

## REGULAR ARTICLE

## PHYSICOCHEMICAL, MICROBIOLOGICAL, ANTINUTRITIONAL AND PHYTOCHEMICAL CHARACTERIZATION OF HONEY PRODUCED AND CONSUMED AT FOUR DIFFERENT LOCATIONS

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## ABSTRACT

The floral source, physicochemical, microbiological, anti-nutritional and phytochemical characterization of four different honey samples obtained from different locations in Ondo State were determined. This was with the view of authenticating their nutritional makeup as was as determine their potential to pose health hazards amongst the consumers. Physicochemical analysis gave moisture ( $13.5 \pm 0.02$ - $15.0 \pm 0.03\%$ ), Ash ( $0.5 \pm 0.02\%$ - $1.5 \pm 0.01\%$ ), pH ( $4.33 \pm 0.03$ - $5.28 \pm 0.02$ ) Titratable acidity ( $0.19 \pm 0.01$ - $0.47 \pm 0.02\%$ ), total sugar ( $0.21 \pm 0.01$ - $0.03 \pm 0.03\%$ ), dextrose ( $0.21 \pm 0.01$ - $0.29 \pm 0.02\%$ ), fructose ( $0.22 \pm 0.03$ - $0.31 \pm 0.01\%$ ), hydrated lactose ( $0.28 \pm 0.1$ - $0.04 \pm 0.02\%$ ), hydrated maltose ( $0.34 \pm 0.03$ - $0.48 \pm 0.01\%$ ), anhydrous lactose ( $0.27 \pm 0.01$ - $0.48 \pm 0.01\%$ ), electrical conductivity ( $0.39 \times 10^2 \pm 0.02$ - $5.21 \times 10^2 \pm 0.02$ ms/cm). Total phenol content from (1.45 to 1.66 mg GAE/100g), Total flavonoid content from (0.04-0.07mg QE/100g) and not detected in samples EF3 and GH4. Alkaloid content ranged from (0.06-0.24%), Phytate content ranged from (2.06-3.91mg/100g), Tannin content ranged from (1.37-1.76 mg/100g), Oxalate content ranged from (0.18-0.27mg/100g), Saponin content ranged from (0.06-0.225) and the Ferric Reducing Antioxidant Power ranged from (0.04-0.05mg/100g) and not detected in sample CD2 and GH4. Also, the microbial analysis results revealed that the honey samples had bacterial count between  $2.0 \times 10^3$ -  $9.0 \times 10^3$  CFU/ml, the total yeast/mold count had a count of  $1.0 \times 10^3$ ,  $3.0 \times 10^3$ ,  $10.0 \times 10^3$  and  $5.0 \times 10^3$  CFU/ml for the four samples respectively. The coliform count indicates the absence of coliform bacteria in all the samples. Isolates from the honey samples were seven different genera of bacteria (*Streptococcus*, *Enterococcus*, *Pseudomonas*, *Bacillus*, *Klebsiella*, *Micrococcus* and a trace of *Clostridium* spp) which could be hazardous to health if not properly handled.

**Keywords:** Honey characterization, phytochemicals, floral source, microbiological, anti-nutritional factors

## INTRODUCTION

Honey is used in the food industry due to its nutritive, therapeutic, and dietetic quality. Honey is the sweetest natural product, obtained by processing flowers nectar or plants manna (Marghitas, 2008). Honey is defined by the Codex Alimentarius Commission as the natural sweet substance produced by honeybees from the nectar of blossoms or from the secretion of living parts of plants or excretion of plant-sucking insects living on the plants, which honeybees collect (Anonn, 2003). In main features depending on the floral origin or the nectar foraged by bees. The composition and quality of honey also depend on several environmental factors. Bacteria spores and less often *Clostridium* spp. may be present, but honey possesses antibacterial activities that do not support bacterial growth and/or production (Anonn, 2004). However, fungi have been reported to grow and ferment or spoil unprocessed honey (Anonn, 2003). Therefore, the microbiological quality of honey may serve as an indicator of the hygienic conditions under which the product was processed, handled, and stored (Ramirez, 2000).

