EVALUATION OF THE PHYSICO-CHEMICAL PROPERTIES OF SOME HONEY SAMPLES FROM ENUGU, ENUGU STATE, NIGERIA

Ugwu, S.C., Onyenso, A.I. and Nwobodo, K.A.
Department of Forestry and Environmental Management
Michael Okpara University of Agriculture, Umudike, P.M.B. 7267, Umuahia, Abia State
*Corresponding Author Email: onyensoanthony@gmail.com; Phone: +2347032213426

ABSTRACT
Honey is a sweet and viscous substance produced by honeybees (*Apis mellifera adansonii*) and its quality varies with its origin. Three honey samples were sourced in Enugu State Southeastern Nigeria: a. honey harvested from an apiary (Sample A); b. honey purchased from a roadside honey vendor (Sample B) and c. honey purchased from Shoprite supermarket (Sample C); and were assessed for physico-chemical properties in a laboratory. SPSS version 25.0 was used for data analysis. The ANOVA results of the physico-chemical properties of the studied honey samples Results showed significant differences ($p<0.05$) in (a) physical properties (b) proximate compositions (c) minerals contents and phytochemical properties except for phytate. The physical properties were: total solids, 73.56±0.05-82.08±0.04%; soluble solids, 70.23±0.03-81.23±0.04 (^{o}Brix); specific gravity, 0.75±0.02-1.54±0.04; free acidity, 0.93±0.01-1.35±0.00 (meq/kg); pH, 3.52–4.32. For proximate composition: moisture content, 9.15±0.03-17.12±0.07%; crude protein, 0.92±0.02 - 2.88±0.06%; crude fat, 0.15±0.04-4.59±0.01%; ash, 0.28±0.08-3.51±0.09%; crude fibre, 1.05±0.02-2.09±0.07%; carbohydrates, 77.79±0.09-80.53±0.73%. The mineral contents: sodium (Na), 2.87±0.08-4.59±0.04mg/100g; potassium (K), 32.09±0.01-45.66±0.05 mg/100g; calcium (Ca), 2.58±0.07-4.13±0.03mg/100g; magnesium (Mg), 1.74±0.03-2.87±0.05mg/100g; and iron (Fe), 0.42±0.02-0.83±0.03mg/100g. For the phytochemical parameters: Phytate, 0.23±0.02- 0.36±0.03mg/100g; Tannin, 0.23±0.02-0.58±0.04mg/100g; and HCN, 0.00±0.00- 0.05±0.01mg/100g.There was no presence of Oxalate in the honey samples. The physicochemical properties of the studied honey samples were within the reference standards of Codex Alimentarius and United State Department of Agriculture (USDA).
Keywords: Honey; physico-chemical properties; honey quality standards; Enugu State.

INTRODUCTION
Honey is the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature (Codex Alimentarius, 2001). It is a complex mixture and presents very great variations in composition and characteristics due to geographical and botanical origin of nectar foraged by bees (Crane, 1990; Onyenso and Akachuku, 2011). A fundamental factor that influences the commercial value of honey includes botanical and floral origin, season, beekeeper's handling and environmental factors (Kaskonienes *et al*., 2010; EL-Metwally, 2015). Freshly collected honey is viscous and of greater density than water (Akachuku, 1995; Onyenso and Akachuku, 2011). It has strong hygroscopic character, relatively low heat conductivity and low surface tension. Although honey varies in colour depending on the types of flowering plants visited, the colour variation is within golden yellow to yellowish brown (Akachuku and Onyenso, 2009).

Honey has an outstanding history of human consumption as a natural food material and is of great use in nutrition, medicine and various industrial purposes. It is the best natural health food with many curative and nutritional properties and its collection throughout most parts of Africa has been through the traditional means (Eleazu *et al*., 2013; Osuagwu *et al*., 2020).

The constituents of honey are primarily sugars such as monosaccharides, disaccharides, oligosaccharides, and polysaccharides. It contains enzymes such as glucose oxidase, diastase, invertase, catalase, and peroxidase. Honey also contains other bioactive constituents such as organic acids, ascorbic acid, trace elements, vitamins, amino acids, proteins, and Maillard reaction products (Bogdanov *et al.*, 2008). Ancient Egyptians, Assyrians, Chinese, Romans and Greeks have traditionally used honey as a medicinal remedy for wound healing, treatment of skin ailments and various gastrointestinal diseases (White *et al*., 1962). Modern research has shown that honey may possess anti-inflammatory activity and stimulate immune responses within a wound; this has been attributed to its secondary metabolites of antibacterial potency (Gheldof *et al*., 2002). This study aimed at assessing the physico-chemical properties of some honey samples from Enugu in Enugu State, Nigeria.

MATERIALS AND METHODS
Study area
Enugu State is within the tropical moist rainforest (Keay, 1959) on latitude 06°30^{I}N and longitude 07°30^{I}E with derived savannah vegetation (Obi,

2014). The land area coverage of the Enugu State is approximately 7,161km^2; its rainfall and temperature are seasonal with yearly variations. The annual rainfall range is between 937.20mm to 2243.30mm, while mean temperature range is between 26.80^{0}C to 32.5^{0}C. Two distinct seasons are observed, dry and wet seasons. The dry season extends over a period of about 6 months, from October to March while the wet season extends over a period of about 5 months, from April to September (Agbaogun, 2020). The mean monthly relative humidity ranges from 59.97% to 94.23%, and high throughout the year. The soil status of the State is very good and well drained during its rainy seasons (Ikagwu *et al*., 2020).

