European Journal of Sustainable Development (2012), **1**, 2, 271-286 ISSN: 2239-5938

NUTRITIONAL AND MICROBIOLOGICAL COMPONENTS OF HONEY SAMPLES OBTAINED FROM OGUN STATE, SOUTHWESTERN NIGERIA.

By

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ABSTRACT

There are no detailed studies on the nutritional and microbiological characteristics of honey produced in Ogun State in the Southwestern part of Nigeria. This paper investigated these components in honeys produced from different parts of this state. A total of 10 honey samples per year were collected for the years 2008 – 2010. These were separately analyzed for their physical properties, nutritional and microbiological components in the laboratory. The results of the physical properties showed that honey samples obtained from Ago-Iwoye has the lowest pH of 3.48, which was significantly different from the pH values of 5.06, 5.21 and 4.06 obtained from honey samples from Abeokuta, Ibefun and Ilisan honey samples respectively. There was significant difference in moisture contents of honey samples obtained from Ogere (16.19 %), Otta (19.14 %) and Ijebu-Ode (18.21 %), while the percentage ash contents of 0.78 % obtained from honey samples collected from Abeokuta was not significantly different from the value of 0.75 % obtained from Ago-Iwoye honey ($P \leq 0.05$). However, the value of 1.11 mg 100 g^{-1} for hydroxymethylfurfural obtained from Ago-Iwoye honey samples was not significantly different from the value of 0.32 mg 100 g^{-1} in honey samples obtained from Sagamu.

The value for glucose ranged from 18.42 – 30.16 g 100 g^{-1}, while fructose sugar varied between 25.42 – 38.21 g 100 g^{-1}. Minimum protein value of 0.02 % was obtained from Ijebu-Ode honey, while the maximum of 0.51 % was obtained from honey samples from Ilisan. Results of the elemental nutrient showed that potassium was the most abundant element in honey samples with the range value of 14.78 – 17.42 mg 100 g^{-1} followed by calcium, which varied from 2.13 – 11.25 mg 100 g^{-1}. However, result of microbiological properties showed that the total plate count varied from 0.2 – 3.4 cfu g^{-1}, whereas total coliforms were not detected in honey samples collected from Ago-Iwoye, Otta, Ibefun, Ife and Sagamu. *Clostridium* spp, *Bacillus* spp and yeast were also detected at low count in all honey samples investigated.

Moreover, honey samples obtained from different parts of Ogun State were found to be contaminated with *Pseudomonas* spp., *Xanthomonas* spp., *Bacillus* spp., *E. coli* and *Clostridium* spp, while fungi spores intercepted were *Pennicillium oxalicum, Aspergilus niger, A. Flavus* and *Fusarium oxysporum* at different levels and counts.

Key words: **Hydroxymethylfurfural, Proline, *Apis mellifera*, *Clostridium* spp.**

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INTRODUCTION

Honey is the sugary substance produced from the nectar of flowers by worker bees (Alfred, 2004). It is the most important primary product of beekeeping both from a quantitative and economic point of view. It was the first bee product used by mankind in ancient times (Crane, 1980).

China, Turkey and the United States of America are the top producers of natural honey (Krell, 1996). US honey production in 2007 was 148 million pounds, while Canada had an annual honey production of 62 million pound in 2008 (USDA, 2008). Honey contains mainly sugars (NHB, 2008). It contains more carbohydrates (82.3 %) than any other animal product (Lawal *et al*., 2009). According to McNulty (2002), the glucose and fructose component averages to about 76 – 80 percent of the honey, while water accounts for about 17 – 20 percent. The amount of amino acids and proteins in honey are relatively small (0.7 percent); thus having relatively small nutritive effects. However, these components can be important for judging honey quality (Cottee, 2004).

Several investigations have shown that the trace element contents of honey depend mainly on the botanical origin of the honey (Feller *et al*., 1989). Honey contains varying amount of mineral substances ranging from 0.02 – 1.03 g 100 g^{-1}. The main element found in honey is potassium with an average of about one-third of the total (Bogdanov *et al*., 2007). The acid content of honey is relatively low ranging from pH value of 3.3 – 4.6, but it is important for honey taste (Echugo and Takenaka, 1974).