Honey is a very concentrated sugar solution with a high osmotic pressure, making impossible the growth of any micro-organisms. It contains less micro-organism. It contains fewer micro-organisms than other natural food, especially there no dangerous *Bacillus* species. *Bacillus* bacteria, causing the dangerous bee past, but these are not toxic for humans. That is why to prevent bee pests, honey should not be disposed in open places, where it can easily be accessed by bees. However, several bacteria are present in honey, most of them being harmless to man. Recent extensive reviews covered the main aspect of honey microbiology and the possible risks. To establish the standards that ensure honey quality, microbiological properties must be taken into consideration. A large interest was shown in the last few years for the composition of Romanian honey and its prospective value on the market regarding the U.E standards (Bratu et al., 2001). The presence of *C. botulinum* spores in honey was reported for the first time in 1976. Since then, there were many studies in honey all over the world. In some of them no botulinum was found, in others, few kinds of honey were found to contain the spores. Honey does not contain the botulinum toxin, but the spores can theoretically build the toxin after digestion (Floris 2006). The presence of yeast in honey is due to its introduction in the hive by the bees. In a case where the quality of the honey is not affected, it comes from the extraction and manipulation spaces, from the machines etc. and it is dangerous because of its quantity and acidophilus.

Due to its chemical composition, the honey favors the development of osmophilic yeast that produces the fermentation, the decrepit aspect, and the crystallization of the honey (Clemansa, 2004). Also, honey has been incriminated as a source of spores of *Clostridium botulinum* responsible for causing the infant botulism. A low percentage of the contamination with *B. cereus* and fungi has been proved: yeast, *Mucor* spp., *Penicillium* spp. and other species from the *Aspergillus* type *A. candidus* (Martins, 2003). The microbiological contamination during and after the processing of the honey has been demonstrated through the absence of micro-organisms from the collected samples from the primary sources and the sources and the presence of a type of bacterium (*Bacillus* spp.) and types of fungi (most frequently *Candida*, *Aspergillus*, *Geotrichum* and *Rhizopus*) can be collected from samples from local markets. This indicates the contamination from secondary sources while further manipulation and processing. The contamination with fungi and bacteria indicates improper hygiene conditions during the collection, manipulation, processing, and storage (Tchoumboue et al., 2003). The aim of the study is to characterize and describe the floral origin of honey obtained from four locations in Ondo state Nigeria using their physicochemical, microbiological, anti-nutritional and phytochemical compositions. This was with a view of authenticating their nutritional makeup as was as their potential for health hazards amongst the consumers at those locations and beyond.

## MATERIALS AND METHODS

## Collection of Samples

The sample (honey) were bought from four different locations in Ondo State, from Akure (mainly for industrial use), from Owo (Honey from roadside), Oyinmitemi (which is a consumer-ready product) and lastly, Ile-Oluji (gotten from beekeepers). The samples were kept in a dry clean place, safe from dirt and rodent infestation.

## Floral Source Determination

This was determined by using the method of Erdtman (1986). 10 g of the honey sample was dissolved in 10 ml of distilled water, the mixture was concentrated by centrifuging at 1500 rpm for 30 minutes. 10ml of acetocycin (9.1) acetic acid (1 ml) and  $H_2SO_4$  (9 ml) was added and it was placed in a water bath at  $100^\circ C$  for 30minutes. Then it was centrifuged and decanted again the sediment or precipitate was washed with 12 ml of distilled water and 12 ml of 7% KOH was added and stirred thoroughly and decanted. Finally, the sediment was stunk with

basic fuchsin solution and on a glass slide and it was checked under the microscope.

- Predominant and pollen (p, more than 45% of pollen grain counted)
- Secondary pollen (S, 16-45% pollen grain)
- Important minor (L, 3-16% pollen grain)

#### Physicochemical Characterization of Honey

Physicochemical parameters were determined using the standard method of AOAC (1990).