Samples collection

Three 50cl each of honey samples were procured from different honey sources within Enugu in Enugu State, Southeastern Nigeria. The honey samples were stored in clean airtight bottles at an ambient temperature to avoid moisture absorption. Honey sample A was obtained from a private beekeeper (apiary); honey sample B obtained from a roadside honey vendor and honey sample C obtained from a Shoprite supermarket.

Laboratory analysis

The honey samples were taken to the Central Services Laboratory Division of National Root Crops Research Institute Umudike for physico-chemical analysis.

Physical properties: the specific gravity was determined by the pycnometer gravimetric method described by Onimawo and Egbulem (1998); pH was determined by electrode metre method (Pearson, 1976); Acidity of honey samples was determined according to QSAE (2005); the amount of total soluble solids (°Brix) was determined using a refractometer (Q767-B, Tokyo, Japan) at 20 °C while total solids content (%) was computed following the equation described by Saxena *et al.* (2010).

Proximate composition: the moisture content of honey was determined by following the procedure of AOAC (1990) and expressed as the percentage; crude protein was determined by the microkjeldal method (James, 1995); crude fibre was determined by Weeden method; ash content was determined by impurities technique (Pearson, 1976; Ojiako and Akubugwo, 1997); The carbohydrate content of the test sample was determined by estimation using the arithmetic difference method described by Pearson (1976) and James (1995).

Mineral analysis: calcium (Ca), magnesium (Mg), sodium (Na), potassium (K) and iron (Fe) were determined through the method described by Novozamsky *et al.* (1983)

Phytochemicals: Phytate was determined according to the method described by Norhaizan and Nor Faizadatul Ain (2009); oxalate was determined by method described by Day and Underwood (1986); tannin was determined according to Van-Burden and Robinson method (1981); and hydrogen cyanide (HCN) by following Draft method OTM-29.

Statistical analysis

Data on physico-chemical properties of honey were analyzed using one-way Analysis of Variance (ANOVA). All posthoc tests were carried out using Tukey-test and the standard level of significance was $p<0.05$. We used the software Statistical Package for Social Sciences (SPSS) version 25 (2017) for windows for statistical analysis.

Results and discussion

Physical properties

There were significant variations ($P<0.05$) between the mean values of the physical properties of the investigated honey samples obtained from Enugu State, Nigeria (Table 1). Roadside honey sample showed significantly ($p<0.05$) higher total solid contents (82.08±0.04%) than the apiary (73.56±0.05%), and Shoprite (76.37±0.07%) honey samples. These values are below the international limit of >83% (USDA, 2019); but aligned with the findings of Babarinde *et al*. (2011) and Saxena *et al*. (2010), who found 72.2 – 76.5% and 78.4 – 82.8%, respectively. Babarinde *et al*. (2011) and Kamal *et al*. (2019) reported that glucose and fructose comprised the total solids present in honey accounting for about 85%. However, the obtained results were higher than the results recorded by Olugbemi *et al*. (2013) and Osuagwu (2020) who reported a range value of 11.33% to 20.34%, in the study of honeys from Umuahia, Abia State, Nigeria. The observed variations obtained in values across the parameters of the studied honey samples could be ascribed to differences in multifloral origin of plant bearing nectar which honeybees visited, climatic variation, extraction and storage methods (Osuagwu *et al*., 2020). The soluble solid (^{o}Brix) is closely connected to the amount of sugars existing in honey, making it an essential marker of conceivable adulteration (Kamal *et al*., 2019). Data presented in Table 1 showed that the ^{o}Brix of the studied honeys extended from 70.23 to 81.23. The findings are in line with the report by Dele (2017); Kamal *et al*. (2019); Souza *et al*. (2006); Saxena *et al*. (2010); de Sousa *et al*. (2016).

Specific gravity of honey is moisture content and floral source dependent (USAD, 1985; EU, 2001). The obtained specific gravity (S.G.) of the investigated honey samples were: apiary (0.75±0.02); roadside (1.54±0.04) and Shoprite (1.08±0.03). The specific gravity of the apiary and Shoprite honey samples were below the USDA (2019) standard range limit of 1.38 – 1.45; Ndife, *et al*. (2014) range of 1.42±0.15 to 1.44±0.52 and Olugbemi, *et al*. (2013) range of 1.3415 to 1.3432. This is an indication of higher viscosity (Onyenso *et al*., 2020; Osuagwu *et al*., 2020). Roadside honey sample recorded specific gravity of 1.54±0.04, above the international tolerance range limit of 1.38 to 1.45 (USDA, 1985; EU, 2001). Differences in the specific

gravity of the investigated honey samples could be ascribed to the water content and floral source of the studied honey samples (Osuagwu *et al*., 2020).