On account of the nutritional value and fast absorption of its carbohydrate, honey is a food suitable for humans of every age (Blasa *et al*., 2006). Honey is particularly recommended for children and sportsmen because it can help to improve the physiological activities immediately it is consumed and absorbed (Blasa *et al*., 2006).

The beneficial role of honey is attributed to its anti-bacterial and anti-inflammatory properties with regard to its high osmolarity, acidity, and content of hydrogen peroxide (H_2O_2) components (Weston, 2000). The anti-microbial agent in honey is predominantly hydrogen peroxide of which the concentration is determined by relative levels of inhibine and glucose oxidase (Weston, 2000).

Honey is farmed and used allover Nigeria. Initially, local farmers harvested honey from the wild, but today, apiculture is a growing industry in many

parts of the country. Some studies on the healing effects and anti-microbial activity of Nigerian honey on burns and wounds have been reported (Adesunkanmi and Oyelami, 1994). However, there is no detailed study on the nutritional and microbiological characteristics of the honey produced in Ogun State. This study therefore investigated the nutritional and microbiological components of honey samples obtained from different parts of Ogun State, Southwestern Nigeria.

MATERIALS AND METHODS

Sample Collection

Honey samples used for this study were collected from different locations in Ogun State, Southwestern Nigeria. Ten (10) samples of honey were separately collected in sterilized glass bottles in 2008, 2009 and 2010 respectively. These samples were kept in dark cupboard and separately analyzed at the Biochemistry and Microbiology laboratories of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State. Nigeria.

Physicochemical Analyses

The pH of honey samples obtained from different locations in Ogun State were measured with the pH meter (UNICAM 9450). 10 ml of honey sample was taken and mixed in a beaker with 10 ml of ultra-pure water and shaken for 20 min and allowed to stand for 15 – 20 min before the electrode was inserted. Readings were taken for all honey samples in triplicates to allow for statistical analysis (AOAC, 1990).

Moisture Content Analysis

One-hundred gramme of honey sample each was weighed accurately in a pre-weighed platinum dish and gently heated in a muffle furnace at 105 ^{0}C until the sample turned black and dried. This was allowed to cool in a dessicator and re-weighed again until a constant weight was obtained.
The weight loss in respect of 100 g represented the moisture contents of the honey sample. The percentage moisture content (MC) was calculated for all samples using the formula below:

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$$\% \text{ MC} = \frac{M_1 - M_2}{M_1 - M_0} \times 100$$

Where M_0 = wt (g) of dish
M_1 = wt (g) of dish of honey sample before drying
M_2 = wt (g) of dish of honey sample after drying

Determination of Ash content

Honey sample (2g) was ashed by calculation in a furnace at 600 ^{0}C to a constant weight. Ash percentage was calculated for all honey samples as:

$$\% \text{ Ash} = \frac{\text{(Wt. of crucible + ash) – Wt. of empty crucible}}{\text{Wt. of honey sample}} \times 100$$

Measurement of Refractive Index (RI) **and Acidity**

Refractive Index of honey samples was measured using the Refractometer at a constant temperature of 20 ^{0}C (AOAC, 1990). Honey acidity was determined by the trimetric method. 10 g of each honey sample was accurately dissolved in 75 ml CO_2 free distilled and titrated with 0.1 m NaOH solution. Total acidity was obtained by adding free and lactonic acidities (Malika *et al.*, 2005). Results were expressed as milli-equivalent of acid per kilogram (meq kg^{-1}) of honey.

Determination of Hydroxylmethylfurfural (HMF)

Ten (10) grammes of each unheated sample was dissolved in 20 ml ultra-pure water and thereafter transferred to 50 ml volumetric flask and made up to 50 ml. 2 ml of each sample was introduced into 2 test tubes and 5.0 ml solution was added to each tube. The blank was prepared by adding 1 ml barbituric acid to a tube with 1 ml of ultra-pure water. The absorbance of the test sample was read against the blank at 550 nm wavelength using the spectrophotometer (Spectronic 20 D model). The HMF was calculated using the equation proposed by Winker (1955).