##### Determination of Moisture Content

Petri-dishes were washed and dried in an oven at 105 °C for 1 hour; the petri dish was removed and cooled in the desiccator. 2 g of the honey sample was accurately weighed into the Petri-dish and it was spread properly, and the petri-dish was placed in an oven, and it was heated at 105 °C for 3 hours, they are cooled in the desiccator and weighed.

##### Ash Content Determination

Ashing crucible was washed and heated in the muffle furnace for 15 minutes and cooled in a desiccator. The crucible was weighed, and 2 g of the honey sample was weighed into the crucible was then heated in the muffle furnace for 3 hours at 550 °C until a trace of carbon could be seen.

##### Determination of pH

pH meter was used to determine the pH of honey, at a room temperature, the electrode of the pH meter was dripped into a beaker containing 100 ml of buffer solution (pH 9) in order to calibrate the instrument, after calibration, it was turned on the electrode was immersed in the honey for a period and the reading was taken.

##### Determination of Reducing Sugar

10 ml of the mixed freshly solution was pipetted into a 250 cm<sup>3</sup> conical flask, the solution was boiled on asbestos covered gauze with a Bunsen burner, and 1ml of the sugar solution was added to the boiling liquid at an interval of 100 of seconds until the blue color was nearly discharged. The 4 drops of aqueous methylene blue solution (1%) was added and titration continued until the indicator became completely decolorized. The titration was repeated adding before boiling almost all the sugar solution, required to effect reduction of the topper, the solution was boiled gently for 2 minutes, and 4 drops of methylene blue indicator was added and titration completely within a total boiling time of 3 minutes. At the point, the entire blue color gets discharged and the liquid turned the orange-red volume of the sugar solution used was recorded as the liter value and their factors obtain from invert sugar table.

##### Determination of Non – Reducing Sugar

7 ml of concentrated hydrochloric acid was added to 100 ml of sugar, the mixed sample contained in the flask was immersed in a bath water at 60 °C FOR 12 minutes, rotating the flask for three minutes. The flask was removed at exactly 12 minutes, and it was immersed in cold water for 15 minutes the content of the flask was neutralized with phenolphthalein with 5N sodium hydroxide.

##### Determination of Electrical Conductivity

The electrical conductivity was turned on and the probe was calibrated using a standard solution of known conductivity. The honey sample was collected in a glass container. Enough of the honey sample was collected so that the probe tip can be submerged into the sample, the probe was rinsed with deionized water and blot dry. The probe was submerged into the sample until the electrical conductivity on the water stabilizes the electrical reading is stable.

#### Phytochemical and anti-nutritional content determination

##### Extraction of phenolic

For the extraction of phenolics from the honey samples, a modified method (Yao et al., 2004) using column chromatography was applied. A 100 g honey sample was totally dissolved in 500 ml acidified distilled water using a magnetic stirrer the pH value was adjusted with HCl to pH to 2.0 at laboratory temperature. The solution obtained was filtered through a lump of cotton wool in a funnel to remove the solid particles. The filtrate was mixed with 150 g of Amberlite XAD 2 (pore size 9 nm, particle size 0.3-1.2 mm) and stirred for 10 min with a magnetic stirrer. This mixture was transferred into a glass column and eluted with 250 ml of acidified distilled water (pH 2.0 adjusted with HCl) followed by 300 ml of distilled water for removing all saccharides. Phenolics absorbed on the solid phase were eluted with 400 ml methanol and the methanolic extract was subsequently evaporated to dryness in a rotary vacuum evaporator at 40 °C. The solid was dissolved in 5 ml of distilled water and extracted three times with 5 ml of diethyl ether, subsequently, the diethyl etheric extracts were combined, dried

with anhydrous sodium sulphate and diethyl ether was removed using a nitrogen flow. The dry extract obtained was stored in a refrigerator (4 °C for the analyses).