A higher free acidity value (>50mEq/kg) in honey is an indication that fermentation has taken place (EU, 2001; Osuagwu *et al*., 2020). The acidity level (Free acidity; is the acidity titratable with sodium hydroxide up to the equivalence point) of the analyzed honey samples ranged from 0.93±0.01 meq/ kg to 1.35±0.00 meq/kg, which was found within the specified Codex, 2001(≤40 meq/kg) and USDA, 2019 (>50 meq/kg); suggesting that the investigated honeys were well processed, stored and stable against fermentation. Ndife, *et al*. (2014), obtained a similar range of free acid value from 1.30±1.04 meq/kg to 1.55±1.10 meq/kg. Other studies stated a higher range of acidity of honey, 35.7–40.5meq/kg (Azonwade *et al*., 2018). According to Baroni *et al*. (2009), the acidity of honey varied from 24.4 to 25.4meq/ kg, and changes with the source of nectar (Sahinler *et al*., 2004). Inappropriate processing, early harvesting, immature honeycombs and broods, the action of microorganisms (Xerotolerant yeast) can speed up the rate of honey fermentation, which increases the level of total acidity (Sahinler *et al*., 2004).

Generally, honey is acidic in nature disregarding its geographical origin. It can be seen that the investigated honey samples were acidic (pH 3.52-4.32) and remained within the recommended limit (pH 3.40-6.10) of the Codex Alimentarius Commission (2001), which ensures honey freshness. Low pH in the acidic range is an indication of good shelf life and excellent stability of honey against microorganisms and natural flavour. pH values of analyzed honey samples corroborated with the reported range of 3.80 to 4.13 for Southeast Nigeria honey (Olugbemi *et al*., 2013); pH range of 3.01 to 4.21 for honey samples from Ceara State, Northeast Brazil (Selene, *et al*., 2013), and a pH mean range of 4.10±2.01 to 4.47±1.93 (Ndife, *et al*., 2014). Variations in pH values of honey could be due to different acids found in different floral bearing nectar honeybees visited, extraction and storage methods.

Table 1: Physical properties of the honey samples

Parameters	SampleA±SE	SampleB±SE	SampleC±SE	Reference standards
Total solids (%)	73.56^{c}±0.05	82.08^{a}±0.04	76.37^{b}±0.07	>83.0
Soluble solids (^{o}Brix)	70.23^{c}±0.03	81.23^{a}±0.04	75.66^{b}±0.04	Not available
Specific gravity	0.75^{c}±0.02	1.54^{a}±0.04	1.08^{b}±0.03	1.38 – 1.45
Free acidity (meq/ kg)	0.93^{b}±0.01	1.35^{a}±0.00	1.24^{a}±0.04	≤40 or <50.0
pH	4.11^{b}±0.01	3.52^{c}±0.02	4.32^{a}±0.02	3.5- 4.5

$^{a-c}$ means superscripts within a row are significantly different (P<0.05). Sample A =honey from apiary; Sample B = honey from roadside vendor; Sample C = honey from Shoprite supermarket; SE = standard error. Reference standards = Codex Alimentarius (2001); United State Department of Agriculture for Honey Grading (USDA 2019).

Proximate composition

The mean values of proximate compositions of the studied honey samples were presented in Table 2. Apiary honey sample showed significantly (p<0.05) higher moisture content (17.12±0.07%) when compared to Shoprite supermarket (15.35±0.03%) and roadside (9.15±0.03%) samples. Moisture content of honey is an important factor for consideration in relation to stability, storage, exportability, fermentation and granulation. Low moisture content of less than 18% confers on honey longer period of preservation and against osmophilic bacterial activities (EU, 2001; Osuagwu *et al*., 2020). The moisture content of the studied honey samples was found well below the imposed limit (≤ 20%) of the regulatory commissions (Codex Alimentarius, 2001; EU, 2001) and ranged from 9.15 to 17.12% (Table 2). However, the obtained honey moisture content values were analogous to the report by Onyenso *et al*. (2020) who had range value of 12.12±0.00 to 14.33±2.12%, in a study on physicochemical properties of honey harvested from different Langstroth hives in Umudike. Honey with over 20% moisture content will ferment (White *et al*., 1962). Also, honey with carbohydrate content greater than 83%, moisture content less than 17.1% and storage temperature less than 11^{o}C, will not ferment (EU, 2001). A high amount of moisture is responsible for the undesirable fermentation of honey during storage, where osmotolerant yeast takes advantage to form C_2H_6O and CO_2. This alcohol further oxidized to CH_3COOH and H_2O, and gives a sour taste of honey (Imtara, *et al*., 2018; Kamal *et al*., 2019).

Significant differences (P<0.05) in protein content were observed among the samples. Roadside sample showed significantly (p<0.05) higher protein content (2.88±0.06%) when compared with Shoprite supermarket (1.79±0.03%) and apiary (0.92±0.02%) samples. The crude protein values of the studied honey samples: apiary (0.92±0.02%), roadside (2.88±0.06%) and Shoprite (1.79±0.03%) were all beyond the international limit of 0.3% (USDA 2019). Osuagwu (2020) observed crude protein value range of 0.04 to 1.06% on honeys produced in the Guinea Savannah Zones of Nigeria and Ndife *et al*. (2014), 0.90±0.28% to 1.10±0.41% on honeys produced from various apiary units of University of Ilorin,

Nigeria. Differences in the values of crude protein in honey could be linked to the differences in soils composition, locations and floral origin Osuagwu (2020).

