RVT) and the sample was allowed to move down the U-tube with the aid of the thumb to the other side of the viscometer. The movement was timed using a stopwatch. The viscosity was calculated using the Hagen Bach Poiseni equation as shown below:

$$\mu = \frac{\Pi P r 4 t}{8 V L}$$

Where:
μ = Viscosity (centipoise)
P = Pressure
r = Radius of the tube
T = Time taken for the sample to move
V = Volume of sample
L = Length of the tube
Π = 22/7

Determination of sugar content/brix: The brix content of each sample was determined using the hand refractometer. Few drops of the sample were mounted on the lip of the refractometer and the level of clearness was taken and recorded as the degree brix for the sugar concentration.

Determination of the moisture content: The moisture content of each sample was determined as follows; 5 g of the sample was weighed and placed into a pre-weighed aluminium drying dish. The sample was dried to constant weight in an oven at 105°C for 4 h under vacuum (AOAC, 1990).

$$\text{Moisture content} = \frac{M_1 - M_2}{M_1 - M_0}$$

Where:
M_0 = Weight of the aluminium dish
M_1 = Weight of the fresh sample + dish
M_2 = Weight of the dried sample + dish

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

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

Determination of the ash content: Five gram of each honey sample was separately weighed out 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 were then placed in the muffle furnace and maintained at 600°C for 6 h. They were then cooled in a desiccator and weighed immediately (AOAC, 1990).

The percent Ash was calculated as:

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

Determination of the hydroxymethyl furfural (HMF): This was carried out using the method of Winkler (1955). Ten grams of each honey sample was dissolved in 20 mL oxygen-free cold water and thereafter transferred to 50 mL volumetric flask and made up to 50 mL. Two milliliter of each sample was introduced into 2 test tubes and 5.0 mL solution was p-toluidine was added to each tube. Thereafter, 1 mL barbituric acid was added to a tube and 1 mL water was added to the other tube (blank). The absorbance of the test sample was read against the blank at 550 nm using the spectrophotometer (Spectronic 20 D model). The HMF was calculated using the equation:

$$\text{HMF (mg kg}^{-1}) = \frac{\text{Absorbance} \times 19.2}{\text{Cell path length}}$$

Microbiological analysis: This was carried out using the method of Harrigan and McCance (1976) as follows: 1 mL of each sample was aseptically introduced into 9 mL of sterile distilled water. This was shaken and serially diluted. From an appropriate dilution, 0.1 mL was introduced into sterile plates and agar poured on it by the pour plate method and incubated appropriately as follows:

- **Plate count agar:** The plates were incubated at 37°C for 24-48 h
- **MacConkey agar:** The plates were incubated at 35°C for 24-48 h
- **Potato dextrose agar:** The plates were incubated at 30°C for 24 h for yeast isolates and 3-5 days for moulds

RESULTS AND DISCUSSION

Honey samples from Akwa-Ibom, Ibadan, Ogun, Ondo, Shaki and Yola had pH values of 4.95, 4.67, 5.14, 4.65, 4.90 and 5.06, respectively (Table 1). The brix content of the honey samples from Akwa-Ibom, Ibadan, Ogun, Ondo, Shaki and Yola were 28.00, 34.00, 35.00, 29.00, 30.00 and 37.00, respectively (Table 1). The total titrable acidity calculated as % lactic acid of the honey samples showed that honey samples from Akwa-Ibom, Ibadan, Ogun, Ondo, Shaki and Yola had percentage lactic acid of 0.19, 0.15, 0.07, 0.05, 0.03 and 0.04, respectively (Table 2). The honey samples also had percentage total solids of 82.00, 80.60, 80.80, 84.00, 80.00 and 80.40, respectively (Table 2). The percentage ash content of the honey samples from Akwa-Ibom, Ibadan, Ogun, Ondo, Shaki and Yola were 0.70, 0.76, 0.60, 0.76, 0.84 and 0.80, respectively (Table 3). While the percentage moisture content of the honey samples from Akwa-Ibom, Ibadan, Ogun, Ondo, Shaki and Yola were 18.00, 19.40, 19.20, 16.00, 20.00 and 19.60, respectively (Table 3). The colour results showed absorbance of 0.133, 0.448, 0.139, 0.256, 0.286 and 0.140 for honey samples from Akwa-Ibom, Ibadan, Ogun, Ondo, Shaki and Yola respectively. Physical observation showed colours ranging from light amber, amber, light amber, amber, amber, light amber for honey samples from Akwa-Ibom, Ibadan, Ogun, Ondo, Shaki and Yola, respectively (Table 4). Viscosity results for the honey samples showed samples from Akwa-Ibom, Ibadan, Ogun, Ondo, Shaki and Yola having values of 3.142, 3.221, 2.828, 2.514, 3.692 and 2.985 centipoise, respectively (Table 4). The results showed honey samples from Akwa-Ibom, Ogun and Ondo had hydroxymethyl furfural content of 1.29, 1.23 and 1.21 mg/100 g while samples from Ibadan, Shaki and Yola had hydroxymethyl furfural content of 0.43, 0.43 and 0.38 mg/100 g (Table 5). Microbiological analysis of the honey samples revealed that coliform counts were absent from all honey samples from the different locations. Total viable counts were only observed in samples from Ibadan, Shaki and Yola. While samples from Akwa-Ibom, Ibadan, Ogun, Ondo, Shaki and Yola had mould and yeast counts of 1.1×10^1, 1.4×10^1, 1.3×10^1, 1.2×10^1, 1.5×10^1 and 1.8×10^1, respectively (Table 6).

