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Research Article

Determination of the physico-chemical composition, microbial quality and free radical scavenging activities of some commercially sold honey samples in Aba, Nigeria: 'The effect of varying colours'

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Abstract

The effect of the colour of five samples of honey (A,B,C,D,E) on their physico-chemical composition, microbial quality and antioxidant activities was studied using standard techniques. The samples had low pH, moderate moisture, ash contents but significant quantities of phenols, flavonoids with higher scavenging activities on 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical than quercetin ($P < 0.05$) and their colour decreased in the order: E>A>B>C>D based on their absorbance at 560nm. The mean coliform counts of the samples ranged from 0 to 5.0×10^3cfu/ml for bacteria, 0 to 1.5×10^3cfu/ml for fungi while *Staphylococcus*, *Fusarium* and *Penicillum* were isolated. There was a positive correlation between colour versus pH (0.879), phenol (0.85), ash (0.622), inhibition of DPPH radical (0.769) and flavonoid contents (0.157) but a negative correlation between the colour versus reducing sugars (RS) (-0.707), bacteria (-0.252) and fungi counts (-0.170). We obtained a positive correlation between the scavenging activities of the samples on DPPH versus flavonoids (0.621) and phenols (0.859), a negative correlation between the bacterial load of the samples versus pH (-0.448), phenol (-0.556), ash (-0.443), RS (-0.441) and flavonoids (-0.790) as well as a negative correlation between the fungi counts of the samples versus pH (-0.097), phenol (-0.171), RS (-0.783), ash (-0.371) and flavonoids (-0.732). Darker honeys have lower RS but higher pH, phenols and antioxidant activities than lighter honeys. The honey samples could be useful in the treatment of diseases that implicate free radicals while the sterile quality of honey A confers very high therapeutic properties on it.

Keywords: Colour, Effect, Honey, Composition, Classification

1. Introduction

Honey is the natural sweet substance that is produced by honeybees (*Apis mellifera*) from the nectar of blossoms or from the secretions of living parts of plants [25, 44] which these honey bees use and store as food. The geographical, climatic conditions, floral origin or the nectar foraged by bees tend to affect the physicochemical, phenolic and antioxidant activities of honey [2, 3, 4, 11, 24, 38, 48]. Therefore, different honey types have different composition.

Natural honey is composed mostly of carbohydrates, lesser amounts of water and a great number of minor constituents such as: proteins, minerals, phytochemicals and antioxidants. These minor constituents confer medicinal/biological properties on honey such as: treatment of burns, wounds, cataracts, ulcers, etc [36].

According to James *et al*.[28], artificial (adulterated) honeys can be produced from carbohydrate sources and their glucose-fructose composition are often close to the range of natural honeys. These artificial honeys often have similar taste and physical appearance as natural honeys but they (artificial honeys) lack the biological properties of natural honeys because of the absence of these minor constituents of natural honeys. In addition, some indigent Nigerians who cannot afford the cost of proper medication, use honey for various therapeutic purposes. Therefore the need for the characterization of honey samples from different sources to determine their properties cannot be overemphasized .

The various types of antioxidant components in honey have been reported to synergistically contribute to the anti-oxidative/radical scavenging activities of honey[24]. These antioxidant systems include: enzymatic (e.g. catalase and glucose oxidase) and non-enzymatic (example: organic acids, maillard reaction products, amino acids, proteins, flavonoids, phenolics, α-tocopherol, ascorbic acid and carotenoids) substances[43]. The presence of these different antioxidant components in plants makes it relatively difficult to measure each component separately[30]. The antioxidant activity (total antioxidant capacity) of plants and plant extracts can be determined by several *in vitro* methods. However, two methods are widely employed for antioxidant studies. The first set of assays involve electron or radical scavenging and they include: the 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical, Trolox equivalent antioxidant capacity (TEAC), and FRAP assays. They are based on reduction reaction. The second set are associated with lipid peroxidation and they include: the thiobarbituric acid and β-carotene bleaching assays[35].

The DPPH assay is used to predict antioxidant activities by mechanisms in which antioxidants in a system act to inhibit lipid oxidation by scavenging of DPPH radical and this gives an idea of the free radical scavenging capacity of the system/substance being investigated. The method is widely used due to relatively short time required for the analysis.

