Physicochemical, microbiological and sensory properties of honey from Yewaland, South-West Nigeria

ABSTRACT

Consumers are concerned about the authenticity and general quality of honey in Yewaland, Nigeria. Physicochemical (moisture, protein, ash, fructose, sucrose contents; total titratable acidity, total soluble solids, specific gravity, viscosity, electrical conductivity, refractive index and pH), microbial tests (total plate and identification), and sensory properties of honey samples were evaluated. Samples were collected from bee breeders in the different areas of the region. The results ranged: moisture (13.6-15.0%), protein (0.2-5.2%), ash (0.24-0.38%), fructose 40.5-45.6%), sucrose (3.0-5.8%), specific gravity (1.40-1.47), viscosity (1607-2037), electrical conductivity (6.3-14.9 μS/cm), total soluble solids (40.3-75.5mg/l), pH (3.60-4.25), titratable acidity (0.39-0.46) and refractive index (1.34-1.35). *Staphylococcus, shigella,* and Bacillus *spp* were identified in some samples. The appearance of the samples was lighter. Samples from Oja-Odan and Iwoye had the best overall acceptance scores of 8.7 and 8.5 respectively. Thus honey samples were of good quality compared to the international standard and their acidic pH values reveal that they are unadulterated and have good shelf stability potentials. Their characterization will make it possible to obtain Nigerian quality labels.

Keywords: Acidity, honey, physicochemical properties, sensory quality

Comment [MF1]: The results ranged from 13.6-15.05 moisture, 0.2-5.2% protein, 0.24-0.38% ash…. in that order till you get to the last parameter 1.34-1.35 refractive index

1. INTRODUCTION

Honey is a popular natural product that is consumed for its sweetness and ability to confer health benefits to humans [8] after prolonged ingestion. Besides these qualities, honey has sufficient antimicrobial and antioxidant potentials [8, 10]. However, consumers of honey are always concerned about the authenticity and overall quality of the product. Generally, some chemical tests are among the techniques of authenticating the quality of honey, geographical and floral origins [10]. The quality and composition of honey vary, and these depend primarily on the source of the nectar and locations from which it originates, the climatic conditions and beekeeping practices in removing and extracting honey [3]. The characterization of natural honeys could enhance the understanding of their properties and potential applications, notably in foods and medicine. Although, there are a large volume of data on the characterization of honey in literatures, nutritional, health, and clinical databases but information is insufficient in Nigeria.

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Honey is reportedly to contain large array of substances, notably are carbohydrates and water, aside from minor constituents including minerals, proteins, free amino acids, enzymes, vitamins, and other phytochemicals [3]. In the past, honey was harvested by local farmers from the bush as an art, but nowadays apiculture is a vast growing sector as a small-and-medium enterprise among the unemployed youth in Nigeria. The quality of honey is determined by its physicochemical, microbiological, and sensory properties. The physicochemical quality of honey is expressed in terms of moisture, electrical conductivity, ash, fructose and sucrose, acidity and diastase activity content [3].

Studies on the quality assessment of natural honey from some locations in Nigeria have been established [11, 13, 8, 10]. Ndife et al. [10] investigated the comparative quality of honey obtained from different floral in Nigeria. The authors found that there is variability in some quality characteristics of honey samples from different regions of Nigeria, and that those from the northern region had the best overall acceptability ratings. Oyeyemi [13] studied the quality assessment of honey sourced from natural and artificial apiaries in Ekiti State, Nigeria. He found that honey from artificial and natural apiaries is of good quality in terms of some important physicochemical parameters (pH, ash, glucose, fructose contents, glucose/fructose ratio) which were within the international standard limit. Durugbo et al. [8] characterized six Nigerian (northern and southern regions) honey samples for authenticity. The authors found that the honey samples were all multifloral and those from South-eastern Nigeria were dominated by rainforest species, while those from the savannah regions were dominated by savannah species. In addition, chemical analysis showed that the honey samples were of nearly good quality compared to the international standard. Ikegbunam et al. [10] characterized of honey samples from South-eastern Nigeria. The authors found that the pollen spectra of the honey samples showed the floral used by the bees for honey production, which indicates that the honeys were pure and unadulterated. The microorganisms in honey are possibly those that are resistant to high sugar concentration and low pH condition [12]. Thus, for both quality control and sensory assessments of honey from this locality, the characterizations of honey are needed.