$$\text{HMF (mg 100 g}^{-1}) = \frac{\text{Absorbance}}{\text{Cell path length}} \times 100$$

Determination of sugar content

The phenol-sulphuric acid method of Majnard (1970) was used. 10 ml of each honey sample ultra-pure water in a calibrated and centrifuged to

obtain a supernatant solution for the analysis. 1 ml of the diluted solution was pipette into test tube and 1 ml of 52 % phenol was added to each test tube. 5 ml of 96 % H_2SO_4 was also added in drops. The test tube was then allowed to stand for 10 min before the content was transferred into clean grease-free cubettes. Stock glucose was prepared as standard. The values of the reducing sugar (fructose and glucose) present in each honey sample were read on a spectrophotometer at a wavelength of 490 nm.

Determination of crude protein (CP)

Honey samples were analyzed for crude using the routine Kjeldahl nitrogen method (Joslyn, 1970). 10 g of the homogenous honey sample was weighed into digestion flask and dissolved with 10 ml of ultra-pure water. The diluent was transferred into the volumetric flask, while Kjeldahl catalyst tablet (potassium sulphate) was added and thoroughly shaken. 20 ml of concentrated H_2SO_4 was added and fixed into the digester. The flask was cooled and the digest transferred into 100 ml volumetric flask. 5 drops of bromocresol (indicator) and 75 ml of ultra-pure water were added. 10 ml of the digest was pipette into the Kjeldahl distillation flask and titrated with 0.05 N of HCl, while the percentage total nitrogen was calculated using the Joslyn (1970) equation.

$$\% \text{ Total nitrogen} = \frac{14.0\ (\text{sample titre} - \text{blank titre})}{10 \times \text{wt of sample}} \times N$$

Where N = normality of acid

% CP was obtained for all the honey samples by:

% CP = % total nitrogen x 6.25

Mineral Element Analysis

The honey samples were analyzed for mineral elemental determination using Atomic Absorption Spectrophotometer (AAS) and Flame Photometer according to AOAC (2005). 10 g of honey sample was weighed and dissolved in ultra-pure water after thorough mixing; the solution was stirred for 15 min on a mechanical stirrer at 1550 rpm. A solution of Parkloric acid and nitric acid were added and mixed thoroughly. This homogenous solution was dispensed into the AAS in order to determine the concentration of K, Ca, Mg, Cu and P at different wavelengths. A standard was prepared for each of these elements.

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Microbiological analysis

The total plate count method was used for culturing and isolating the different microbes intercepted in the honey samples. MacConkey agar was used as the medium for microflora culture, while Potato dextrose agar was used for growing fungi (Marshall, 1987). Bacteria colonies resulting from the first culture after incubation at 35 ^{0}C for 48 hrs were transferred to fresh media, streaked and incubated again. This sequential streaking, based on the principle of dilution of culture helped to produce discrete bacterial colonies after incubation. After successive transfer, the resulting pure isolates were gram-stained and identified based on colour, size and shape with reference to the manual of Determinative Biology (Beckatt and Stelanke, 1986).

For fungi, incubation was at room temperature (25 – 27 ^{0}C) for 4 days. The resulting mycelia were extracted and grown respectively on malt extract and potato dextrose agar until pure cultures were obtained. In addition to structural morphology of fungi, other identification characteristics such as cultural, surface appearances of colonies and the colour of the colonies were also used. Other parameters considered were the nature of mycelia: septate or non-septate, conidia heads, nature and arrangement of spores (Bradshaw, 1979).

Statistical analysis

Data generated from the laboratory analyses were subjected to statistical analyses using Analysis of Variance (ANOVA), while the sample means were separated with the aid of Duncan Multiple Range Test at $P \leq 0.05$ (SAS, 1999). All values were expressed as the mean standard deviation.