The composition of honey affects the growth and survival of microorganisms through bacteriostatic or bactericidal actions[27]. Similarly, the low pH, phenolic and high sugar contents of natural honey tends to prevent the growth of microorganisms in it. However, there are indications that some species of micro-organisms could withstand the concentrated sugar and acidity of honey[36]. The presence of these micro-organisms in honey, gives an idea of its sanitary or commercial quality [42].

There are indications that the colour of honey could play some important roles in classifying/ grading different samples of honey[7, 45]. Meda *et al*[34]reported that darker honeys have a higher phenolic content than lighter honeys. Similarly, in our previous studies[20], we reported that lighter honeys could contain higher amounts of sugars than the darker honeys. This shows that there could be a strong correlation between the colour of honey and its composition. Originally, the colour of honey was determined using a simple optical device, the Pfund colour grader which compares honey with a fixed amber glass wedge and the measurements were incorporated in various standards. However, it has been reported that the spectrophotometric method is just as applicable and does not suffer from instrument to instrument variability shown by Pfund graders. Thus a simple measurement of absorbance at 560nm enables a colour classification for honey to be established (Biochrom Partners in Science). However, to the best of our knowledge, there is paucity of information on the association of the colours of different honey samples and their compositions. Information generated from this study could serve as a baseline for classifying various honey samples as well as determining their quality.

This therefore led to the study above which was designed to investigate the effect of varying colours on the physicochemical properties, microbial quality and radical scavenging activities of some commercially sold honey samples in Aba, Nigeria.

2. Materials and Methods

Five different honey samples were randomly purchased from some markets in Aba, Abia State, Nigeria. Six ml of each sample was dissolved with 2 ml of methanol, made up to 60 ml with water, and left overnight. The mixtures were centrifuged (Labtech Model Centrifuge) at 3000 x g and the supernatant was analyzed for their phenolic and flavonoid contents. The honey samples were grouped into A, B, C, D and E.

2.1 Physical properties of honey samples: The moisture, dry matter and ash contents of the various honey samples were determined using the AOAC methods[5].

2.2 Colour analysis: The undiluted honey samples were filtered with Whatman filter paper and two ml of each filtrate was measured directly with a UV spectrophotometer (Genesys UV Spectrophotometer) at 560nm[7, 45]. The colour of the samples were also visually observed in a 1cm cuvette before each reading and compared with a colour chat.

2.3 pH: The pH was measured by making a 10% v/v suspension of the samples in distilled water. The suspensions were thoroughly mixed and the pH was measured with a Hanna pH meter (Model HI1270).

2.4 Reducing sugar assay: The volumetric method of Lane and Eynon[32] as modified by the Joint FAO/WHO Codex Alimentarius Commission[29] and the International Honey Commission[26] was used to determine the reducing sugar contents of the honey samples. Results were expressed as percentage of invert sugar per 100 ml of honey.

2.5 Antioxidant assays

2.5.1 Phenolic assay: The method of Singleton and Rossi [41] was used. Briefly, to 0.1ml each extract of the different honey samples was added 50 µl of Folin-ciocalteu reagent and the whole set up was shaken for thorough mixing. After 3 minutes, 0.3 ml of 20% Na_2CO_3 was added to the reaction mixture and the whole setup was shaken and incubated for 15 minutes at room temperature.

One ml of distilled water was added to the reaction mixture and the absorbance was read at 725nm using a UV spectrophotometer (Genesys 10 VIS Thermo Electron Corporation) against the reagent blank. The total phenolic content of the samples was determined using the standard curve of gallic acid and results were expressed as mg gallic acid equivalent / 100g.

2.5.2 Assay of total flavonoids: The method of Meda et al.[34] was used. To 0.5 ml of the extract were added 0.5 ml of methanol, 50 µl of 10% $AlCl_3$ (in ethanol), 50 µl of 1mol/l of potassium acetate and 1.4 ml of water. The mixture was incubated at room temperature for 30mins and the absorbance read using an Ultra-violet spectrophotometer at 415 nm against the reagent blank. The total flavonoid contents of the samples were determined using the standard curve of quercetin and results were expressed as mg quercetin equivalent/100g.