##### Total phenol content determination

Total phenol content (TP) of the samples was determined according to according to (Lachman et al., 2006). Dry extract of phenolics was dissolved in 5 ml of methanol; 0.5 ml of the sample solution was pipetted into a 10 ml volumetric flask and diluted with distilled water. Subsequently, 0.5 ml Folin ciocalteau reagent was added to the solution and after stirring 1.2 ml 20% sodium carbonate solution was added. After refilling with distilled water to mark and through agitation the reaction mixture was left standing for 20min and was measured on the spectrophotometer at wavelength 725 nm against the black. Total phenol was expressed as mg gallic acid equivalent in 100 g of honey (mg GAE 100g).

##### Total flavonoid content determination

This was determined according to the method described by Spilkova et al., (1996). Dry extract of phenolics was dissolved in 5ml of methanol; 1ml of the sample solution was pipetted into a 10 ml volumetric flask and diluted with distilled water. Subsequently, 1.2 ml of 0.2 mol/H<sub>2</sub>SO<sub>4</sub>, 1.2ml of 3 mol/NaNO<sub>2</sub> and 1.2 ml of 10% NaOH were added to the solution. After refilling with distilled water to mark and through agitation the reaction mixture was left standing for 15min and was measured on the spectrophotometer at wavelength 395nm against the black. Total phenol was expressed as mg quercetin equivalent in 100g of honey (mgQE/100g).

##### Alkaloids content determination

Alkaloids of the samples were determined according to the method of Harborne (1973). 5g of each sample was weighed into a separate 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hrs. This was then filtered, and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was completed. The whole solution was allowed to settle, and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. Residual alkaloid was dried and weighted.

##### Phytate content determination

4 g of the samples were soaked in 100 cm<sup>3</sup> 2% HCl for 3 hours and then filtered. 25 cm<sup>3</sup> of the filtrate was placed in a 100 cm<sup>3</sup> conical flask and 5 cm<sup>3</sup> of 0.03% NH<sub>4</sub>SCN solution was added as an indicator. 50 cm<sup>3</sup> distilled water was then added to give it the proper acidity. This was titrated with ferric chloride solution which contained about 0.05 mg of Fe per cm<sup>3</sup> of FeCl<sub>3</sub> used, the equivalent was obtained and from this, the phytate content in mg/100 g was estimated.

##### Tannin content determination

Method of Makkar and Goodchild (1996) was adopted. 200 mg of the sample was weighed into a 5 ml sample bottle. 10 ml of 70% aqueous acetone was added and properly covered. The bottle was put in an ice bath shaker and shaken for 2 hours at 30 °C. Each solution was then centrifuged, and the supernatant stored in ice. 0.2 ml of each solution was pipetted into test tubes and 0.8 ml of distilled water was added. A standard tannic acid solution was prepared from a 0.5 mg/ml stock and the solution made up to 1ml with distilled water. 0.5 ml Folin reagent was added to both sample and standard followed by 2.5 ml of 20% Na<sub>2</sub>CO<sub>3</sub>, the solutions were then vortexed and allowed to incubate for 40 minutes at room temperature after which absorbance was read at 725 nm against a reagent blank concentration of the samples from a standard tannic acid curve.

##### Oxalate content determination

Oxalate was determined according to the method of Day and Underwood (1986) as taken 1 g of the sample into a 100 ml conical flask of 4.5ml and 1.5N H<sub>2</sub>SO<sub>4</sub> was added. The solution was carefully stirred intermittently with a magnetic stirrer for about 1 hour and then filtered using Whatman No. 1 filter paper. 25 ml of sample filtrate was collected and titrated hot (80 °C– 90 °C) against the 0.1N KMnO<sub>4</sub> solution to the point when a faint pink color appeared that persisted for at least 30 seconds.

##### Saponin content determination

Obadoni and Ochuko (2001) method were used to estimate the saponin value. 5g of the sample was measured into a conical flask and 100 cm<sup>3</sup> of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55 °C. The mixture was then filtered, and the residue re-extracted with another 200 ml 20% ethanol. The combined extract was reduced to 40 ml over the water bath at about 90 °C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the other layer

was discarded. The purification process was repeated, 60 ml of n-butanol was added, and the combine n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath and after evaporation, the samples were dried in the oven to a constant weight and the saponin content was calculated in percentage.

