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Impact of Geographical Variations on the Physicochemical Properties, Antioxidant Activities and Viscosity of Honey from Ondo State, Nigeria

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Abstract

Evaluation of the physicochemical properties, antioxidant activities as well viscosity of honey from the three main geographical areas (south, central and north) of Ondo State, Nigeria, was carried out in order to access how geographical variations affect honey qualities. Honey was collected from the six local government areas; two from each geographical area (Okitipupa and Irele, Akure South and Ondo West, and, Owo and Akoko South West Local Government Areas from southern, central and northern geographical areas respectively). The proximate and minerals composition; specific gravity, viscosity, colour, antioxidant properties, pH, sugar contents, Vitamin C, and Fourier Transform Infrared Spectroscopy (FTIR) of the honey samples were analysed. The results obtained showed that the moisture content varied from 10.85% to 12.43%. Honey from Akure South Local Government had the highest moisture content while that from Okitipupa Local Government had the least. The ash content varied from 0.47% to 0.59%; protein content ranged from 0.13% to 1.41%, while carbohydrate content ranged from 76.92% to 88.36%. Akoko South West Local Government had honey with the highest phenolic content while honey from Owo Local Government Area had the highest Ferric reducing antioxidant power assay (FRAP), and Akure South Local Government honey had the highest DPPH scavenging activity All honey samples were acidic in nature, with pH values varying between 4.0 and 4.6. Vitamin C content ranged from 19.65 to 32.15 mg/100g. Increased temperature reduced the viscosity content of all the honey samples. Honey from the various geographical areas exhibited different physicochemical and antioxidant properties.

Keywords: *Antioxidants activities, Geographical variations, Hone quality, Nigeria, Ondo State, Physicochemical properties, Viscosity*

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Introduction

Honey is a sweet, viscous, natural food product formed from the nectar of flowers by honeybees. The conversion of nectar to honey consists of collection of the nectar from flowers by honey bees, ripening by partial enzymatic digestion in the stomach of the honeybee, maturation by moisture evaporation through fanning by the bees, which leaves moisture content of honey to between 13 and 18% (Olaitan *et al.*, 2007). Honey possesses therapeutic and nutritional benefits cosmetic, industrial, and traditional uses (Olaitan *et al.*, 2007) making it to be used as sweeteners in beverages (Honeywonders, 2013) and cosmetics. Factors that affects honey composition includes its floral source, seasonal and environmental. (Kato *et al.*, 2012).

The chemical compositions of honey are fructose (38.2%), glucose (31.3%), sucrose (0.7%**),** maltose (7.1%), water (17.2%) and ash (0.2%), according to White (1975). Several studies have shown that the antioxidant potential of honey is strongly correlated not only with the concentration of total phenolic present, but also with the color; the dark coloured honeys being reported to have higher total phenolic contents and, consequently, higher antioxidant capacities (Alvarez-Suarez et al., 2010 and Alvarez-Suarez *et al.*, 2012). In addition, honey has been reported to be carriers of plants medicinal properties (Alvarez-Suarez *et al.*, 2010) since these properties are embedded in the plants nectars, which would be converted to honey through honey bees. Some of the medicinal properties could be antimicrobial, bioactive or nutraceuticals

(Olabiran *et al*., 2023; Awolu and Oladeji, 2021). Vitamin C has been reported to play important role as an antioxidant by protecting against oxidative stress. Functional fruit developed from blends of *Hibiscus sabdariffa* extract, pineapple, carrot and orange fruits have been found to be rich source of vitamin C (Ogundele *et al.*, 2016).

Ondo state is one of the largest producers of honey in Nigeria. The state is blessed with different geographical varieties including very mangrove swamp forest near the Bright of Benin (in the south geographical area), tropical rain forest in the central and wooded savannah in the north geographical area (Fig. 1). This study seeks to establish the effect of the various geographical areas (with different vegetation) on the chemical quality and antioxidant activities of honey produced from the different regions. The functional groups of the honey from the different regions was also established by the use Fourier-Transform Infrared Spectroscopy (FTIR).

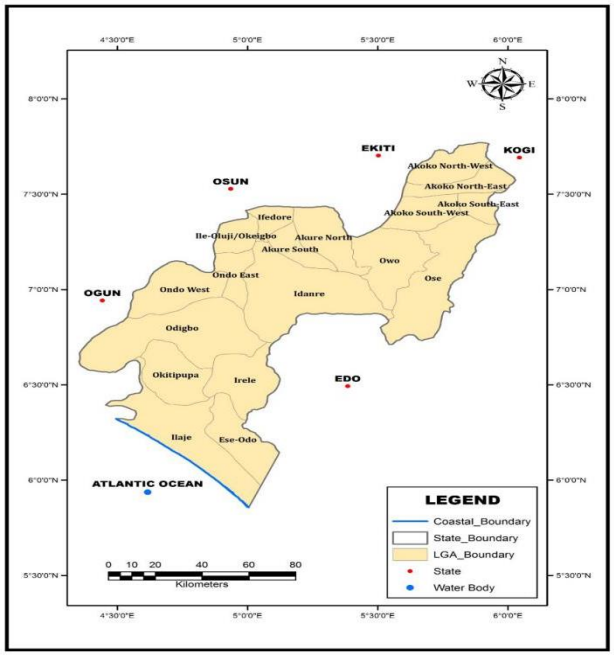

Figure 1: Geographical map of Ondo State
Source: https://www.gamers.com.ng/map-of-ondo-state-nigeria/

Materials and Methods

Materials

Honey samples were collected from beekeepers in six local governments of Ondo State representing the three geographic areas: Akoko South-West and Owo Local Government Areas (northern geographical area); Akure South and Ondo East Local Government Areas (central geographical area); Okitipupa, and Irele Local Governments Areas (southern geographical area) of Ondo State. All chemicals used were of analytical grades.