Similarly, roadside sample showed significantly ($p<0.05$) higher fat content (4.59±0.01%) than the apiary (0.24±0.00%) and Shoprite supermarket (0.15±0.04%) samples. There was no significant difference between the fat contents of apiary and Shoprite supermarket honey samples. Food of a higher fat content stands the risk of rancid spoilage during storage (Estevinho *et al*., 2012). Fat contents of the apiary honey (0.24±0.00%) and Shoprite honey (0.15±0.04%) samples were in the range reported by Ndife *et al* (2014), 0.12±0.01% to 0.20±0.03% while roadside honey sample had high fat 4.59±0.01% content. Osuagwu *et al*. (2020) reported 0.31% to 0.35% fat content range for honey produced in the Guinea savannah zones of Nigeria while Leticia (2013) reported value of between 0.37% and 0.39%. The differences in the values of fat could be due to variation in pollen that bees visited (Osuagwu *et al*., 2020).

Roadside honey sample showed significantly ($p<0.05$) higher ash content (3.51±0.09%) when compared to apiary (0.36±0.01%) and Shoprite supermarket (0.28±0.08%) samples, while no significant difference occurred between apiary and Shoprite supermarket samples. The botanical source of honey is assessed by its minerals, that is, ash content (Kamal *et al*., 2019). It is a quality criterion for botanical and geographical origin of honey (Osuagwu *et al*., 2020). The ash contents of the studied honeys: apiary (0.36±0.01%) and Shoprite (0.28±0.08%) honey samples were within the CODEX and European Food Commission standard (< 0.6%) and also agree with the findings of Onyenso *et al*. (2020) while that of the roadside (3.51±0.09%) was higher. The higher ash content of the roadside honey could be that it contained higher quantities of essential inorganic minerals or be attributed to the soil where the honeybee plants grew (Ndife *et al*., 2014; Osuagwu *et al*., 2020). The amount of ash contained in the investigated honey samples showed they could aid as ample sources of dietary minerals. However, variation in the ash content of honey might be due to beekeeping practices, harvesting and processing methods, the nectar source and geographical location (Saxena *et al*., 2010; Kamal *et al*., 2019).

Fibre content of the analyzed honey samples varied significantly ($P<0.05$). Roadside sample showed significantly ($P<0.05$) higher fibre content (2.09±0.07%), compared with apiary (1.10±0.01%) and Shoprite supermarket (1.05b±0.02%) honey samples. No significant difference occurred between apiary and Shoprite supermarket honey samples. There were significantly differences ($P<0.05$) among carbohydrates contents of the studied honey samples. Shoprite supermarket honey sample showed significantly ($p<0.05$) higher carbohydrates content (80.53±0.73%) compared with apiary (80.27±0.09%) and roadside (77.79±0.09%) samples. The main constituents of honey are the carbohydrates which constitute about 95% of honey dry weight (Onyenso, *et al*., 2020). The main sugars found in honey are the fructose and glucose (White and Doner, 1980; Onyenso and Akachuku, 2011). Osuagwu (2020) reported that honey is a high energy carbohydrate food and that the sugar content in honey is digestible similar to the sugars found in fruits. Also, honey with carbohydrate content greater than 83%, moisture content less than 17.1% and storage temperature less than 11^0C, will not ferment (EU, 2001). Regarding the carbohydrate (%) contents of the investigated honey samples, the apiary (80.27±0.09%) and Shoprite (80.53±0.73%) samples had values closed to the international limit of >83% (USDA, 2019). Aneni *et al*. (2023) obtained 79.77g/100g carbohydrate content value for NIFOR honey.

Table 2: Proximate composition

Parameters	SampleA±SE	SampleB±SE	SampleC±SE	Reference standards
Moisture content (%)	17.12^{a}±0.07	9.15^{c}±0.03	15.35^{b}±0.03	17.1 - ≤20
Crude Protein (%)	0.92^{c}±0.02	2.88^{a}±0.06	1.79^{b}±0.03	0.27
Crude Fat (%)	0.24^{b}±0.00	4.59^{a}±0.01	0.15^{b}±0.04	0
Ash (%)	0.36^{b}±0.01	3.51^{a}±0.09	0.28^{b}±0.08	0.2 - < 0.6
Crude Fibre (%)	1.10^{b}±0.01	2.09^{a}±0.07	1.05^{b}±0.02	0.2
Carbohydrates (%)	80.27^{ab}±0.09	77.79^{b}±0.09	80.53^{a}±0.73	> 83

$^{a-c}$ means superscripts within a row are significantly different ($P<0.05$). Sample A =honey from apiary; Sample B = honey from roadside vendor; Sample C = honey from Shoprite supermarket; SE = standard error. Reference standards = Codex Alimentarius (2001); United State Department of Agriculture for Honey Grading (USDA 2019).