**Ferric Reducing Antioxidant Power (FRAP) content determination**

1 ml of each dilution of phenolic extract were dispensed into different test tubes and 2.5 ml phosphate buffer was added followed by 2.5 ml FeCN solution and incubated at 50 °C for 20 minutes. 2.5 ml trichloroacetic acid (TCA) solution was added to stop the reaction. The reaction mixture was separated into 2.5 ml, and each was diluted with 2.5 ml of distilled water. 0.5 ml of ferric chloride (FeCl<sub>3</sub>) solution was added into each tube and allowed to stand in the dark for 30 minutes for color development and the absorbance was read at 700 nm against a reagent blank.

**Pollen Analysis- Determination of the floral Source of the Honey Samples**

The samples were subjected to qualitative pollen analysis as per Erdtman's acetolysis method (Erdtman, 1986). 10 ml of each of the samples were dissolved in distilled water and the sediment was concentrated by repeated centrifuging for 30 minutes at 1500 rpm. About 10 ml of acetolysis mixture (9:1 C<sub>2</sub>H<sub>5</sub>O<sub>2</sub>: H<sub>2</sub>SO<sub>4</sub>) was added and the tubes were incubated in a water bath (100 °C for 30 mins). It was stirred vigorously and centrifuged and then decanted. About 12 ml of water-free acetic acid was added. Stirred thoroughly, centrifuged, and decanted. The precipitate was washed in about 12 ml of distilled water, Centrifuged and decanted. About 12 ml of 7% KOH was added, stirred thoroughly centrifuged and decanted. Finally, the pollen grains were stained with a solution of Basic Fuchsin and mixed with glycerin. The examination of the pollen slides was carried out with an optical microscope at 400X and 1000X to count the pollen grains.

**Microbiological characterization**

*Preparation of Culture Media*

The media employed in this project work includes nutrients agar, MacConkey Agar, and Potato Dextrose Agar. Appropriate gram measurement of the medium to be used were obtained and dissolved in the appropriate volume of distilled water. All culture media was autoclaved at 121°C for 15 minutes.

*Preparation of Serial Dilution*

A series of test tube each containing 9 ml of sterile distilled water was set up, then a sterile pipette was used to transfer 1ml from the stock sample, then it was introduced to the first test tube labelled 10<sup>-2</sup> from the first test tube and mixed, the same procedure was repeated fill the last test tube (Cheesbrough et al., 2000).

*Culturing*

The pour plate method was used in culturing bacteria of the honey samples. 1 ml of dilution from the test tube labelled 10<sup>-3</sup> was pipette into a sterile petri dish labelled accordingly. 15 ml-20 ml of the already prepared agar was poured into each sample dilution. It was allowed to solidify. The plate was incubated at an inverted position at 37 °C for 24-48 hours.

*Isolation of Micro-organisms*

After the culture for total plate count has been incubated for 24 hours, the pour plate was examined for the growth of isolated colonies strains of the organism was transferred from growth substrate into another plate using streak method to obtain pure culture of each colony, which is the population of cell from single

**Table 1** Physicochemical properties of the honey sample

Sample	Physicochemical Properties										
	Moisture %	Ash (%)	pH	Titrate acidity (%)	Total Sugar (%)	Dextrose	Fructose (%)	Hydrate Lactose (%)	Hydrate Maltose (%)	Anhydrous Lactose (%)	Electrical Conductivity (ms/cm)
MN2	13.5±0.02	1.00±0.02	4.55±0.03	0.47±0.02	0.21±0.01	0.21±0.01	0.22±0.03	0.28±0.01	0.34±0.03	0.27±0.01	3.95X 10 <sup>2</sup> ±0.01
OP3	15.0±0.03	0.5±0.02	4.33±0.03	0.32±0.02	0.30±0.03	0.29±0.02	0.31±0.01	0.40±0.01	0.48±0.01	0.38±0.01	5.21X10 <sup>2</sup> ±0.02
QR4	13.5±0.02	0.5±0.02	4.58±0.03	0.19±0.01	0.28±0.03	0.27±0.03	0.29±0.02	0.38±0.03	0.45±0.03	0.48±0.01	2.64x10 <sup>2</sup> ±0.03
ST5	13.5±0.02	1.5±0.01	5.28±0.02	0.26±0.01	0.30±0.01	0.29±0.03	0.31±0.02	0.40±0.02	0.36±0.01	0.38±0.01	0.39X10 <sup>2</sup> ±0.02