Therefore, this study presents the results of our preliminary investigations of natural honey samples collected from different honey breeders in different locations in Yewaland, Southwest

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region of Nigeria. This investigation entails the physicochemical, microbiological, and sensory properties of the honey samples.

2. MATERIALS AND METHODS

2.1 Sample preparation

Natural honey samples used for this study were harvested from different locations of the bee breeders within the Yewaland, comprising Yewa-North (Oja-Odan, Ibese and Ibayun towns) and Yewa-South (Ilaro, Idogo and Iwoye towns). Yewaland is in the west of Ogun state, Nigeria border the Republic of Benin. All the samples collected were separately strained to remove unwanted materials like wax sticks, dead bees and remains of honey bees combs. Cleaned honey samples were then packaged in a sterile glass jar, labelled with 3-digit codes, place and date of collection, and kept at room temperature for analysis.

2.2 Analysis

2.2.1 Physicochemical tests

Physicochemical tests were conducted on honey samples collected from bee breeders. Samples were analysed using the methods proposed by Bogdanov [6]. The physicochemical properties determined were based on moisture, protein, ash, fructose and sucrose contents, specific gravity, viscosity, electrical conductivity, total soluble solids, total titratable acidity, refractive index and pH.

2.2.1.1 Moisture, protein, ash, fructose, and sucrose content determination

The determinations were carried out following standard procedures [2]. For the specific gravity, viscosity, electrical conductivity and refractive index: The specific gravity of the honey samples was obtained as the ratio of the weight of sample to that of equal volume of water. Viscosity was determined using a viscometer with a spindle 62 at 20 rpm. Results of the test were obtained directly in m.Pa units.

2.2.1.2 Electrical conductivity determination

The electrical conductivity at one-fifth was determined by using a conductometer [5]. The measurements were conducted at 25 °C in a 20% aqueous solution (dry matter of the honey). The value of conductivity (μS/cm) was directly determined by the cell in the solution after immersion.

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2.2.1.3 Total soluble solids and refractive index determination

The total sugar and water content of honey was determined using abbe refractometer. The refractive indices of the honey samples were measured at room temperature using an Abbe Refractometer. Two (2) drops of honey were dropped on the inner prism of the refractometer.

2.2.1.4 Total titratable acidity and pH determination

About 10 g of the honey was dissolved in 75 ml of CO_2-free water in a 250 ml beaker. Then, titrated with 0.1 M NaOH (pH 8.3). The readings were recorded to the nearest 0.2 ml when using 10 ml burette. The results were expressed as follows:

Acidity (mill moles acid/kg honey) = $(C_{NaOH} \times V_{NaOH})/M$.

Where C = concentration of NaOH (mill moles/ml); V = volume of NaOH (ml); M = mass of honey (kg).

Exactly 10 g of honey was dissolved in 75 ml of CO_2-free water in a 250 ml beaker and stirrer to achieve a homogeneous mixture. The pH electrodes were immersed in the solution and the pH was read.

2.2.2 Microbial tests

The determination of microbial contamination (bacteria) in the honeys was performed by the method described by Adadi and Obeng [1].

2.2.2.1 Media and sample preparations

The media (sterilized at 121 °C, 15 min, cooled to 45 °C) used in the study included MacConkey agar, nutrient agar, *Salmonella*, and *Shigella* agar. The media is poured into sterile petri dishes to solidify. Serial dilution (10 ml of honey in 90 ml of sterile 0.1% Peptone water) of the honey samples was carried in the non-turbulence flow hood.

2.2.2.2 Inoculation and incubation

The processes of inoculation and incubation were conducted as described by Adadi and Obeng [1]. Exactly 1 ml each of 10^{-4} and 10^{-5} dilutions was inoculated on solidified nutrient agar for total plate count. The inoculated plates were inverted and incubated (37 °C for 24 h). After 24 h of incubation, plates with countable colonies were removed and counted (cfu/ml) using the colony counter.