Mineral content

The mean results of the mineral elements of the investigated honey samples were presented in Table 3. There were statistical significance differences ($P<0.05$) in the obtained values across the mineral nutrients. Roadside sample showed significantly ($p<0.05$) higher sodium (Na) content (4.59±0.04mg/100g) when compared with Shoprite supermarket (3.69±0.06mg/100g) and apiary (2.87±0.08mg/100g) samples. The results agreed

with the range value of 0.9 to 26.7mg/100g reported by Escuredo *et al*. (2011), on their study of Blossom honey and Honeydew honeys from Northwest Spain. The Recommended Dietary Allowance (RDA) of Sodium for men ranged between 400mg to 420mg and for women varied from 310mg to 320mg (Osuagwu *et al*., 2020). The average amount of Sodium per 100g honey is 2.85mg and per one tablespoon serving 21g is 0.6mg (Sweeteners, Desserts, 2005). Minerals in honey vary according to the botanical origin and soil composition (Onyenso and Akachuku, 2011).

Similarly, Roadside sample showed significantly ($p<0.05$) higher potassium (K) content (45.66±0.05mg/100g) when compared with apiary (37.30±0.00mg/100g) and Shoprite supermarket (32.09±0.01mg/100g) samples. Potassium is the most abundant mineral present in the honey samples among the mineral elements in this study and values ranged between 32.09±0.01 to 45.66±0.05mg/100g. The reported values in this investigation though lower than the specified standard limit of 52mg/100g for honey (USDA, 2019) agreed with the values obtained by Escuredo *et al*. (2011) who documented a range of Potassium values from 32.8 to 312.1mg/100g on the study of Blossom honey and Honeydew honeys from Northwest Spain. Differences in Potassium values could be due to the nature of soils on which nectar plants thrive and variation in locations. However, the Recommended Dietary Allowance (RDA) of Potassium for men and women ranged from 1600mg to 2000mg (Pamplona, 2006). The average amount of Potassium per 100g honey is 50.0mg and per one tablespoon serving 21g is 11.0mg (Sweeteners, Desserts, 2005).

Significant difference ($P<0.05$) in calcium (Ca) content were observed among the analyzed honey samples. Roadside sample showed significantly ($p<0.05$) higher Ca content (4.13±0.03mg/100g) when compared with apiary (3.13±0.03 mg/100g) and Shoprite (2.58±0.07mg/100g) samples. The mean Calcium content obtained from the studied honey samples were lower than the standard limit of 6mg/100g prescribed by USDA (2019), and agreed with results reported by Escuredo *et al*. (2011), who documented mean range from 2.8mg/100g to 16.6mg/100g, on the study of Blossom honey and Honeydew honeys from Northwest Spain. The average amount of Calcium per 100g honey is 4.8mg and per one tablespoon serving 21g is 1.0mg (Sweeteners and Desserts, 2005). Minerals in honey vary according to the botanical origin and soil composition (Franchini *et al*., 2007; Pohl, 2009; Onyenso and Akachuku, 2009).

Similarly roadside honey sample showed significantly ($p<0.05$) higher magnesium contents (2.87±0.05mg/100g) when compared with apiary (1.74±0.03mg/100g) and Shoprite supermarket (2.05±0.08mg/100g) samples. There was no significant difference ($p<0.05$) in Mg contents of apiary and Shoprite supermarket honey samples. The Magnesium content of the investigated honeys ranged from 1.74±0.03 to 2.87±0.05mg/100g. Differences in Magnesium content is ascribed to differences in soils composition and different floral nectar honeybee visited. The obtained Magnesium results in this study is in agreement with the values reported by Escuredo *et al*. (2011), who reported mean range from 1.4mg/100g to 30.7mg/100g, on the study of Blossom honey and Honeydew honeys from Northwest Spain. The average amount of Magnesium per 100g honey is 2.0mg and per one tablespoon serving 21g is 0.4mg (Sweeteners and Desserts, 2005).

Iron (Fe) results indicated significant differences ($P<0.05$) in the values obtained from various honey samples. Roadside sample showed significantly ($p<0.05$) higher Fe contents (0.83±0.03 mg/100g) than apiary (0.59±0.02) and Shoprite supermarket (0.42±0.02 mg/100g) honey samples. The mean iron contents obtained from the studied honey samples agreed with results reported by Escuredo *et al*. (2011), 0.0 – 1.1mg/100g, obtained on the study of Blossom and Honeydew honeys from Northwest Spain. Differences in the obtained values of iron in this investigation could be due to soils composition and floral origin. In an average amount of 100g honey, the amount of Iron is 0.25mg and an average amount of 21g honey of one tablespoon, the amount of Iron is 0.05mg (Sweeteners and Desserts, 2005).

Table 3: Mineral contents

Parameters	SampleA±SE	SampleB±SE	SampleC±SE	International Standard
Na (mg/100g)	2.87^{c}±0.08	4.59^{a}±0.04	3.69^{b}±0.06	4
K (mg/100g)	37.30^{b}±0.00	45.66^{a}±0.05	32.09^{c}±0.01	52
Ca (mg/100g)	3.13^{b}±0.03	4.13^{a}±0.03	2.58^{c}±0.07	6
Mg (mg/100g)	1.74^{b}±0.03	2.87^{a}±0.05	2.05^{b}±0.08	2
Fe (mg/100g)	0.59^{b}±0.02	0.83^{a}±0.03	0.42^{c}±0.02	0.42

$^{a-c}$ means superscripts within a row are significantly different ($P<0.05$). Sample A = honey from apiary; Sample B = honey from roadside vendor; Sample C = honey from Shoprite supermarket; SE = standard error. Reference standards: United State Department of Agriculture for Honey Grading (USDA 2019).