Note: Results are expressed as mean values+standard deviation

The pH values of honey are of great importance during extraction and storage, since acidity can influence the texture, stability, and shelf life of honey. All kinds of honey are acidic, the pH of the honey sample range from 4.33± 0.03 to 5.28± 0.02 honey sample collected from ST5 has the highest value compared to honey collected from Akure 1 honey which has the lowest value. Sample OP3 and QR4 have the same mean value 0.02 which mean they are not significantly different.

colony, and it was later isolated and kept on agar slant with the aid of a sterile loop (Ogbulie et al., 1998; Fawole et al., 2001).

*Total Viable Count*

The total yeast count of the honey samples was done with a potato dextrose agar using the pour plate. The plates were incubated at 37 °C. All visible colonies appearing at the end of the incubation period were counted.

*Total Coliform Count*

Multiple tubes of MacConkey broth were incubated with different volume of the honey sample (10 ml, 1.0 ml and 0.1 ml) respectively. The number of coliforms in 100 ml can be computed based on the various combinations of positive and negative results. Standard tables for computing these values were used to determine the number of coliforms per 100 ml of the honey sample.

**Biochemical Test -Identification and Characterization of the Isolates**

The pure cultures of the isolates were identified using biochemical tests, as described by Cheesbrough (2003).

*Catalase Test*

A smear of the test organism was made on a slide and 3 drops of 3% hydrogen peroxide solution was added to release oxygen bubbles as effervescence which indicates a positive reaction which signifies the production of catalase enzymes which break down the peroxide to release oxygen.

*Coagulase Test*

Three separate loops of saline were placed on a clean slide and a loopful of test colony was suspended in two of these and a loopful of control organism in the third one. A drop of citrated rabbit plasma was added to one test with a sterile loop and the controlled suspension clumped occur in some which indicate a positive result (Ogbulie et al., 1998).

*Motility Test*

This test was carried out using young culture (24 hours old) of the bacterial isolates which were placed on a coverslip of petroleum jelly. The cover slide was then inverted and placed on the Vaseline container.

**RESULTS AND DISCUSSION**

Summarize the results obtained from the physicochemical analysis of the honey sample is presented in Table 1.

The amount of moisture content in the honey is an important factor in determining the quality of honey in the world honey trade. It is also an important factor in determining its storage quality. Moisture content varied from 13.5±0.02% to 15.0± 0.03%. Honey sample OP3 has the highest value, honey collected from Akure II honey (MN2), Owo (QR4) and Ile-Oluji (ST5) have the same value of 13.5 ± 0.02%. This is an indicator of premature extraction of honey which was obtained by Bogdanov, (1999). The ash content varied from 0.5± 0.02% to 1.5± 0.01%, the highest ash value was observed and lowest was observed in the sample collected from (OP3) and Owo (QR4), the mean value along four samples. It was observed in this analysis that light colors honeys usually have low ash content compared to dark color which usually has higher ash content. This also corroborates the same results reported by Bodganor (1999).

The acidity of honey is due to the presence of organic acid and inorganic ion. Honey, in general, is acidic in nature irrespective of its variable geographical origin. The titratable acidity of honey samples was observed in the study 0.19±0.01% to 0.47±0.02%, it was observed in the study that the honey samples collected from different locations are varied in value. The honey sample was collected from Akure II honey (MN2) has the highest value and sample at QR4

has the lowest value. The variation of this factor is the source of nectar and climatic condition of this is related to the result of **Asit et al. (2002)**.