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2.2.2.3 Isolation, identification, and confirmation of bacteria

Colonies selected in randomized pattern from sampled nutrient agar plates were streaked on fresh nutrient agar plates and were then incubated (37°C, 24 h). On the solidified McConkey agar (MA), 1 ml each of 10^{-4} and 10^{-5} dilutions was also inoculated. Then, they were incubated (37 °C, 48 h). Unique colonies were selected and streaked on fresh MA plates to obtain pure cultures. Morphological characteristics and biochemical tests were conducted on species of pure cultures. Exactly 1 ml each of 10^{-4} and 10^{-5} dilutions was again inoculated on salmonella and shigella agar and incubated (37 °C, 48 h). Similarly, unique colonies were selected and streaked on fresh *salmonella shigella* agar plates to obtain pure culture. Morphological characteristics and biochemical tests were used to identify and confirm the pure cultures.

2.2.2.4 Gram staining and biochemical tests

Gram staining and all biochemical tests were performed according to the procedures described by Adadi and Obeng [1].

2.2.3 Sensory evaluation test

Sensory evaluation of the honey samples was carried out using volunteers (n=45) comprising 27 males and 18 females (aged between 25 and 46 years) from the polytechnic community. The evaluation was based on a 9-point hedonic scale (1= dislike extremely to 9= like extremely) for different parameters including colour, aroma, taste, flavour, appearance, after taste and overall acceptability.

2.2.4 Statistical analysis

Data are presented as the mean and standard deviation of replicate determinations. The data from the experiment were statistically analyzed using the analysis of variance (ANOVA) and the Duncan Multiple range test was used to test the significance level at $p<0.05$. The statistical package for social sciences version 23.0 for windows (SPSS Inc., USA) was the software employed to analyse the data.

3. RESULTS AND DISCUSSION

3.1 Nutritional composition of honey samples

The nutritional composition of the honey samples under investigation is presented in Table 1 ~~below~~. While the moisture, ash, and fructose contents of the samples were not significantly ($p>0.05$) different, the protein and sucrose contents differed significantly ($p<0.05$) with sample

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location. Moisture contents of the honey samples analysed varied from 13.7 to 14.6% in Yewa-North and 13.6 to 15.0% in Yewa-South. The moisture content of the samples was far below 21% specification recommended by the Codex Alimentarius Commission (2001) standard. The moisture content of honey is also one of the criteria that determines the shelf stability of natural honey [12]. This suggests that a higher moisture content honey may be prone to fermentation upon storage by osmo-tolerant yeasts [11]. In addition, high moisture content of honey could be an indicator of adulteration [4].

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The protein, ash contents, fructose and sucrose contents ranged from 0.25% to 0.38%, 0.24% to 0.38%, 40.5% to 45.6% and 3.0% to 5.8% respectively. Similarly, the Codex Alimentarius Commission Standard [7] recommended that the ash content of not greater than 0.6% for normal honey. However, the variability in the observed ash content could be explained by the floral origin, geographical location, and level of maturity of the honey [4]. All samples have met the standard limits of honey in terms of ash content.

The sugar content ranged from 3.0% to 5.8% for honey samples. The amount of sucrose is a very crucial parameter in evaluating the maturity of honey and is analysed with the aim of identifying any adulteration of honey [4]. High concentration may be an indication of adulterations, notably the addition of low-quality sweeteners like cane sugar or refined beet sugar. To guide against this adulteration, the Codex Alimentarius Committee [7] recommended 5 g of total sugar/100 g of floral honey. From the present study, the total sugar content obtained from Oja-Odan, Ilaro, and Iwoye exceeded the limits set by codex, which indicated adulteration in all the samples collected from these areas.