Methods

Determination of proximate composition of the honey

The moisture, ash, crude protein, crude fibre and fat contents were evaluated using AOAC (2005) methods. Carbohydrate contents, on the other hand was evaluated by subtracting the percentages compositions of moisture, ash, crude fibre, crude protein and fat contents from 100%.

Carbohydrate content = 100 – (crude fat + moisture + total ash + crude fibre + crude protein).

Minerals Determination

About 2 g of sample was placed in a crucible, ashed in a muffle furnace at 550 ^{o}C for 5 h and then transferred to the desiccators to cool. The ashed sample was dissolved with 1mL nitric acid and 1ml HCl and then made up to 50 ml. Iron, cupper, calcium, lead and cadmium were determined by the use of atomic absorption spectrometer (Bulk Scientific, Moderl 210VGP), while phosphorus was determined using phosphovanado-molybdate method.

Determination of % glucose, fructose and sucrose

Percentage sugar was determined using high performance liquid chromatograph (HPLC). Two grammes of raw honey were placed in a 10 mL volume-metric flask, and a water/methanol solution (3:1) was added to make a final volume in the flask (9 mL), and the flask contents were mixed thoroughly. Then the honey sample was manually stirred, and delivered into the volumetric flask using a 0.5 mL micropipette. In order to include a surrogate standard, 0.2 mL of a solution composed of 5 g of xylose and 25 mL solution of water/methanol (3:1) was added. This gave a final xylose concentration of 4 mg/mL. A 0.2 mL volume of Carrez I reagent (distilled water solution of potassium hexa cyano ferrate (II),$K_4Fe(CN)$ 6·3H2O, 15 g/100 mL) was added and mixed. Subsequently, a 0.2 mL volume of Carrez II reagent (distilled water solution of zinc acetate, $Zn(CH_3COO)_2$·2H2O, 30 g/100 mL) was added and mixed. The flask was then filled to 10 mL volume with the water/methanol solution, and mixed. The solution was transferred to a 10 mL glass centrifuge vial and centrifuged at 4000 rpm for 15 min to remove the protein fraction. An aliquot of 5 mL of the supernatant was transferred to a 10 mL glass vial (provided with cap) that had been pre-washed with dichloromethane. About 5 mL of dichloromethane was added, the vial was capped and shaken and then vortexed vigorously for two min. The two layers were allowed to separate and the upper layer (containing the sugar fraction) was collected with a Pasteur pipette. Two additional extractions were performed on this sugar fraction as described above. Following the third extraction, the sugar fraction was filtered through a 0.45 µm disposable syringe filter (Whatman Puradisc 25AS, polysulfonefilter media). The sample was refrigerated and used for HPLC injection within 3 days.

Determination of specific gravity, Vitamin C and pH

Specific gravity: Specific gravity was determined by using pycnometer meter (AOAC, 2005).

Vitamin C: The vitamin C content was determined by the spectro-photometric method using ascorbic acid as a reference compound according to the method reported by Awolu *et al.* (2013).

pH: Exactly ten grammes of the honey samples were dissolved in 75 ml of distilled water in a 250 ml beaker and stirred with magnetic stirrer. The pH was measured with the meter (Inolab, Germany), calibrated at pH 4.0 and 7.0 buffer solutions (OSAE, 2009).

Aqueous extracts of honey for determination of antioxidants properties

Aqueous extracts of honey was prepared using the method described by Oboh *et al.* (2010). About 2.5 g of honey was soaked in 100 ml of distilled water for about 24 h at 37 ºC. The homogenates were filtered, centrifuged at 2,000 rpm for 10 min. The supernatant was stored in the refrigerator for subsequent analysis.

Determination of total phenol

The total phenolic content was determined according to the method described by Singleton *et al.* (1999). Appropriate dilution of the extracts was oxidized with 2.5 ml 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45 ºC, and the absorbance was measured at 700 nm using spectrophotometer. Gallic acid was used as standard phenol and the total phenolic content was subsequently calculated as gallic acid equivalent (GAE, 2009).

Determination of total flavonoid content

The total flavonoid content of the extract was determined using colorimetric assay (Bao, 2005). The extract (0.2 ml) was added to 0.3 ml of 5% $NaNO_3$ at zero time. After 5 min, 0.6 ml of 10% $AlCl_3$ was added and after 6 min, 2 ml of 1 M NaOH was added to the mixture followed by the addition of 2.1 ml of distilled water. Absorbance was read at 510 nm against the reagent blank and flavonoid content was expressed as mg rutin equivalent.

Ferric reducing antioxidant power assay (FRAP)

The reducing power assay was conducted by method described by Wang *et al*. (2008) and Oyaizu (1986) with ascorbic acid (AA) and tert-butyl-4-hydroxyanisole (BHA) being used as the positive controls. In brief, 2.5 ml of individual deionized water diluted grain extracts (ranged from 0.1 to 1 mg/ml) was sequentially mixed with equal volume of phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1% w/v). After incubation at 50 ºC for 20 min, 2.5 ml of trichloroacetic acid (10% w/v) was then added to the mixture followed by centrifuging at 3000 rpm for 10 min. Consequently, 5 ml of the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1% w/v). After 30 min of incubation at room temperature in the dark, absorbance of the resulting solution was measured at 700 nm using a Thermo Spectronic Helios Spectrophotometer. The ferric reducing power capacities of the grain extracts and standard antioxidants were expressed graphically by plotting absorbance against concentration. Samples for the assay were prepared in triplicate.

Determination of DPPH free radical scavenging ability

The free radical scavenging ability of the extracts against DPPH (1,1-diphenyl-2 picrylhydrazyl) radical was evaluated as described by Gyamfi *et al.* (1999). An appropriate dilution of the extracts (1 ml) was mixed with 1 ml of 0.4 mM/L methanol solution containing DPPH radicals. The mixture was left in the dark for 30 min and the absorbance was measured at 516 nm in the spectrophotometer. The DPPH free radical scavenging ability was subsequently calculated with respect to the reference, which contained all the reagents without the test sample.