RESULTS AND DISCUSSION

Physicochemical Characteristics

The mean physicochemical components of the samples of honey obtained from different parts of Ogun State in 2008, 2009 and 2010 are presented in Tables 1 and 2. The mean pH values of the honey samples ranged from 3.48 – 5.21. There was a significant difference in the mean pH value of 5.06 ± 1.11 obtained from Abeokuta and the mean value of 3.77 ± 0.61 obtained from Sagamu, but was not different significantly from the pH mean values of 5.21 and 5.16 obtained from Ibefun and Ayetoro honey

samples respectively. The mean pH range obtained in this study was however closer to the range of 4.31 – 6.0 reported for Nigerian honey from other locations (Adebuyi *et al.*, 2004). The acidic pH of honey has been shown to promote

Table 1:**Mean physical characteristics of honey samples obtained from some locations in Ogun State Western Nigeria for 2008, 2009 and 2010**

Source	pH value	MC (%)	Ash (%)	RI
Abeokuta	5.06 ± 1.11^{a}	21.41 ± 1.31^{a}	0.78 ± 0.21^{b}	1.02 ± 0.01^{c}
Ijebu-Ode	4.62 ± 0.31^{b}	18.21 ± 2.11^{b}	0.54 ± 0.11^{c}	1.31 ± 0.01^{b}
Ago-Iwoye	3.48 ± 0.35^{d}	19.17 ± 2.16^{a}	0.75 ± 0.12^{b}	1.18 ± 0.01^{b}
Ayetoro	5.16 ± 0.71^{a}	20.13 ± 1.47^{a}	0.84 ± 0.22^{a}	1.86 ± 0.13^{a}
Ogere	4.31 ± 0.43^{b}	16.19 ± 1.32^{c}	0.36 ± 0.06^{d}	1.77 ± 0.11^{a}
Otta	3.97 ± 0.14^{c}	19.14 ± 1.31^{a}	0.41 ± 0.11^{c}	1.10 ± 0.02^{b}
Ibefun	5.21 ± 0.23^{a}	21.27 ± 3.16^{a}	0.34 ± 0.14^{d}	1.13 ± 0.11^{b}
Ilisan	4.06 ± 1.34^{c}	16.15 ± 1.27^{c}	0.32 ± 0.01^{d}	1.21 ± 0.01^{b}
Ifo	3.74 ± 0.21^{c}	20.16 ± 2.16^{a}	0.77 ± 0.03^{b}	1.24 ± 0.01^{b}
Sagamu	3.77 ± 0.61^{c}	16.28 ± 1.71^{c}	0.96 ± 0.21^{a}	1.07 ± 0.03^{c}
Range	3.48 – 5.21	16.15 – 21.41	0.32 – 0.96	1.02 – 1.86

MC – Moisture content
RI – Refractive index

Table 2: **Mean HMF, free lactone and free total acidity of honey samples obtained from some locations in Ogun State for 2008, 2009 and 2010**

Source	HMF (mg 100 g^{-1})	Free acidity (meq kg^{-1})	Lactonic acid (meq kg^{-1})	Total acidity (meq kg^{-1})
Abeokuta	1.27 ± 0.31^{b}	20.1 ± 2.11^{c}	11.4 ± 1.14^{b}	31.5^{c}
Ijebu-Ode	0.39 ± 0.04^{c}	18.6 ± 1.67^{c}	5.7 ± 0.21^{c}	24.3^{f}
Ago-Iwoye	1.11 ± 0.06^{b}	25.7 ± 3.14^{b}	9.1 ± 1.15^{b}	34.8^{c}
Ayetoro	0.48 ± 0.11^{c}	20.8 ± 1.21^{c}	9.7 ± 1.10^{b}	30.5^{c}
Ogere	1.37 ± 0.14^{b}	29.4 ± 1.75^{a}	10.2 ± 0.94^{b}	39.6^{b}
Otta	0.34 ± 0.06^{c}	14.3 ± 3.11^{d}	6.4 ± 1.23^{b}	20.7^{e}
Ibefun	1.17 ± 0.15^{b}	19.7 ± 1.32^{c}	8.9 ± 0.41^{c}	28.6^{d}
Ilisan	1.63 ± 0.16^{a}	27.5 ± 2.17^{a}	12.1 ± 2.11^{b}	39.6^{b}
Ifo	1.25 ± 0.03^{b}	27.8 ± 1.42^{a}	11.7 ± 1.44^{b}	39.5^{b}
Sagamu	0.32 ± 0.12^{c}	30.4 ± 2.97^{a}	14.3 ± 1.30^{a}	44.7^{a}
Range	0.32 – 1.63	14.3 – 30.4	5.7 – 14.3	20.7 – 44.7

healing by causing oxygen release from haemoglobin (Leveen *et al.*, 1973). The pH of honey is low enough to prevent the growth of many species of pathogenic organisms.