Total sugar is the main constituent of honey, the sugar spectrum of honey depends on the sugar present in nectar and enzyme present in bees. Sample OP3 and ST5 have the highest value to be 0.30±0.03% while sample MN2 has the lowest value to be 0.21±0.01%. Dextrose ranges from 0.21±0.01% to 0.29±0.02%. 0.29±0.02% is the highest value and it was the sample. ST5 and sample OP3 and ST5 have the same value of 0.29±0.02%. The fructose varied in value except OP3 and ST5 with the same value 0.31±0.01% which is the highest value while 0.22±0.03% is the lowest value within the range of 0.22±0.03%-0.31±0.01%. The hydrated lactose ranges from 0.28±0.01% to 0.40±0.02% and it was obtained from (MN2). Among the value discussed above, it was observed

**Table 2** Phytochemicals and antinutritional composition of the honey samples

Sample	Phytochemicals and antinutritional							
	Total Phenol (mgGAE/100g)	Total Flavonoid (mgQE/100g)	Alkaloid (%)	Phytate (mg/100g)	Tannin (mg/100g)	Oxalate (mg/100g)	Saponin (mg/100g)	FRAP (mg/100g)
MN2	1.57±0.07	0.04±0.01	0.06±0.01	3.71±0.58	1.76±0.07	0.18±0.01	0.13±0.03	0.04±0.01
OP3	1.57±0.07	0.07±0.01	0.13±0.01	3.91±0.29	1.43±0.07	0.23±0.06	0.22±0.02	ND
QR4	1.45±0.04	ND	0.24±0.01	3.71±0.58	1.37±0.01	0.18±0.01	0.19±0.01	0.05±0.01
ST5	1.66±0.04	ND	0.13±0.01	2.06±0.58	1.58±0.07	0.27±0.01	0.06±0.01	ND

ND-Not Detected, GAE-Gallic Acid Equivalent, QE-Quercetin Equivalent. Note: Results are expressed as mean values+standard deviation.

Total phenol content ranged from 1.45 mg GAE/100 g to 1.66 mg GAE/100 g. This range is like those obtained from the same type- monofloral honey reported by **Beretta et al., (2005)**. Total flavonoid content is 0.04 mg QE/100g to 0.07 mg QE/100 g and was not detected in QRT4 and ST5. **Makawi et al., (2009)** opined that flavonoid has potential to impound metal ions by forming complexes thus preventing the formation of free radicals. Alkaloid ranged between 0.06 to 0.24, phytate content ranged from 2.06 mg/100 g, tannin content ranged from 1.37 mg/100 g to 1.76 mg/100 g, oxalate content ranged from 0.18 mg/100g to 0.27 mg/100g, saponin ranged from 0.06 to 0.22 and Ferric Reducing Antioxidant Power (FRAP) are 0.04 mg/100g to 0.05 mg/100 g and was not detected in MN2 and ST5. This shows OP3 has both adequate phytochemicals and antinutritional content. Pollen grain counts, kinds of honey are always classified as unifloral, monofloral and multi floral depending on poly frequency. Unifloral kinds of honey were considered those with predominant pollen type over 45%. In this study, the pollen grain count of ST5 was classified as monofloral since it has the value of 1057 and it is extra light Amber in Color. MN2 honey has the value of 1548; OP3 has 1640 and as well has amber color and same with MN2. QR4 has the value of 1221 and it was light amber in color, therefore, the pollen grain count range from 1057 to 1640. Generally, multi-floral honey has mixed flavor and aroma compared to unifloral honey which has unique flavor and aroma. Table 3 shows the result of the floral sources, samples have a pollen count of 1548, 1640, 1221 and 1057 respectively. According to Conti, 2000, the bee producing unifloral honey is said to collect its nectar from a single type of flower. On the contrary, polyfloral honey is made from any nectar sources available to bees.