FTIR-ATR Spectroscopy Measurements

FTIR-ATR spectroscopy measurements were performed using the Vertex 70 (Bruker) spectrometer applying the Attenuated Total Reflectance (ATR) technique with a diamond crystal. The selected infrared radiation was the average IR range (400-4000 cm^{-1}). The spectral resolution was 2 cm^{-1} and 64 scans were used. For the reference (ripe pollen sample) and for each honey sample, the absorption bands corresponding to chemical compounds were identified in the respective samples. Moreover, each measurement was performed in triplicates. For all spectra obtained, baseline correction and normalization were applied using the OPUS 7.0. Software. In all the figures the average of the three obtained spectra for each sample are presented

Viscosity measurements

The viscosity of the honey samples were determined using Rion Visco-meter (VTO4F), as described by Awolu *et al.*, (2013). Rotor speed was 62.5 rpm. For each analysis, the filled sample beaker and the spindle were temperature equilibrated. The temperature of the samples ranging from 20 ℃ to 100 ℃ was regulated using a water bath.

Colour evaluation

Colour was measured using a Colour Meter PCE-CSM 2 (Deutschland GmbH) connected to a CQCS3 software. The spectrophotometer was calibrated against a white plate before the reading was taken.

Statistical Analysis

The data for the analysis were generated in triplicates, and subjected to one way analysis of variance (ANOVA), SPSS 19.00 while means were separated using Duncan multiple range test (DMRT) at $p<0.05$.

Results and Discussion

Proximate Composition of Honey

The results of the proximate composition of the honey samples are presented in Table 1. While honey from the southern region (samples E and F) had the least moisture contents (10.87 and 12.05%), honey from the central region (samples C and D) had the highest moisture contents. A moisture content of less than or equal to 17.8% has been reported for an ideal for honey. In this case, honey from the southern and northern regions fall within the acceptable range. Honey from sample D was also acceptable. Lazarevic *et al.* (2012) reported moisture content of 20% for honey samples originating from the entire territory of Serbia. Honey harvested from northwest Spain had average water content of 17.6% (Escuredo *et al.*, 2014). Honey have been found to be hygroscopic; the lower the moisture content the better. So honey from the northern regions (samples E and F) of Ondo State would have better shelf life compared to honey from other regions. In fact, honey from the northern regions were better in terms of moisture contents than honey from Serbia and Spain reported above. High moisture contents of honey would lead to honey quality deterioration (Singh and Singh, 2018). Codex Alimentarius Commission stipulated ash content of less or equal to 0.6% for honey.

Table 1: Proximate composition of Honey

Samples	Moisture (%)	Ash (%)	Protein (%)	Carbohydrate (%)
A	10.85±0.23^{e}	0.52±0.04^{b}	0.26±0.06^{b}	88.36±0.22^{a}
B	15.59±0.02^{c}	0.54±0.01^{b}	0.23±0.06^{b}	83.62±0.06^{c}
C	17.23±0.10^{b}	0.59±0.02^{a}	0.13±0.06^{b}	82.04±0.070^{d}
D	22.43±0.11^{a}	0.47±0.02^{c}	0.16±0.06^{b}	76.92±0.09^{e}
E	12.05±0.11^{d}	0.53±0.01^{b}	0.23±0.12^{b}	87.18±0.23^{b}
F	10.87±0.06^{e}	0.52±0.03^{b}	1.41±0.42^{a}	87.29±0.47^{b}

Values represent means of triplicates. Values in a column with same superscript are not significantly different at $p \leq 0.05$.
A: Okitipupa local government, **B:** Irele local government, **C**: Ondo east local government, **D**: Akure south local government, **E**: Owo local government, **F**: Akoko south west local government.

The ash content of the honey ranged from 0.47% to 0.59% for all the samples. The ash content of a food material could be used as an index of mineral constituents of the food (Sanni *et al.*, 2008). Standard Organization of Nigeria (SON) also reported ash content of not more than 0.6% for honey.

Protein content ranged from 0.13% to 1.41%. Sample F had the highest protein content. Honey has been reported to contain about 0.5% proteins which are mainly enzymes and free amino acids (Bogdanov *et al.*, 2008). It was also reported that the contribution of the available protein to human protein intake was marginal.

Carbohydrate content ranged from 76.92% to 88.36%. Sample A had highest content while sample D had the least. The high carbohydrate content could be attributed to high glucose and fructose content present in honey. Honey is above all a carbohydrate material, with 95 to 99.9 percent of the solids being sugars. Carbohydrate composition depends mainly on the honey's botanical and geographical origin, and is affected by climate, processing and storage (Tornuk *et al.*, 2013, Escuredo *et al.*, 2014).

Mineral Contents of Honey

Mineral ions play vital roles in metabolism as transport co-factor and stabilizes enzymes (Chima *et al.*, 2012). Some of the functions of mineral elements include body metabolism, skeletal strength and maintenance of acid-base equilibrium in body. The mineral content of honey from the different geographic areas indicated that calcium was the most abundant (Table 2). Honey from the central geographic areas were the most abundant in calcium contents. Calcium has been reported as one of the most abundant mineral elements in honey after potassium. Sample A had the highest calcium content while sample E had the least. Honey from the central geographical area, specifically, Akure south local government was the best in terms of calcium and iron contents. This could only indicate the geographical condition of Akure south local government supports the minerals. While calcium and phosphorus are major elements, iron and cupper are trace elements. Ndife *et al.*, (2014) reported an iron content of 0.52 mg/100g to 1.12 mg/100g for different florals of honey in Nigeria. Lead and cadmium are toxic elements, hence the non-presence on the honey samples are welcome developments.