Phytochemical contents

The mean results of the phytochemical contents of the investigated honey samples were presented in Table 4. The result showed no significant differences (P>0.05) between the phytate, HCN contents of apiary, roadside and Shoprite supermarket honey samples. There was no presence of oxalate in the three honey samples. Phytate is an anti-nutritional component of honey and other food substances. It is the molecule that is formed when phytic acid binds to a mineral. The mean phytate contents of the studied honey samples aligned with 0.38 mg/100g reported for honey sample from Biase Southern of Cross River State, Nigeria (Igbang *et al.,* 2018) and less than 22.29 - 961.20 mg/100g reported for honey samples from four Northern states of Nigeria (Oriolowo *et al.,* 2019). Phytate in human diets significantly lowers cholesterol and the risk of coronary diseases (Klevay, 1974). It also helps in the management and prevention of diabetes as well as growth of different cancer line (Shamsuddin *et al.,* 1996). However, at higher consumption rate, phytate has been associated with nutritional diseases such as ricket in children and osteomalacia in adults (Adeniyi *et al.,* 2016). Tannin is nontoxic and can generate physiological responses in animals (Scalbert, 1991). The tannin contents of honey samples studied ranged were lower than 278.48-426.14 mg/100g reported for four honey samples from Northern state of Nigeria (Oriolowo *et al.,* 2019). Tannin has a number of nutritional and health benefits such as being an anti-oxidant, cardio-protective, anti-inflammatory, anticarcinogenic and anti-mutagenic (Kumari and Jain, 2012). Similarly, plants containing tannin have been reported to be used for healing of wounds, varicose ulcers, hemorrhoids, frost bile, burn in herbal medicine and selectively inhibit HIV replication (Onyenso, 2018). However, at higher concentrations, tannins may complex with food thereby rendering digestive enzymes like trypsin, chemotrypsin, amylase and lipase less effective (Felix and Mello, 2000). The hydrogen Cyanide contents of the studied honey samples were below the critical level of 500mg/kg (Onwuka, 2005; Onyenso, 2018).

Table 4: Phytochemicals contents

Parameters	SampleA±SE	SampleB±SE	SampleC±SE	International Standard
Phytate (mg/100g)	0.23^{a}±0.02	0.36^{a}±0.03	0.28^{a}±0.03	Not available
Tannin (mg/100g)	0.32^{b}±0.02	0.58^{a}±0.04	0.23^{b}±0.02	Not available
HCN (mg/100g)	0.02^{a}±0.01	0.05^{a}±0.01	0.00^{a}±0.00	Not available
Oxalate (mg/100g)	Nil	Nil	Nil	Not available

[a-c] means superscripts within a row are significantly different (P<0.05). Sample A =honey from apiary; Sample B = honey from roadside vendor; Sample C = honey from Shoprite supermarket; SE = standard error.

Conclusion

This study evaluated the physicochemical properties of some honey samples from Enugu State, Nigeria. Results of two major parameters moisture and ash contents which are used in determining honey quality however indicated that the three honey samples studied recorded values within the known standards thereby meeting the requirements of quality honeys as described in Codex Alimentarius; the U.S. Department of Agriculture (USDA) and European Regulatory Commissions for bee honeys. The highest moisture content of all the studied honeys is 17.12% and this confers on the samples stability, against osmophilic bacterial activities, long period preservation. Honeys from Enugu State, Nigeria, are of good quality.

References

Adeniyi, K. A., Olayemi, I. K., Shittu, K. O., Busari, M. B., Mohammed, S. S., Bashir, L., & Yusuf, R. S. (2016). Comparative phytochemical and antinutritional constituents of Nigeria sweet and bitter honey varieties. *World Journal of Pharmaceutical Research*, *5*(3), 255-267.

Agbaogun, S. O., & Akintunde-Alo, D. (2020). Dynamics of land use land cover change in Enugu City of Enugu State, Nigeria.

Akachuku, C.O. and Onyenso, A.I. (2009). Honey yield in different hives used in modern beekeeping in the rainforest area of Nigeria. *Journal of Tropical Forest Resources*. 25(1): 17-24.

Akachuku, C.O. (1995). Plant species of some wetlands in Nigeria visited by honeybees for nectar and pollen collection. In: sustaibale utilization of aquatic/wetland resources (S.O. Otubusin, G.N.O. Ezeri, O.A. Ugwumba, A.A.A. Ugwumba, Eds). Published by Nigeria Association for Aquatic Science. pp. 279-297.

Aneni, T. I., Adaigbe, V. C., Ogbebor, C. O., Okere, C. I., Aghayedo, C. O., Odoemelam, V. K., and Adeoye, O. O. (2023). Proximate analysis of honey samples: NIFOR apiary and open market. *International journal of food science and agriculture*, 7(1), 33-40.

Azonwade, F.E., Paraiso, A. and Dossa, C.P.A., *et al*. (2018). Physicochemical characteristics and microbiological quality of honey produced in Benin. Journal of food quality, 2018: 1-13.

Babarinde, G. O., Babarinde, S. A., Adegbola, D. O., &Ajayeoba, S. I. (2011). Effects of harvesting methods on physicochemical and microbial qualities of honey. *Journal of food science and technology*, *48*(5), 628-634.