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The moisture content (MC) of honey samples varied from 16.15 – 21.41 % (Table 1). The highest MC of 21.41 % was obtained from the honey sample collected from Abeokuta, which was significantly different from the lowest value of 16.15 % obtained from Ilisan, but not significantly different from 19.17, 20.13, 21.27 and 20.16 % obtained from honey samples collected from Ago-Iwoye, Ayetoro, Ibefun and Ifo respectively. The MC in honey samples collected from Ogun State for the years under study was comparable to the values obtained by Omafuvbe and Akanbi (2009) for Nigerian honey and differs slightly from US honey as reported by White (1975b). The variation observed from the mean MC can be explained by the composition and floral origin in honey samples collected from different locations in Ogun State. An increase in MC is indicative of adulteration. Low MC in honey also forms an important part of the system which protects honey from the attack by microorganisms.

There were significant differences in the mean ash content in honey samples collected from various locations of Ogun State, Nigeria. The ash content ranged between 0.32 – 0.96 % (Table 1). The mean ash content of 0.84 % obtained from Ayetoro was significantly different from 0.36 and 0.34 % obtained from Ogere and Ibefun honey samples respectively. Ash represents a direct measure of inorganic residue after honey carbonation. The variability in the ash content observed could be explained by the floral origin, geographical location and level of maturity of the honey. However, the mean ash content obtained value of 0.61 % is closer to 0.66 % obtained for honey in the Sudano-Guinea zone of western Cameroun (Tchoumborne *et al*., 2007).

The highest mean refractive index (RI) of 1.86 was obtained from the honey samples collected from Ayetoro, which was significantly different from the lowest mean value of 1.02 obtained from Abeokuta honey sample, but not significantly different from the mean value of 1.77 obtained from the honey samples collected from Ogere. These values are similar to 1.46 – 1.48 reported by Adebiyi *et al.* (2005).

The results showed that honey samples from Abeokuta, Ijebu-Ode, Ago-Iwoye and Ayetoro had mean hydroxymethylfurfural (HMF) content of 1.27, 0.39, 1.11 and 0.48 mg 100 g^{-1}, while samples from Ogere, Otta, Ibefun, Ilisan, Ifo and Sagamu had mean HMF constituents of 1.37, 0.34, 1.17, 1.63, 1.25 and 0,32 mg 100 g^{-1} respectively (Table 2). The mean value of HMF obtained showed that it occurred only in trace amounts as

reported by Thrasyvoulou (1997). The HMF measures the quality of honey formation of 5-hydroxymethyl-furfuraldehyde by acid hydrolysis of its sucrose with the formation of red colour.

The values for free lactone and total acidities are summarized in Table 2. Mean free acidity ranged between 14.3 – 30.4 meq kg^{-1}, lactone acidity values were between 5.7 – 14.3 meq kg^{-1}, while total acidity values varied from 20.7 – 44.7 meq kg^{-1}. The total acidity of 39.6 meq kg^{-1} obtained from Ilisan honey, which was not significantly different from the mean values of 39.5 and 44.7 meq kg^{-1} obtained from the honey samples collected from Ifo and Sagamu respectively, but was different significantly from the mean values of 31.5, 20.7 and 28.6 meq kg^{-1} obtained from the honey samples collected from Abeokuta, Otta and Ibefun respectively (Table 2).

The mean values obtained for total acidity of honey samples obtained from the honey samples collected from different locations in Ogun State falls within the range reported for Moroccan honey (Malika *et al.*, 2005). The acidity of honey contributes to its stability against microorganisms and flavor (Omafuvbe and Akanbi, 2009).