Table 2: Mineral Contents of Honeys (ppm)

Samples	**Ca**	**Fe**	**Cu**	**P**	**Pb**	**Cd**
A	3763.3±56.86^{b}	7.53±0.50^{c}	0.27±0.12^{a}	143.15±2.75^{a}	NIL	NIL
B	85.66±4.509^{d}	11.69±2.10^{b}	0.38±0.10^{a}	24.370.55^{f}	NIL	NIL
C	2140.0±52.92^{c}	5.50±0.70^{de}	0.00±0.00	132.33±2.52^{b}	NIL	NIL
D	5150.0±18.02^{a}	25.03±0.61^{a}	0.00±0.00	95.23±2.04^{c}	NIL	NIL
E	47.20±2.96^{e}	4.53±0.45^{e}	0.27±0.06^{a}	77.36±0.93^{d}	NIL	NIL
F	71.46±6.17^{d}	7.03±0.06^{cd}	0.27±0.12^{a}	36.56±2.27^{e}	NIL	NIL

Values represent means of triplicates. Values in a column with same superscript are not significantly different at $p<0.05$.
A: Okitipupa local government, **B:** Irele local government, **C**: Ondo east local government, **D**: Akure south local government, **E**: Owo local government, **F**: Akoko south west local government.

The differences in mineral contents of the honey for the different geographical areas could be attributed to the different cultivars, soil in which the original nectar bearing plant was located (Alvarez-Suarez *et al.*, 2010; Amril and Ladjama, 2013) and botanical origins. The percentage mineral content is considered as a quality criterion indicating the possible botanical origin of honey (Vanhanen *et al.*, 2011).

Sugar Content

The sugar contents is of the honey samples are presented in Table 3. Fructose content had highest value followed by glucose and sucrose. El-Sohaimy *et al.*, (2015) also reported low sucrose content and high fructose content for honey obtained from different origins (Egypt, Yemeni, Saudi and Kashmiri samples). Glucose content ranged from 31.36% to 36.26% with sample F having the highest value while sample B had the least. Monosaccharaides make up about 75% of the sugars found in honey, along with 10-15% disaccharides and small amounts of other sugars (da Silva *et al.*, 2016). Sucrose content ranged from 1.0% to 1.67%.The values of sucrose obtained from this study are with the acceptable level of not more than 5% reported by Codex Alimentarius. There were significant ($p \leq 0.05$) differences in the sugar contents of the honey samples across the local government areas and geographical areas. Honey from the northern geographical areas and Akure south local government area had the best fructose and glucose contents, making them best in terms of honey (and sugar) qualities.

Table 3: Sugar contents of Honey from different senatorial districts

Samples	Glucose (%)	Fructose (%)	Sucrose (%)
A	34.23±0.25^{d}	40.35±0.56^{a}	1.13±0.12^{cd}
B	31.36±0.15^{de}	39.23±0.25^{b}	1.36±0.06^{b}
C	34.30±0.17^{d}	40.26±0.06^{c}	1.67±0.12^{a}
D	35.13±0.15^{b}	37.16±0.06^{c}	1.33±0.06^{b}
E	34.64±0.14^{c}	40.36±0.23^{a}	1.00±0.00^{d}
F	36.26±0.15^{a}	39.16±0.15^{b}	1.25±0.07^{bc}

Values represent means of triplicates. Values in a column with same superscript are not significantly different at $p \leq 0.05$.
A: Okitipupa local government, **B:** Irele local government, **C**: Ondo east local government, **D**: Akure south local government, **E**: Owo local government, **F**: Akoko south west local government.

The Specific Gravity, Vitamin C and pH of Honey Samples

The results of the specific gravity, vitamin C and pH of the honey samples are presented in Table 4. All of the tested honey samples were acidic in nature, with pH values that varied between 4.0 and 4.6. These values were similar to the values (pH 3.49 – 4.70) reported for honey samples from Algeria, Brazil, India, Spain and Turkey (Azeredo *et al.,* 2003; Kayacier and Karaman, 2008; Ouchemoukh *et al.*, 2007). A highly acidic honey sample indicates the possible fermentation of sugars into organic acids. None of the investigated samples exceeded the allowed limit, which may be considered as an index of freshness of all honey samples (Khalil *et al.*, 2012). According to Codex Alimentarius standards (Codex Alimentarius 1981) and the United States National Honey Board reference guide (National Honey Board 2005) pH range should be between 3.4 and 6.1.

Table 4: pH, Specific Gravity and Vitamin C of Honey from Different Geographical Areas in Ondo State

Samples	**pH**	**Specific gravity**	**Vitamin C (mg/100g)**
A	4.6	0.85±0.009^{a}	21.47±0.045^{c}
B	4.3	0.72±0.017^{d}	32.15±0.020^{a}
C	4.2	0.72±0.005^{cd}	19.65±0.017^{d}
D	4.0	0.74±0.010^{b}	19.60±0.036^{d}
E	4.2	0.73±0.005^{bcd}	19.65±0.012^{d}
F	4.3	0.74±0.005^{bc}	25.07±0.043^{b}

Values represent means of triplicates. Values in a column with same superscript are not significantly different at $p \leq 0.05$.
A: Okitipupa local government, **B:** Irele local government, **C**: Ondo east local government, **D**: Akure south local government, **E**: Owo local government, **F**: Akoko south west local government

The specific gravity ranged from 0.72 to 0.85. Sample A had the highest specific gravity while sample B had the least. Mohammed *et al.*, (2017) reported specific gravity range of 1.42–1.46. The difference in pH of honey could be attributed to climate or difference in locations. Popov-Raljić *et al*., (2015) reported that the altitude and climate affect the physiochemical properties and quality of honey (Khalil, 2011) Vitamin C content of the honey samples ranged from 19.65 mg/100g to 32.15 mg/100g. Sample B had the highest vitamin C content while sample E had the least vitamin C content. Studies have indicated possible role of vitamin C in the protection of certain types of chronic diseases including certain types of cancer (Passmore and Eastwood, 1986). Vitamin C acts as an antioxidant in the blood and other body fluids. It regenerates the active antioxidants form of vitamin E and enhances non haem iron absorption by keeping iron in its more readily absorbable forms (Onimawo and Akubor, 2012). Simultaneous presence of iron and vitamin C in the gut improves bioavailability of iron from non- haem foods (Onimawo and Akubor, 2012).