Baroni, M.V, Arrua, C. Nores, M.L., *et al* (2009). Composition of honey from Córdoba (Argentina): assessment of north/south provenance by chemometrics. *Food chem*., 114:727-733.

Bogdanov, S., Jurendic, T., Sieber, R., &Gallmann, P. (2008). Honey for nutrition and health: a review. *Journal of the American college of Nutrition*, *27*(6), 677-689.

Codex Alimentarius (2001). Revised Codex Standard for Honey. Codex Stan 12-1981.

Crane, E. (1990) Bees and beekeeping. Scientific, Practice and World Resources. 1st ed., Heinemann Newnes, Halley Court, Jordan Hill, Oxford OX2 8EJ, London, UK, pp 388–451

de Sousa, J.M.B., de Souza, E.L., Marques, G. *et al* (2016). Sugar profile, physicochemical and sensory aspects of monofloral honeys produced by different stingless bee species in Brazilian semi-arid region. LWT Food Sci Technol 65:645–651.

Dele, O. S. (2017). Quality Assessment of Honey Sourced from Natural and Artificial Apiaries in Ekiti State, Nigeria. *Turkish Journal of Agriculture-Food Science and Technology*, *5*(10), 1125-1129.

Eleazu, C.O., Iroaganachi, M., Okoronkwo, J. (2013). Determination of the physicochemical composition, microbial quality and free radical scavenging activities of some commercially sold honey samples in Aba, Nigeria: The effect of varying colors'. *J. Nutr Food Sci*. 3(2): 189.

EL-Metwally, A.A.E. (2015). Factors Affecting the Physical and Chemical Characteristics of Egyptian Bee honey. Ph. D. Thesis, Fac. Agric. Cairo Univ. pp. 320.

Escuredo, O., Seijo, M. and Fernandez-Gonzalez, M. (2011). Descriptive analysis of rubus honey from the north-west of Spain. *International Journal of Food Science and Technology* 46: 2329 – 2336

Estevinho, L.M., Feas, X., Seijas, J.A., and Vazquez Tatol, M.P. (2012) Organic honey from palynological, Microbiological and bioactive compounds Characterization. Food and Chemical Toxicology 50: 258-264.

EU (2001) European Union Council Directives 2001/110/CE relating to honey. *Official Journal of European Community* L10: 47-57.

Felix, J. P. and Mello, D. (2000). Farm Animal Metabolism and Nutrition. United Kingdom: CABI Publishing Wallingford, UK. Pp 450.

Franchini, R.A., Ferraz de Souza, A., Colombara, C., Matos R. MAR, and Matos, R.C. (2007). Rapid determination of hydrogen peroxide using peroxidaseimmobilized on Amberlite IRA-743 and minerals in honey. *Journal of Agriculture and Food Chemistry* 55(17): 6885-6890.

Gheldof, N., Wang, X. H., & Engeseth, N. J. (2002). Identification and quantification of antioxidant components of honeys from various floral sources. *Journal of agricultural and food chemistry*, *50*(21), 5870-5877.

Igbang, O. J., Abang, O, Eneji, E. G. (2018). Selected Antinutrients and Proximate Compositions of Honey (Apismellifera) Sample Collected from Biase, Southern Senatorial District of Cross River State, Nigeria. Biochemical Pharmacolology (Los Angel), 7 (4): 261.

Ikagwu, P., Oluka, I. and Nweke, E. (2020). Determination of Soil-Crop Suitability for Enugu State using Geographic Information System (GIS) & Multi-Criteria Evaluation (MCE). International Journal of Engineering Research & Technology (IJERT), 9 (1): 274 – 281.

Imtara, H., Elamine, Y., and Lyoussi, B. (2018). Physicalchemical characterization and antioxidant activity of Palestinian honey samples. *Food Sci. Nutri*., 6 (8):2056-2065.

Kamal, Mostafa Md., Rashid, Harun Ur Md., Mondal, Chandra Shakti, Hasan Fuad El Taj and Chuleui Jung (2019). Physicochemical and microbiological characteristics of honey obtained through sugar feeding of bees. *J. of Food Sci. Technol*., 56(4): 2267-2277.

Kaskonienes, V., Venskutonis, P.R., and Ceksteryte, V. (2010.) Carbohydrate composition and electrical conductivity of different origin honeys from Lithuania, LWT-Food Sci Technol 43: 801-807.

Klevay, L. M. (1974). Coronary heart disease: The Zinc/Copper hypothesis. American Journal Clinical Nutrition, 28:764–774

Leticia, M.E. (2013). Comparative study of the physicochemical and palynological characteristics of honey from *Melipona subnitida* and *Apis Mellifera*. *International J of Food Science and Technology* 48: 1698-1706.

Ndife, J., Kida, F., and Makarfi, T. (2014). Quality Assessment of Nigerian honey sourced from different floral locations. *Journal of Food and Nutrition Sciences* 2(4): 162-167.