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Table 1. Nutritional composition of honey samples from different sampling locations

Sampling Location	Nutritional composition (%)				
	Moisture	**Protein**	**Ash**	**Fructose**	**Sucrose**
Yewa-North					
Oja-odan	13.7±0.1	5.2±0.1^{a}	0.32±0.13	44.2±1.3^{ab}	5.2±0.1^{a}
Ibese	14.6±0.3	4.7±0.2^{a}	0.37±0.01	44.9±1.5^{a}	4.7±0.3^{ab}
Ibayun	14.2±0.2	5.1±0.3^{a}	0.30±0.01	44.9±2.0^{a}	3.0±0.1^{c}
Yewa-South					
Ilaro	14.9±0.1	0.3±0.1^{b}	0.25±0.01	41.0±1.6^{b}	5.2±0.2^{a}
Iwoye	15.0±0.1	0.2±0.1^{b}	0.24±0.02	40.5±1.9^{b}	5.8±0.1^{a}
Idogo	13.6±0.3	0.2±0.1^{b}	0.38±0.03	45.6±1.2^{a}	3.9±0.1^{b}
p-value	0.423	**< 0.0001**	0.382	0.052	**0.0001**

Values are means with standard deviations of replicate determination (n=3). Means within the same column with different letter(s) differ significantly ($p<0.05$).

Figures in bold are highly significant

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3.2 Physicochemical properties of honey samples

The specific gravity and viscosity values of all honey samples were not significantly different ($p<0.05$). The specific gravity and viscosity ranged from 1.40 to 1.46 and 1607 to 2057, respectively. The electrical conductivity of honey samples is highly significant ($p<0.05$) between the two regions. The values obtained in the northern and southern parts of both regions ranged between 9.9-14.9 μS/cm and 6.3-14.0 μS/, respectively (Table 2). Electrical conductivity is associated with the concentration of mineral salts, organic acids, and proteins [3]. A probable explanation for the variability in the electrical conductivity of the honey samples could be attributed to the fluctuations in concentrations of mineral salts, organic acids, and proteins [3]. The values obtained in this study did not agree with the European directives standards (≤0.8mS/cm) for nectar honey but agreed with that for honeydew honey (≥0.8mS/cm) [9]. All the samples of honey analyzed have conductivities greater than 0.8mS/cm, thus making it difficult to classify them as honey obtained from nectar of the flowers.

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The pH values of the honey samples under investigation showed that all the samples were within the acidic range of pH 3.60 to 4.25 (Table 2). Generally, honey is adjudged as being acidic in

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nature despite its geographical origin [12]. The pH values were within the acceptable range specified by Codex Alimentarius [7]. In view of the results of pH obtained from this study, it confirms that the honey samples analysed are from plants visited by bees. The pH values obtained in this study agreed with the previous works of Batagarawa and Malumfashi [4], who reported a pH range of 3.95 to 5.12 for seven honeys from Katsina State, Nigeria. Several investigators have made the assumption that honeys are generally acidic, despite their geographical origin [4]. The pH values of honey are crucial during storage since the acidity can influence the texture, stability and shelf life of honey.

Comment [MF8]: indicate the values specified by Codex Alimentarius for clarity

Total soluble solid (TSS), titratable acidity (TTA) and refractive index (RI) values showed no significant differences ($p<0.05$) irrespective of sample location. The TSS, TTA and RI values ranged from 40.3 to 75.5 mg/l, 0.39 to 0.46 and 1.34 to 1.35 respectively.

Table 2: Physicochemical properties of honey samples from different locations in Yewaland

Sample Location	Physicochemical properties						
	Specific gravity	**Viscosity**	**Electrical conductivity**	**Total soluble solid (mg/l)**	**pH**	**Titratable acidity**	**Refractive index**
Yewa-North							
Oja-odan	1.46±0.01	1923±15^{ab}	14.9±1.2^{a}	75.5±2.3	3.70±0.01^{b}	0.41±0.01	1.34±0.01
Ibese	1.47±0.02	1811±12^{ab}	9.9±1.3^{b}	40.3±2.1	4.25±0.02^{a}	0.39±0.01	1.35±0.01
Ibayun	1.40±0.01	1802±11^{ab}	12.1±1.3^{ab}	73.9±3.7	3.95±0.03^{ab}	0.41±0.01	1.35±0.02
Yewa-South							
Ilaro	1.43±0.01	2037±16^{a}	12.4±1.2^{ab}	74.8±4.8	3.90±0.04^{ab}	0.39±0.01	1.35±0.02
Iwoye	1.43±0.02	1998±10^{a}	14.0±1.5^{a}	74.1±5.2	3.60±0.01^{b}	0.46±0.01	1.35±0.01
Idogo	1.41±0.02	1607±11^{b}	6.3±0.1^{c}	72.9±1.3	4.20±0.01^{a}	0.40±0.01	1.35±0.01
p-value	0.847	0.170	**0.003**	0.476	0.053	0.884	0.951

Values are means with standard deviations of replicate determination (n=3). Means within the same column with different letter(s) differ significantly ($p<0.05$).