Antioxidant Properties of Honey Samples

Antioxidant activity is related with compounds capable of protecting a biological system against potentially harmful effects of processes or reactions that cause excessive oxidation, involving reactive oxygen (Cheng *et al.,* 2016). In addition to sugar major components, other minor components of honey Honey is mainly composed of sugars (fructose and glucose), water and also contains small amounts of other constituents like proteins, vitamins, minerals, flavonoids, phenolic acids, enzymes, numerous volatile compounds and other natural products (Khalil *et al.*, 2011).

The total phenolic contents of honey from different geographical areas in Ondo state are shown in Fig. 2. Sample F had the highest phenolic content concentration while sample D had the least value. Similar trend was observed by Bakchiche *et al.*, (2017), who observed differences in the phenolic content of honey from different locations in south of Algeria. The phenolic profile ranged from 0.021 mgGAE/g to 0.031 mgGAE/g. It has been established that the phenolic content is related to antioxidant activities due to its redox properties, which allows them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Wang *et al.*, 2014). Phytochemical antioxidants such as carotenoids, flavonoids and phenols have potential health roles in the reduction of platelet aggregation, **blood pressure,** cardiovascular of disease and a role in modulation of cholesterol synthesis and absorption (Li, 2008). Phenolics or polyphenols are one of the most important classes of compounds found in honey and total concentration of phenols in honey is highly dependent on its plant source (Khalil *et al.*, 2012). The flavonoid content of the honey samples are shown in Fig. 3. Sample F had the highest flavonoid content based while sample D had the least. Flavonoid content ranged from 0.021mg QE/g to 0.043 mg QE/g. Algerian honey had flavonoid content ranged from 0.027–0.072 mg CEQ/g (Moniruzzaman *et al.*, 2013), while Turkish honeys had flavonoid content ranged from 0.028–0.087 mg CEQ/g (Souza *et al*,. 2006). Flavonoids are low molecular weight phenolic compounds that are vital components for the aroma and antioxidant properties of honey (Khalil *et al.*, 2012).

The FRAP contents of honey samples are shown in Fig. 4. The FRAP assay gives a direct estimation of the antioxidants present in a sample based on its ability to reduce the Fe^{3+}/Fe^{2+}

couple (Bakchiche *et al.*, 2017). Sample E had the highest value while sample C had the least.

The DPPH contents of the honey samples are shown in Fig. 5. DPPH scavenging is widely used to test the free radical-scavenging activity of several natural products (Ahn *et al.*, 2007).

The DPPH contents of the samples at different concentrations are shown in Fig. 5. Sample D had the highest DPPH content, thus indication the highest antioxidant potential while sample A had the least.

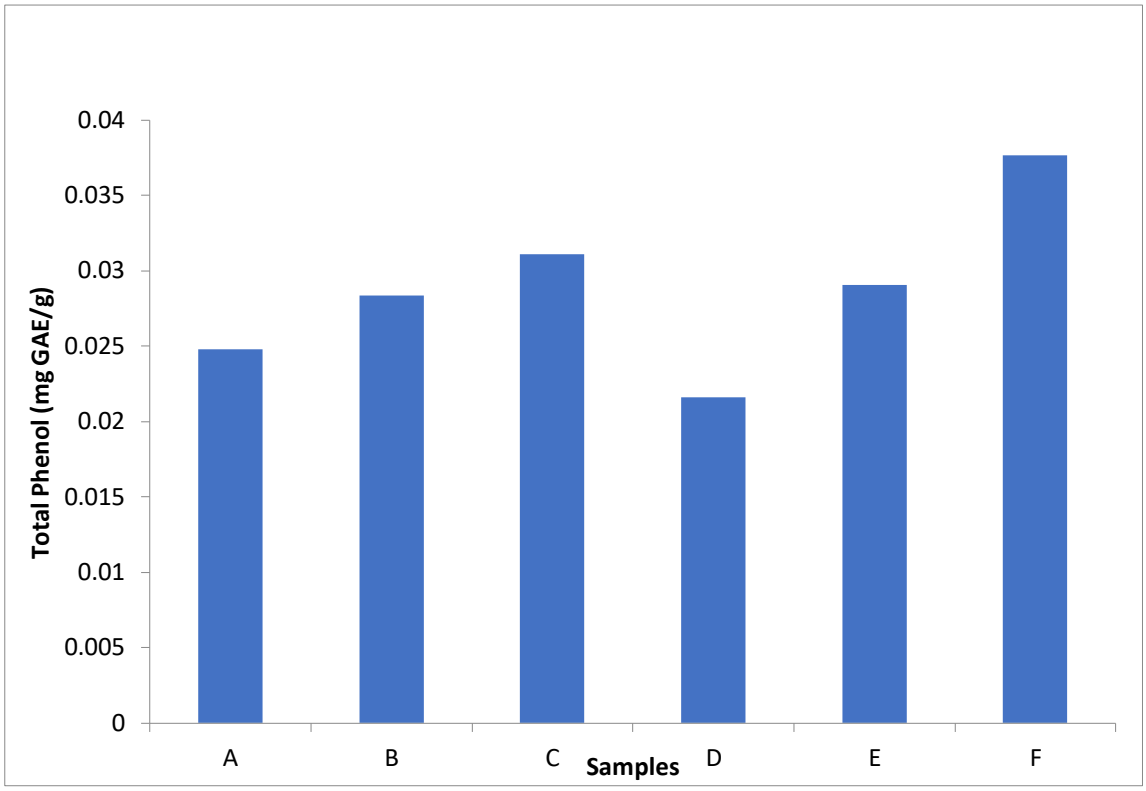

Figure 2: Total phenolic contents of honey from geographical areas of Ondo state.
A: Okitipupa local government, **B:** Irele local government, **C**: Ondo east local government, **D**: Akure south local government, **E**: Owo local government, **F**: Akoko south west local government.