The reason for testing honey for quality control purposes is to verify the authenticity of the product and to reveal the possible presence of artificial components or adulterants, as well as to address processing and market needs (Krell, 1996). This requires not only determining the moisture and mineral content (ash), but also the levels of hydroxymethylfurfural (HMF), acidity, diastase activity, apparent sugars and water insoluble solids (Bogdanov *et al*., 1999). The pH values of the honey samples from various locations (Table 1) revealed that all the honey samples were within the acidic range of pH.

Table 1: pH values and Brix content of honey samples from various locations within Nigeria*

Sample locations	pH values	Brix content
Akwa Ibom	4.95	28
Ibadan	4.67	34
Ogun	5.14	25
Ondo	4.65	35
Shaki	4.90	30
Yola	5.06	37

*Results are average of 2 determinations

Table 2: Total titrable acidity and total solids of Honey samples from various locations within Nigeria

Sample locations	Total titrable acidity (% Lactic acid)	Total solids (%)
Akwa Ibom	0.19	82.00
Ibadan	0.15	80.60
Ogun	0.07	80.80
Ondo	0.05	84.00
Shaki	0.03	80.00
Yola	0.04	80.40

Table 3: Ash content and moisture content of honey samples from various locations within Nigeria

Sample locations	Ash content	Moisture content
	(%)	
Akwa Ibom	0.70	18.00
Ibadan	0.76	19.40
Ogun	0.60	19.20
Ondo	0.76	16.00
Shaki	0.84	20.00
Yola	0.80	19.60

Table 4: Colour and viscosity of honey samples from various locations within Nigeria

Sample locations	Absorbance (660 nm)	Colour	Viscosity (centipoise)
Akwa Ibom	0.133	Light amber	3.142
Ibadan	0.448	Amber	3.221
Ogun	0.139	Light amber	2.828
Ondo	0.256	Amber	2.514
Shaki	0.286	Amber	3.692
Yola	0.140	Light amber	2.985

*Results are average of 2 determinations

Table 5: Hydoxymethyl furfural (HMF) in honey samples from various locations within Nigeria

Sample locations	Hydroxymethyl furfural (mg/100 g)
Akwa Ibom	1.29
Ibadan	0.43
Ogun	1.23
Ondo	1.21
Shaki	0.43
Yola	0.38

Table 6: Microbiological analysis of honey samples from various locations within Nigeria

Type of analysis	Colony forming units (cfu mL^{-1})*/sample locations					
	Akwa Ibom	Ibadan	Ogun	Ondo	Shaki	Yola
Standard plate count	NIL	1.0×10^1	NIL	NIL	1.2×10^1	1.3×10^1
Coliform count	NIL	NIL	NIL	NIL	NIL	NIL
Moulds and Yeast counts	1.1×10^1	1.4×10^1	1.3×10^1	1.2×10^1	1.5×10^1	1.8×10^1

*Results are average of 2 determinations

The pH values were also within the acceptable range of between 3.6 and 5.6 (Kirkwood *et al*., 1960; Adebiyi *et al*., 2004). The significance of pH at acidic range in foods cannot be overemphasized. They prevent the honey samples from constant infection by various species of micro-organisms and thus help to ensure constant shelf life for the honey samples. The acidic pH of all honey samples from various locations indicate they have good shelf life.