Figures in bold are highly significant

3.3 The microbiological quality of honey samples

The microbial counts ranged from $0.2x10^2$ to $0.9x10^2$ cfu/ml for samples from Yewa-North comprising Oja-Odan, Ibese, and Ibayun. Only the sample from Ilaro in Yewa-South had bacteria growth with microbial counts of $0.1x10^2$ cfu/ml. However, samples taken from both

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Iwoye and Idogo locations showed no growth and so no microorganism was isolated. Different genera of bacteria were isolated from honey samples at different production locations. *Staphylococcus* was a common isolate in all honey samples. *Shigella spp.* was isolated from samples from Oja-Odan and Ibese, while *bacillus spp* was isolated from Ibayun and Ilaro. The microbiological quality of honey samples will provide an indication of the sanitary conditions under which honey was processed, handled, and stored [3].

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Table 3: Microbial status (bacteria load and genera) from different locations in Yewaland

Sample Location	Bacteria count (cfu/ml)	Organism isolated
Yewa-North		
Oja-odan	0.9 10^2	*Staphylococcus, Shigella spp*
Ibese	0.3 10^2	*Staphylococcus, Shigella spp*
Ibayun	0.2 10^2	*Staphylococcus, Bacillus spp*
Yewa-South		
Ilaro	0.2 10^2	*Staphylococcus, Bacillus spp*
Iwoye	No growth	No microorganism isolated
Idogo	No growth	No microorganism isolated

cfu = colony forming units

3.3 Sensory attributes

Sensory attributes analyzed between the different types of honey differed significantly ($p<0.05$). Honey samples from Oja-Odan and Ibese had the highest scores of 8.5 and 8.2, respectively, for appearance ~~or colour attribute~~. Similar trends were obtained in panellists' scores on taste and aroma. Colour usually an indication of flavour concentration [12]. Higher pH, phytochemicals, antioxidant activities, and mineral content has been attributed to darker honey [10]. The lowest scores of 6.1 and 6.5 were recorded for texture (smoothness) of the honey samples from Idogo and Ibayun, respectively. The texture of honey is a function of viscosity. Honey samples from Oja-Odan and Iwoye had the best overall acceptance scores of 8.7 and 8.5 respectively.

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Comment [MF11]: Did you determine the colour by mere looking at the samples. I believe that what you determined was appearance because there was no place you showed the procedures of how colour was determined.

Comment [MF12]: Colour is not part of the attributes in sensory results(Table 4)

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Table 4: Sensory analysis scores for honey samples from different locations

Sample Locations	Appearance	Texture (mouth feel)	Viscosity	Taste	Overall acceptability
Yewa-North					
Oja-Odan	8.5	7.3	6.5	8.2	8.7
Ibese	8.2	7.5	6.3	8.3	7.5
Ibayun	6.2	6.5	6.9	7.1	7.4
Yewa-South					
Ilaro	7.3	8.1	6.1	8.3	8.0
Iwoye	7.1	8.3	6.5	8.0	8.5
Idogo	6.8	6.1	6.3	7.6	7.5

4. CONCLUSION

The investigation on natural honey samples from Yewaland, Ogun state of Nigeria was conducted and data obtained clearly established that the samples were not adulterated. Hence, the data were in conformity with the official permissible levels. The majority of the honey samples were of good quality when compared with the Codex standard for honey specifications, notably the moisture and ash contents, pH, total titratable acidity, and refractive index of the entire honey sample. The sucrose or total sugar content for samples from Oja-Odan, Ilaro, and Iwoye exceeded the Codex standard. While the results indicated full compliance to international standard for most of the samples analysed some form of adulteration has also been discovered in some of the samples analysed as indicated. It can be concluded that the samples obtained from Yewa-North proved to be better in terms of quality than those obtained from Yewa-South.

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Comment [MF13]: incomplete. No volume and wrong paging

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