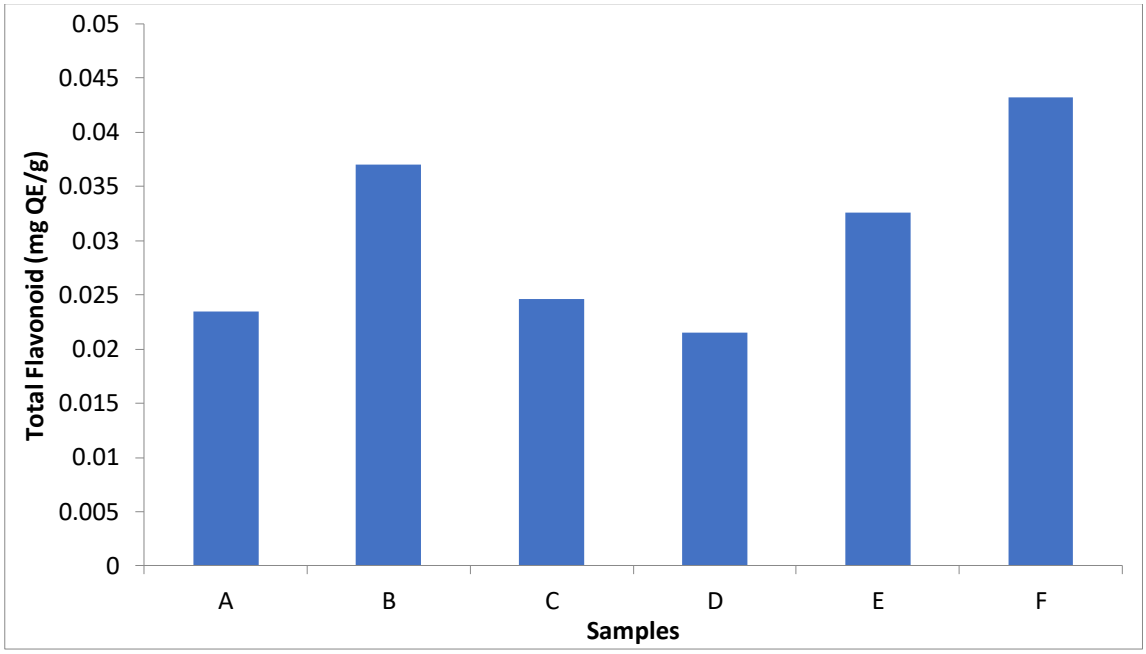

Figure 3: Total flavonoid contents of honey from different senatorial districts of Ondo state.
A: Okitipupa local government, **B:** Irele local government, **C**: Ondo east local government, **D**: Akure south local government, **E**: Owo local government, **F**: Akoko south west local government.

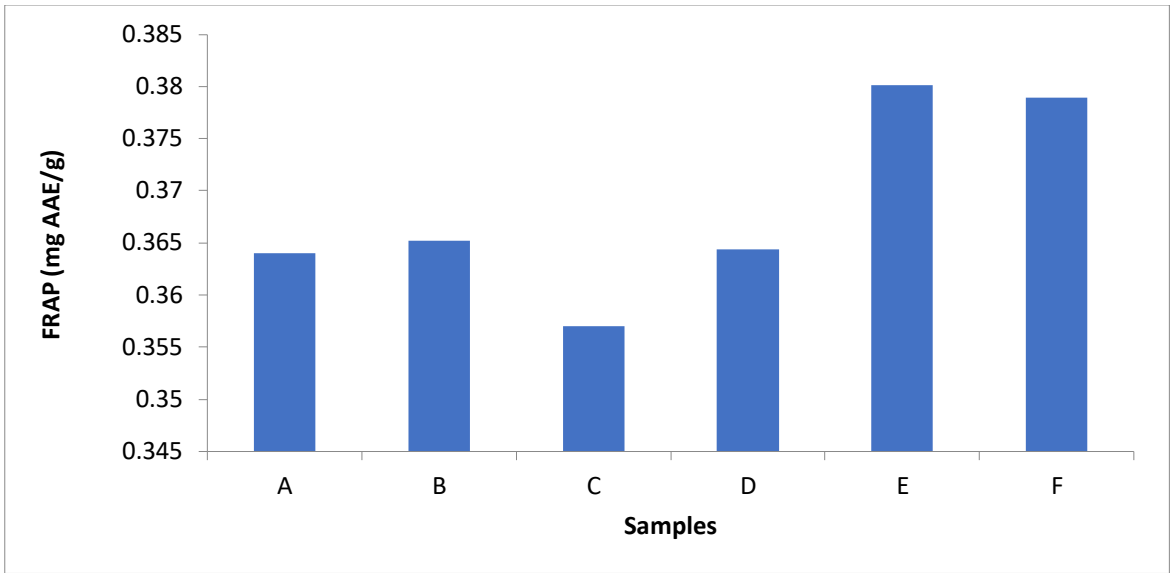

Figure 4: FRAP contentof honey from different senatorial districts of Ondo state.
A: Okitipupa local government, **B:** Irele local government, **C**: Ondo east local government, **D**: Akure south local government, **E**: Owo local government, **F**: Akoko south west local government.