**Table 4** Cultural characteristics and biological reactions of selected honey samples

Sample	Morphological Characteristics	M.T	G.S	Catalase					Coagulase					Probable Organisms	
				A	B	C	D	E	A	B	C	D	E		
MN2	Pinkish strand, opaque cocci colonies	-	+	-	+					+	+				<i>Streptococcus</i> spp. <i>Enterococcus</i> spp.
OP3	Smooth, creamy translucent	+	+	-	+	+	-	+	+	+	+	-	+	+	<i>Bacillus</i> spp.
QR4	Pink, smooth and slightly convex	-	+	-	+	+	+	+	+	+	+	+	+	+	<i>Streptococcus</i> spp. <i>Klebsiella</i> spp.
ST5	Smooth, creamy opaque colonies	+	-	-	+	+	+	+	+	+	+	+	+	+	<i>Pseudomonas</i> spp. <i>Micrococcus</i> spp. <i>Clostridium</i> spp.

MT = Motility test, GS = Gram Staining, A = Isolate from each plate, B= Isolate from each plate, C= Isolate from each plate D= Isolate from each plate, E= Isolate from each plate.

**CONCLUSION**

The comparison of these honey varieties with published International Standard allowed us to have an idea of the quality of honey produced and consumed in some important locations of Ondo state. The floral source obtained was mostly unifloral. The number of aerobic, mesophilic, and thermophilic bacteria, mold and yeast identified from these different locations were of values greater than the published standard yeast and mold load of 10<sup>3</sup> CFU/ml. Although no standard microbiological loads exist for honey, the results obtained give the degree of wholesomeness and safe quality of the honey samples. By implication these

that the highest value of hydrated lactose was obtained from sample OP3 and ST5 and the lowest dextrose was obtained from sample MN2 from a range of 0.21±0.01%-0.40±0.02%. hydrated maltose and Anhydrous lactose of OP3 and QR4 range from 0.27±0.01% to 0.48±0.01% respectively with MN2 0.27±0.01% having the lowest value. The hydrate lactose ranges from 0.34±0.03% to 0.48±0.01, anhydrous range from 0.27±0.01% to 0.48±0.01%. Electrical Conductivity of OP3 has the highest electrical conductivity 5.21x10<sup>2</sup> ± 0.02ms/cm, ST5 has the lowest value 0.39x10<sup>2</sup>. The values of the electrical conductivity depend on the mineral organic acid, polyol content and vary with botanical origin.

Table 2 summarizes the outcome of the phytochemicals and antinutritional screening of the honey samples.

Also, the color of the honey sample gives an indication of the number of pollen grains contained in the honey samples. Samples QRT4, OP3 and MN2 have amber, light amber respectively, while ST5 has extra light amber color.

The microbiological assessment of the honey samples was determined. Table 3 showed the total viable count of the samples where aerobic, mesophilic, and thermophilic organisms (bacteria) were counted. Samples QRT4, MN2 and ST5 have between 7.0 x 10<sup>3</sup> CFU/ml, while sample OP3 has the least bacterial count of 2.0 x 10<sup>3</sup> CFU/ml. According to the study, in Table 3, honey sample MN2 contained the highest yeast count of 10.0 x 10<sup>3</sup> CFU/ml. QRT4 has total yeast count of 3.0 x 10<sup>3</sup> CFU/ml, and ST5 has 5.0 x 10<sup>3</sup> CFU/ml count, OP3 has the least count of 1.0 x 10<sup>3</sup> CFU/ml. The bacteria and yeast count indicate improper hygiene conditions during collection and storage, served as the source of contamination and the nutritional composition of the sample which is mainly sugar in which yeast and mould can easily grow. Table 4 showed the characteristics and the biochemical reaction of the honey samples. Probable bacteria isolated from the samples were *Streptococcus* spp., *Bacillus* spp., *Clostridium* spp. of the gram-positive and *Klebsiella* spp., *Pseudomonas* spp., *Enterobacter* of the family *Enterobacteriaceae* of the gram-negative. The presence of *Streptococcus* spp. and *Bacillus* spp. in these samples could lead to infection of the lung, meningitis, and infection of the lower part of the abdomen, as they survive in honey at low temperatures. These submissions have also been reported in the previous work of **Olaitan et al. (2007)**.

samples must be handled with utmost care for them not to pose any serious potential health hazard especially during sales and handling prior to consumption.

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