Nutritional Constituents

The mean nutrient contents of honey samples obtained from different locations in Ogun State for 2008, 2009 and 2010 are presented in Table 3. The glucose values of 18.42 and 18.91 g 100 g^{-1} obtained from the honey samples collected from Abeokuta and Ijebu-Ode respectively are significantly different from the mean value of 25.32 and 30.1 g 100 g^{-1} obtained from Ogere and Ilisan honey samples respectively. The fructose values obtained in honey samples collected ranged from 25.42 – 38.21 g 100 g^{-1}. This showed that fructose and glucose sugars are the major sugars found in honey. This result is in conformity with the research reported by Krell (1996) that the majority of sugars in honey are the simple sugars, fructose and glucose, which represents about 85 – 95 % of total sugars found in honey. Generally, fructose sugar is greater than glucose sugar for honeys collected from different parts of Ogun State. This is in agreement with the report of Crane (1990) who reported that fructose is more abundant than glucose in US honey.

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Table 3:**Mean nutritional components of honey samples obtained from some locations in Ogun State for 2008, 2009 and 2010**

Source	Glucose	Fructose	CP	K	Ca	Mg	Cu	P
	(g 100 g^{-1})		ns				(meq 100 g^{-1})	
Abeokuta	18.42 ± 2.14^{c}	25.42 ± 1.23^{c}	0.12 ± 0.01	14.78 ± 1.01^{b}	6.31 ± 1.11^{b}	0.19 ± 0.26^{d}	0.03 ± 0.01^{d}	1.06 ± 0.02^{a}
Ijebu-Ode	18.91 ± 1.67^{c}	30.46 ± 2.14^{b}	0.02 ± 0.01	16.51 ± 1.02^{a}	4.50 ± 0.16^{c}	0.15 ± 0.04^{c}	0.06 ± 0.01^{c}	1.18 ± 0.04^{a}
Ago-Iwoye	28.64 ± 3.21^{a}	30.82 ± 1.77^{b}	0.36 ± 0.03	17.42 ± 1.62^{a}	4.10 ± 0.31^{c}	0.23 ± 0.01^{b}	0.03 ± 0.00^{d}	0.96 ± 0.04^{c}
Ayetoro	28.71 ± 1.76^{ba}	37.11 ± 3.60^{a}	0.07 ± 0.02	16.76 ± 2.10^{a}	4.23 ± 0.14^{c}	0.24 ± 0.01^{b}	0.07 ± 0.02^{c}	1.67 ± 0.03^{a}
Ogere	25.32 ± 3.11^{b}	30.06 ± 3.60^{b}	0.38 ± 0.01	16.20 ± 1.67^{a}	3.32 ± 0.12^{c}	0.17 ± 0.01^{c}	0.12 ± 0.01^{b}	1.32 ± 0.04^{a}
Otta	25.41 ± 2.46^{b}	27.15 ± 1.24^{c}	0.17 ± 0.02	16.32 ± 1.20^{a}	3.35 ± 0.11^{c}	0.29 ± 0.03^{b}	0.05 ± 0.01^{c}	0.52 ± 0.02^{b}
Ibefun	26.11 ± 1.21^{b}	37.67 ± 1.36^{b}	0.24 ± 0.01	16.15 ± 1.31^{a}	2.13 ± 0.17^{d}	0.15 ± 0.02^{c}	0.13 ± 0.01^{b}	0.46 ± 0.03^{b}
Ilisan	30.16 ± 1.31^{a}	31.52 ± 1.74^{b}	0.51 ± 0.03	16.24 ± 1.21^{a}	7.74 ± 1.19^{b}	0.20 ± 0.02^{d}	0.21 ± 0.02^{a}	1.32 ± 0.01^{a}
Ifo	28.08 ± 2.17^{a}	38.21 ± 2.30^{a}	0.21 ± 0.02	15.11 ± 1.23^{b}	11.25 ± 1.34^{a}	0.74 ± 0.11^{a}	0.14 ± 0.01^{b}	1.37 ± 0.03^{a}
Sagamu	29.16 ± 1.95^{a}	36.31 ± 1.37^{a}	0.09 ± 0.01	15.34 ± 1.31^{b}	6.31 ± 0.78^{b}	0.92 ± 0.12^{a}	0.09 ± 0.02^{b}	1.24 ± 0.02^{a}
Range	18.42 – 30.16	25.42 – 38.21	0.02 – 0.51	14.78 – 17.42	2.13 – 11.25	0.15 – 0.92	0.03 – 0.21	0.46 – 1.67