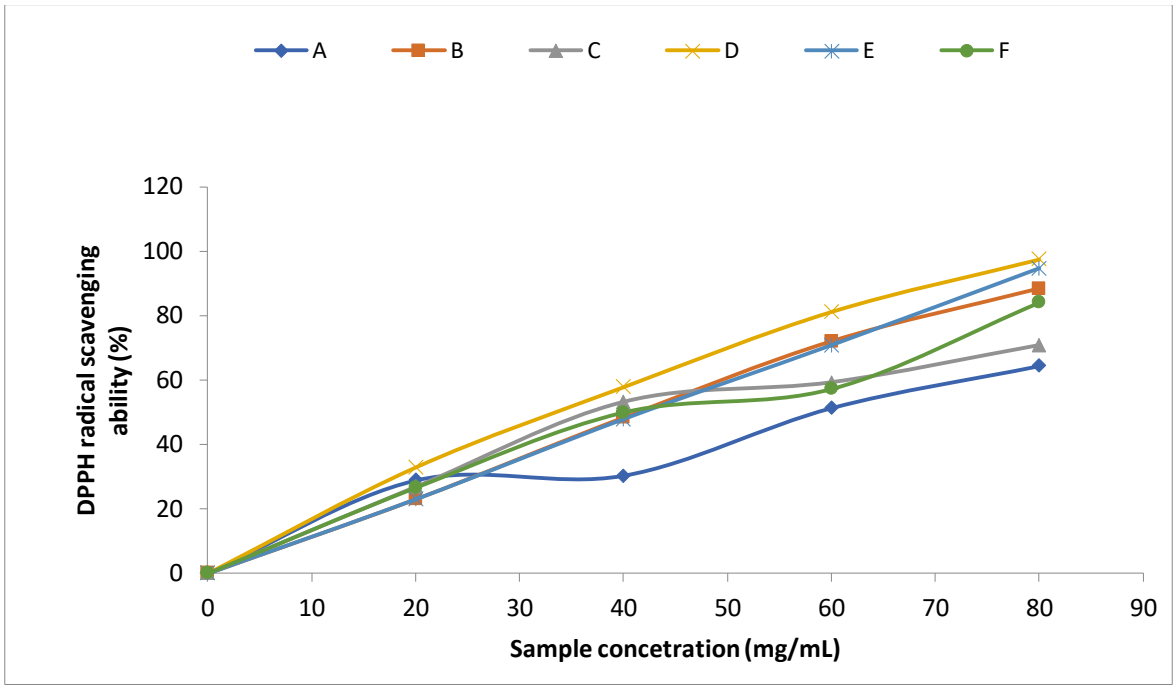

Figure 5: DPPH contents of Honey from the different geographical areas in Ondo State.
A: Okitipupa local government, **B:** Irele local government, **C**: Ondo east local government, **D**: Akure south local government, **E**: Owo local government, **F**: Akoko south west local government.

Fourier Transform Infrared (FTIR) Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy has been described as a nondestructive approach to the identification of chemical and physical constituents of samples. FTIR techniques is gaining momentum in quality and process control applications especially in the food industries for monitoring for food adulteration (Fausan *et al.*, 2018) and also to determine the presence and absence of some certain functional groups (Sacithraa *et al.*, 2013). The regions of the spectra are generalized as follows: The X–H stretching region (4000 – 2500 cm^{-1}), the triple bond region (2500 – 2000 cm^{-1}), the double bond region (2000 – 1500 cm^{-1}) and the fingerprint region (1500 – 600 cm^{-1}) (Sacithraa *et al.*, 2013). The functional groups and wavenumbers present in the samples are presented in Table 5 The major functional group identified for in the samples are O–H (Alcohol), C–H (Alkanes), C≡C (Alkynes) and C=C (Alkenes). While samples A, B and C had all the functional groups, samples D and F lacked 'C–H' functional group, while sample E was lacking in 'C–H' and 'C≡C' functional groups. The 'O–H' functional group represents hydroxyl group which characterizes alcohols or carboxylic acids. Functional groups are specific atoms, ions, or groups of atoms having consistent properties.

Table 5: Identification of Functional Groups of the Honey samples from Different Geographical Areas of Ondo State

Functional Groups	Transmittance (cm^{-1})					
	A	**B**	**C**	**D**	**E**	**F**
Alcohol (O–H)	3365.00	3359.00	3348.00	3179.00	3176.00	3333.00
Alkanes (C–H)	2933.23	2933.00	2934.00	Absent	Absent	Absent
Alkenes (C=C)	1641.51	1642.00	1643.00	1622.00	Absent	1640.00
Alkynes (C≡C)	2122.33	2122.00	2123.78	2125.00	2125.00	2123.73

Effect of Temperature on Viscosity of Honey

The effects of temperature on the viscosity of honey samples has affected by the geographical locations are shown in Fig. 6. Most fluid foods are non-Newtonians, and their viscosities have been reported to vary with temperatures (Awolu *et al.*, 2013; Keshani *et al.*, 2012; Gómez-Díaz_*et al.*, 2009). Understanding of the rheological properties has also been reported as very vital in process design and evaluation, process control and consumers' acceptability of food products (Chuah *et al.*, 2008). In addition, Rheological property is an important physical attribute that could affect texture, quality, sensory attributes and shelf stability of liquid food products (Dak *et al.*, 2007). An inverse relation between temperature and viscosity has been reported (Mossel *et al.*, 2000). Viscosity is an important physical property that correlates with other physicochemical and sensory properties (Gómez-Díaz_*et al.*, 2009). The result of this study also reported a decrease in the viscosity of the honey samples as the temperature increases. At temperature lower than 60 °C, there were variations in the viscosity of the honey samples. This variation decreased significantly at temperatures above 60 °C. Similar result was obtained by Gómez-Díaz_*et al.*, (2009) on the influence of temperature on Galician honeys. The documentation of the effect of temperature on rheological properties had been based on observed variations in temperature encountered during processing and storage of liquid fluids (Rao, 1999).