The brix content of the various honey samples were as shown on Table 1. The results of the brix content revealed that all the samples had brix contents that were within the acceptable range of between 25 and 40. The brix content is a measure of the sugar content in the honey sample (Kirkwood *et al*., 1960; Adebiyi *et al*., 2004). Invertase converts the sucrose in pure honey into fructose and glucose. During the processing of honey, heat destroys the invertase in honey. Adulterated honey therefore contains excess sucrose and low fructose due to the loss in activity of the enzyme invertase. The brix content of the Nigerian honey samples showed that the sugar levels have not been altered. The total titrable acidity of the honey samples from various locations were as shown on Table 2. Total titrable acidity was highest in samples from Akwa-Ibom with 0.19% and least in samples obtained from Shaki with 0.03%. However, all the samples were within an acidity range that allows for an enhanced shelve stability of the product samples and thus prevent spoilage by micro organisms (Williams *et al*., 2009). The total solids of the honey samples from various locations were as shown on Table 2. The total solids were highest in samples obtained from Ondo with 84.00% and least in samples from Shaki with 80.00%. 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 from Nigeria indicate that the honey samples were within the acceptable range of total solids. The ash content of the honey samples from various locations (Table 3) showed that samples obtained from Shaki had the highest with 0.84% and those from Ogun had the least with 0.60%. However, in all the samples from the various locations, the ash contents were within the acceptable range of 0.04-0.93% (White, 1975; Kirkwood *et al*., 1960). However, the ash contents were higher than those found for other Nigerian honeys which were between 0.09 and 0.518 (Adebiyi *et al*., 2004). The ash content is a measure of the mineral elements in the honey samples. The ash content of the Nigerian honey samples indicates good physical property. The moisture content of the various honey samples (Table 3) revealed that samples obtained from locations in Shaki had the highest with 20.00%, while that from Ondo had the lowest with 16.00%. However, all the samples had moisture contents that were within the acceptable range of 15.70 and 26.70% as stipulated by the Honey regulations (1976), White *et al*. (1962), White (1975) and Kirkwood *et al*. (1960). Absorption of moisture during open storage and processing could cause an increase in moisture content. An increase in moisture content of honey is indicative of adulteration. The honey samples from Nigeria had acceptable moisture contents. The colour of the various honey samples obtained from various locations (Table 4) revealed that samples from Akwa-Ibom, Yola and Ogun were the best in terms of their colour grading with extra light amber colour while samples obtained from Shaki, Ondo and Ibadan were amber coloured. However, according to White (1975), the colour of honey varies from almost colourless to nearly black according to its botanical source and to conditions of processing and storage it has undergone. The dark honeys are known to contain more minerals than the lighter ones (White, 1975). The colour of the honey samples from the various regions could not be the single determinant of adulteration. This finding is comparable to values obtained for Nigerian honeys (Adebiyi *et al*., 2004). The viscosity of each of the honey samples from various locations (Table 4) revealed that samples from Shaki had the highest viscosity of 3.692 centipoise and samples from Ondo had the least value 2.514 centipoise. Viscosity of the honey samples is a measure of the quality of the honey samples. Pure honey has high viscosity. A low viscosity indicates a high moisture content or dilution (Cervantes *et al*., 2000). The viscosity values of the Nigerian honey samples therefore shows that all the samples were still in their natural state of production and had not undergone any form of adulteration in terms of dilution with other products. The hydroxymethyl furfural (HMF) of honey samples obtained from various locations were as shown in Table 5. The hydroxymethyl furfural measures the quality of honey via the formation of 5-hydroxymethyl furfuraldehyde by acid hydrolysis of its sucrose with the formation of red colour (Winkler, 1955). The results revealed that samples obtained from Akwa-Ibom, Ibadan, Ogun, Ondo, Shaki and Yola had low hydroxymethyl furfural. The low value indicates that the sugar levels of these samples were not affected during storage. The formation of red colour with acid by samples from Akwa-Ibom, Ogun and Ondo show that these samples had never undergone any form of adulteration. However, samples obtained from locations in Ibadan, Shaki and Yola that had light colouration might have undergone some form of adulteration (Winkler, 1955). The microbiological analysis of the honey samples

from various locations (Table 6) revealed that samples obtained from Akwa-Ibom, Ondo and Ogun were completely free of adulteration. This is shown by the absence of coliforms and total bacteria counts. However, samples obtained from Ibadan, Shaki and Yola might have undergone some level of adulteration due to the presence of some total viable counts in the samples. The result of these physico-chemical studies show that adulteration of honey takes place to some extent in Nigeria though it might have been due to unhygienic handling during processing and storage. Further studies could be done to compare the level of caramelization in Nigerian honey from different plants and regions.

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