The protein content detected in honey samples collected from Ogun State is in small quantities with the highest value of 0.51 % obtained from Ilisan honey sample, while the lowest value of 0.02 % was obtained from Ijebu-Ode honey sample. The mean value of 0.38 % obtained from Ogere honey was significantly different from the mean values of 0.09, 0.07 and 0.12 % obtained from the honey samples collected from Sagamu, Ayetoro and Abeokuta respectively (Table 3). This result is in agreement with the report of Terrab *et al.* (2003) who stated that honey is not intended as a protenacous food as the amount of protein are relatively small and at most 0.7 %.

The results of the atomic absorption spectrophotometric (AAS) analysis of the honey samples are presented in Table 3. Five elements were detected: K, Ca, Mg, Cu and P at different concentrations in honey samples collected from different locations in Ogun State. K was the most abundant element with range values of 14.78 – 17.42 mg 100 g^{-1} followed by Ca with a range of 2.13 – 11.25 mg 100 g^{-1}, while P had value range of 0.46 – 1.67 mg 100 g^{-1}. The values of Mg and Cu were in trace amount, but varied from 0.15 –

0.92 and 0.03 – 0.21 mg 100 g^{-1} respectively. However, there were significant differences in the elemental contents of honey samples collected from different locations in Ogun State (Table 3). These results are in agreement with the research carried out by Adebiyi *et al.* (2004) who reported that K was the most abundant element in Nigerian honey followed by Ca. it then confirms that Ogun State honeys are quite rich in elemental nutrients, which are necessary for major physiological activities in human being.

Microbiological Characteristics

Microbial counts in samples of honey collected from different locations in Ogun State are reported in Table 4. The total bacterial count (TBC) showed that honey samples collected from Ogere had a count of 3.4 x 10^3 cfu g^{-1}, while that of Ilisan was 0.2 x 10^3 cfu g^{-1}. The total plate count (TPC) showed low level for all the honey samples obtained from different locations in Ogun State. The total coliform count (TCC) was also very low with a minimum of 0.3 x 10^3 cfu g^{-1} obtained from the honey samples collected from Abeokuta, while the maximum value of 2.0 x 10^3 cfu g^{-1} was obtained from the honey samples collected from Ogere. There were no coliform count in honey samples collected from Ayetoro, Otta, Ibefun, Ifo and Sagamu areas of Ogun State. This may be explained by the evidence that honey is well preserved against bacteria so that these organisms would not survive unfavourable conditions. This result is in agreement with the report that Moroccan honeys are very low in bacterial and coliform counts.

Table 4: **Mean microbial count** (x 10 Cfu g^{-1}) **of honey samples obtained from some locations in Ogun State for 2008, 2009 and 2010**

Source	TBC	TCC	TF	*Clostridium* spp.	*Bacillus* spp.	TYC
Abeokuta	1.8	0.3	0.6	++	1.4	3.0
Ijebu-Ode	2.3	1.5	0.1	++	0.1	1.0
Ago-Iwoye	1.4	1.2	0.6	1.4	1.7	1.4
Ayetoro	2.6	++	0.3	++	++	2.0
Ogere	3.4	2.0	0.8	0.6	2.0	6.4
Otta	2.4	++	1.4	0.2	1.4	2.8
Ibefun	1.7	++	0.3	0.1	++	2.1
Ilisan	0.2	1.4	0.4	++	0.3	2.7
Ifo	1.6	++	0.3	0.3	1.0	2.7
Sagamu	2.6	++	0.6	++	2.4	2.9

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Results are mean of 3 determinations
TCC - total coliform count
TF- the total fungi
cfu g^{-1} - colony forming unit per gram
TBC - total bacterial count
TYC – total yeast count
+ + = Absence of microbe

Clostridial and bacillus counts were also very low. In fact, there were clostridium count in most of the honey samples collected from Ogun State except those from Ago-Iwoye, Ogere, Otta, Ibefun and Ifo, which had clostridial counts of 1.4, 0.6, 0.2, 0.1 and 0.3 x 10^3 cfu g^{-1} respectively. No bacillus count was also recorded for honey samples collected from Ayetoro and Ibefun (Table 4). Our results are in agreement with the research carried out by Omafuvbe and Akanbi (2009) and Malika *et al.* (2005).