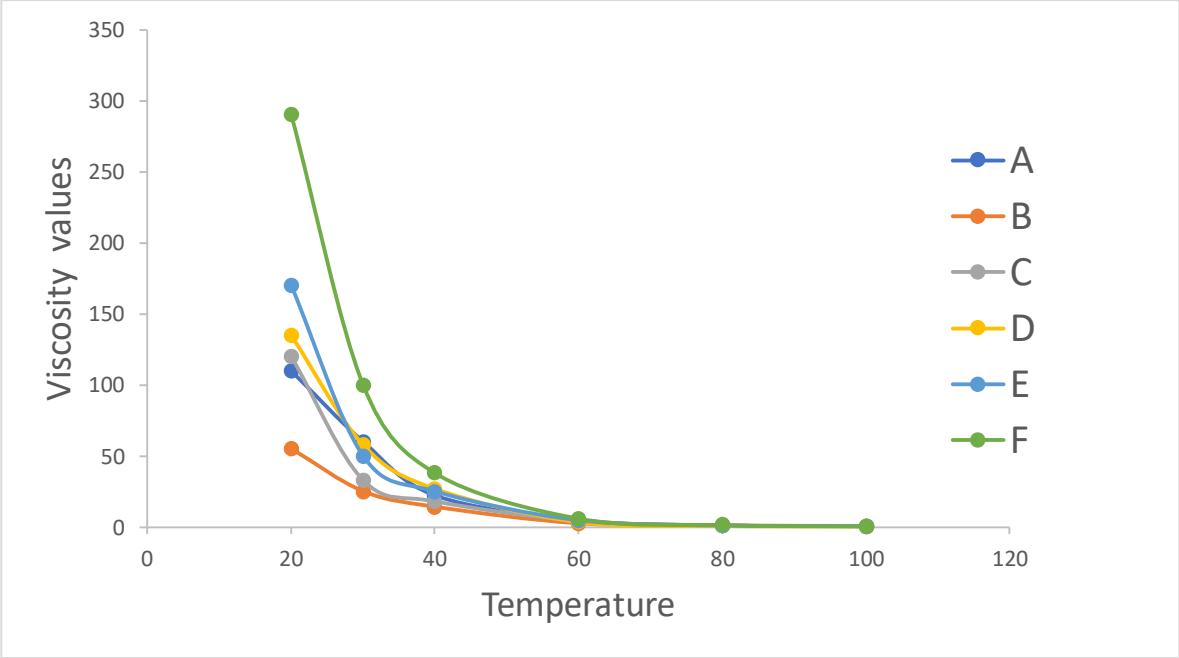

Figure 6: Effects of temperature on Viscosity of Honey

A: Okitipupa local government, **B:** Irele local government, **C**: Ondo east local government, **D**: Akure south local government, **E**: Owo local government, **F**: Akoko south west local government.

Colour of Honey

The colour characteristic of honey samples are presented in Table 7. The primary characteristic for honey classification is color, which is classified according to USDA-approved color standards (1985). The color of untreated honey depends on its botanical origins. Going by the L^* (lightness) of the honey samples colour, they could be characterized as dark honey. Gonzalez-Miret *et al.*, (2005) has reported the characterization of honey into either light honey ($L^* > 50$) or dark honey ($L^* < 50$). While sample C was the darkest, sample F was the least dark. There were however, no significant differences in the level of lightness of samples A, D and E. The variations in the level of lightness could not be unconnected with different harvest time, age or origins of the honey as affected by the geographical variations.

Table 7: Colour of Honey

Samples	L*	a*	b*
A	11.86 ± 1.02^{c}	0.10 ± 0.09^{ab}	0.28 ± 0.31^{a}
B	17.09 ± 3.54^{b}	0.26 ± 0.22^{a}	-0.19 ± 1.08^{b}
C	9.34 ± 0.40^{d}	0.29 ± 0.10^{a}	0.29 ± 0.03^{a}
D	11.79 ± 1.26^{c}	-0.02 ± 0.16^{b}	0.74 ± 0.10^{a}
E	10.55 ± 0.82^{c}	0.33 ± 0.06^{a}	0.53 ± 0.26^{a}
F	20.93 ± 2.01^{a}	0.25 ± 0.06^{a}	0.55 ± 0.54^{a}

All the values are means ± standard deviation of triplicate determination. Means within the same column having the same superscript are not significantly different at ($p<0.05$). L indicate lightness, +a = red, -a = green, +b = Yellow and −b = Blue.
A: Okitipupa local government, **B:** Irele local government, **C**: Ondo east local government, **D**: Akure south local government, **E**: Owo local government, **F**: Akoko south west local government.

Conclusion

The honey significantly differs in physicochemical properties, sugar contents, antioxidant characteristics, and functional across the various geographical divides of the state. However, all the parameters measured conform to International standards. While the northern geographical areas comprising Owo and Akoko south west local government had honey with the best total phenolic content, total flavonoid content and FRAP, the honey from the region lacked 'C–H' functional group. Honey samples A, B and C, however, had four functional groups identified which are, O-H, C–H, C=C and C≡C. At temperatures below 20 ^{o}C, there were wide scattering or non-uniformity of the viscosity of all the honey samples which decreased until the temperature reached 60 ^{o}C. After 60 ^{o}C, the viscosity had uniform behavior. This study therefore concludes that Ondo state had honey rich nutritionally but with variables in their nutritional properties across geographical areas.

Declaration

The authors declare no conflict of interest.

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