Norhaizan, M. E. and Nor Faizadatul Ain, A. W. (2009). Determination of Phytate, Iron, Zinc, Calcium Contents and Their Molar Ratios in Commonly Consumed Raw and Prepared Food in Malaysia. Malaysia. *Journal of Nutrition*; 15(2):213-222

Novozamsky, I., Houba, V. J. G., van Eck, R. and van Vark, W. (1983). A novel digestion technique for multi-element plant analysis. *Communications in Soil Science and Plant Analysis* 14: 239-248.

Obi, N. I. (2014). The influence of vegetation on microclimate in hot humid tropical environment-a case of Enugu urban. *International Journal of Energy and Environmental Research*, *2*(2), 28-38.

Olugbemi, O., Ikeme, C. H., &Dioha, I. J. (2013). Physicochemical analysis of honey from Umuahia, Abia-State, Nigeria. *Research Journal in Engineering and Applied Sciences*, *2*(3), 199-202.

Onwuka, G.I. (2005). Food analysis and instrumentation: theory and practice. Naphthali prints, Nigeria. Pp. 129-144.

Onyenso, A.I. (2018). Phytochemical contents of Allanblackia floribunda Oliv. (Guttiferae) leaves use in as components of human and animal ethno-medicine in Nigeria. *Nigerian agricultural journal*, 49 (2): 248-251.

Onyenso, A.I. and Akachuku, C.O. (2011). Physico-chemical properties of honeys produced by two stinglessbee species - *Trigonacarbonaria* and *Melipona beecheii*in South-Eastern Nigeria. *Journal of Agriculture, Forestry and the Social Sciences (JOAFSS). VOL. 9, No.1, 2011 edition*.

Onyenso, A.I., Nwobodo, K.A. and Onyenweaku, L.N. (2020). Physico-chemical properties of honey harvested from different langstroth hives in Umudike, Nigeria. *Proceedings of the 1st International Conference of the College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture Umudike* on promoting sustainable natural and environmental resources management for economic recovery and growth, pp. 41-46.

Oriolowo, O. B., John, O. J., Abubakar, D. S., Jonah, T. M., &Ismaila, D. (2019). Anti-nutritional composition of honey samples from four northern states of Nigeria. *Nigerian Journal of Basic and Applied Sciences*, *27*(2), 32-38.

Osuagwu, O.S., Oyerinde, A.A., Onipede, A.S. and Ombugadu, A. (2020). **c**omparative Studies of the Physicochemical Properties and Mineral Elements of Honey Produced in the Guinea Savannah Zones of Nigeria. Biomed J. Sci. & Tech. Res., 24(5): 18548-18561.

Pamplona, R. (2006). Encyclopedia of foods and their Healing power. 1: 100-103.

Pearson, D., (1976). The Chemical analysis of foods (7th edition). Churchill Livingston Edinburgh,London.

Pohl, P. (2009). Determination of mineral content in honey by atomic absorption and emission spectrometric. Trends in Analytical Chemistry 28(1): 117-125.

QSAE (2005) - Honey specification: Ethiopian standard, ES 1202: 2005. Quality and Standards Authority of Ethiopia (QSAE), Addis Ababa, Ethiopia, pp. 1-17.

Sahinler, N., Sahinler, S., Gul, A. (2004). Biochemical composition of honeys produced in Turkey. *J Apic Res*., 43:53-56.

Saxena, S., Gautam, S. and Sharma, A. (2010). Physical, biochemical and antioxidant properties of some Indian honeys. Food Chem., 118:391-397. Doi: 10.1016/j.foodchem.2009.05.001.

Scalbert (1991).Antimicrobial properties of tannis. Phytochemistry., 30: 3875-3882

Selena Maria de Morais, Maria da Conceicao Tavares Cavalcanti Liberato, Carlos Emanuel de Carvalho Magalhaes, Islay Lima Magalhaes, Daniel Bomfim Cavalcanti, et al. (2013). Physicochemical properties and mineral and protein content of honey samples from Ceara state, Northeastern Brazil. Food Sci, Technol (Campinas) 33(1) Campinas Jan./Mar. Epub Mar 26.

Shamsuddin, A. M., Yang, G. Y. and Vucenik, I. (1996). Novel anti-cancer functions of IP6: Growth inhibition and differentiation of human mammary cancer cell lines in vitro. Anticancer Research, 16:3287–3292.

Souza, B.A., Roubik, D., Heard, T. *et al*. (2006) Composition of stingless bee honey: setting quality standards. Interciencia 31:867–875.

SPSS (2017). SPSS Statistics Para Windows, version 25.0; SPSS Inc.: Chicago, IL, USA.

Sweeteners, Desserts (2005). American Diabetes Association.

USDA (1985). United States Department of Agricultural Standards for honey grades of extracted honey, (5th Edn.). In Agricultural marketing service fruit and vegetable Division processed products branch. Department of Agriculture. Washington DC, USA.

USDA. (2019). U.S. Department of Agriculture, Agricultural Research Service. Food Data Central, Available at https://fdc.nal.usda.gov/fdc-app.html#/fooddetails/169640/nutrients Retrieved on 14th October, 2021.

White, J.W. and Doner, L.W. (1980). Honey composition and properties: Beekeeping in the United States. Agriculture Handbook No. 335. Revised October p. 82- 91.

White, J.W., Riethof, M.L., Subers, M.H. and Kushnir, I. (1962). Composition of American honey. U.S. Department of Agriculture Technical Bulletin 1261: 124.