The results in this study clearly showed that honey samples collected from different locations in Ogun State have total fungal count between 0.1 – 1.4 x 10^3 cfu g^{-1}. The total yeast count (TYC) obtained were also very low with a range of 1.0 – 6.4 x 10^3 cfu g^{-1} in honey samples collected from Ijebu-Ode and Ogere respectively (Table 4). This variation in TYC may be due to the type of harvesting, processing and freshness of the honey. The low count of fungal and yeast cells are in accord with the report of Tysett *et al.* (1970) who reported that French honey had zero count of yeast and moulds. The absence of fungal spores and yeast cells in some of the honey samples confirms that honey has inherent antimicrobial properties that can delay the growth of many microorganisms. Generally, honey may contain organisms from bees, soil, air and dust that may be introduced during post-harvest handling (Jay, 1992).

The occurrence of bacteria detected in honey samples collected from different locations in Ogun State is presented in Table 5. The bacteria found in the honey samples were identified as *Pseudomonas* spp., *Xanthomonas* spp., *Bacillus* spp., *Escherichia coli*, and *Clostridium* spp. These are plant pathogens, except *Bacillus* spp., *E. coli*, and *Clostridium* spp.; hence, originated from the plant host containing the nectars where the bees visited. The *Clostridium* and *E. coli* bacteria were detected in very small count in the honey samples investigated. This is an indication that the sanitary conditions during extraction and handling in the apiaries were quite efficient. This result is in agreement with the research of Omafuvbe and Akanbi (2009) and Adenekan *et al.* (2010) who reported that commercial honey in Nigeria does not harbour spores of *E. coli* and *Clostridium* spp.

Table 5: **Occurrence of bacteria detected in honey samples obtained from some locations in Ogun State for 2008, 2009 and 2010**

Source	*Pseudomona* spp.	*Xanthomonas* spp.	*Bacillus* spp.	*E. coli*	*Clostridium* spp.
Abeokuta	++	++	++	++	--
Ijebu-Ode	++	--	--	++	--
Ago-Iwoye	++	--	++	++	++
Ayetoro	++	++	--	--	--
Ogere	++	++	++	++	++
Otta	++	++	++	++	++
Ibefun	++	++	--	--	++
Ilisan	++	--	++	++	--
Ifo	++	++	++	--	++
Sagamu	++	++	++	--	--

Results are mean of 3 determinations
++ = presence of bacterial spore
-- = absence of bacterial spore

The results in this study showed that honeys collected from different locations in Ogun State harbor arrays of fungal contamination. The fungal spores found in the honey samples were identified as *Pennicillium oxalicum, Aspergillus niger, A. flavus* and *Fusarium oxysporum* and their occurrence pattern is presented in Table 6. The fungal arrays detected are not known to be pathogenic to human beings and this confirms the report of Tchoumboue *et al.* (2007) that eight fungal species were identified from the western Cameroun honey. It is also in conformity with the report of other researchers that fungi and spore-forming bacteria may be present in honey for a limited period of time (Anon, 2001). The occurrence of these fungi in honey could be an indication of contamination from secondary sources during handling, processing and storage or adulteration (Adenekan *et al.*, 2010).

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Table 6: **Occurrence of fungal and yeast organisms detected in honey samples obtained from some locations in Ogun State for 2008, 2009 and 2010**

Source	*Penicillum oxalicum*	*Aspergillus niger*	*Aspergillus flavus*	*Fusarium oxysporum*	Yeast cell
Abeokuta	++	++	++	++	++
Ijebu-Ode	--	++	++	--	++
Ago-Iwoye	++	++	--	--	++
Ayetoro	++	--	--	--	++
Ogere	++	++	++	++	++
Otta	++	--	++	++	++
Ibefun	--	--	++	++	++
Ilisan	--	++	--	--	++
Ifo	++	--	++	--	++
Sagamu	++	++	--	--	++

Results are mean of 3 determinations
++ = presence of fungal organism
-- = absence of fungal cells

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