2.5.3 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical scavenging assay: The method of Blois[8] was used with modifications. A measured amount (0.5 ml) each of the honey samples was dissolved in 200 ml of methanol to give a concentration of 2.5 mg/ml and the mixture was filtered with Whatman No. 1 filter paper. Then, 0.1, 0.2, 0.3, 0.4 and 0.5 ml of each filtrate was further diluted with methanol to give final concentrations of 125, 250,375, 500 and 625 µg/ml respectively. Finally, 0.1 ml of 0.3mM DPPH in methanol was added to each of the reaction mixtures and the whole setup was well shaken and left in the dark for 30mins before the absorbance was read spectrophotometrically at 517nm against a DPPH control containing only 1 ml of methanol in place of the extract. The same procedure was followed for standard quercetin (2.5 mg/ml in methanol) which was diluted to the concentrations: 125, 250, 375, 500 and 625µg/ml respectively. The percentage scavenging activity was calculated as:

$$\% \text{ Scavenging activity} = \frac{(\text{Absorbance of control-Absorbance of sample})}{(\text{Absorbance of control})} \times 100$$

2.6 Microbial assay

2.6.1 Isolation of microorganisms

One ml of each sample was picked with the aid of a sterile pipette. A plastic rack was arranged with 9 sterile test tubes containing 9 milliliters of sterile distilled water. A ten fold dilution[22] was done by dispersing 1ml of the sample into the first test tube (10^{-1}) which was well shaken. One milliliter was taken again from 10^{-1} dilution and transferred to the next test tube (10^{-2}). The dilution continued to 10^{-9}. Each test tube was shaken vigorously before each transfer.

2.6.2 Inoculation: The pour plate method[40] was used in plating all the samples. One ml from dilution 10^{-3} was dispersed into sterile petridish with the aid of a sterile pipette. Molten nutrient and potato dextrose agars were poured into the plates (10 ml) and isolation of bacteria was carried out using the nutrient agar while the potato dextrose agar was used to isolate fungi. The plates were swirled gently for easy mixing of the samples and the media. All plates were allowed to solidify on the bench and each plated sample was duplicated.

2.6.3 Incubation: The potato dextrose agar plates were transferred to an incubator at 25^{o}C for 3-5 days while the nutrient agar plates were transferred to another incubator at 37^{0}C for 18-48 hours. All incubated plates were examined daily for mycelia and colony growth.

2.6.4 Subculture and purification: After the incubation period, discrete colonies from bacteria were picked with a flamed wire loop and sub cultured into a newly prepared nutrient agar plate. Similarly, a flamed surgical knife was used to subculture different colour of mycelia growth from potato dextrose agar (PDA) plates on newly prepared PDA plates. All nutrient agar plates were transferred into an incubator at 37^{o}C for 18-48 hours while the PDA plates were incubated at 25^{o}C for 3-5 days. Purified colonies and mycelia were transferred into slants and stored in a refrigerator for characterization.

2.6.5 Characterization of purified culture (fungi): Macroscopic examination was done by physical characteristics of the mycelia for colour and structure. Microscopic characteristics were equally done through the morphology structures for separate, non-separate and special organs like rhizoids as described by Bernette and Hunter[6]. A wet mount method[22] was done before viewing the isolates under an x40 compound microscope. Each morphology structure of each isolate was matched with a mycology Atlas[6] for identification.

2.6.6 Characterization of purified culture (bacterium): Each purified bacterium was examined microscopically, macroscopically and gram stained. Biochemical (oxidase, H_2S, coagulase, catalase, methyl, indole) and sugar fermentation tests were carried out and results were matched with a Bacteriology Atlas[10].

2.6.7 Morphology for microbial load (Total Viable Count): One ml of each honey sample was picked with the aid of a sterile 10ml pipette. A plastic rack was arranged with 9 sterile tubes containing 9mls of sterile distilled water. A ten fold serial dilution was carried out by dropping 1ml of sample into the first test tube and the set up was well shaken. From this (10^{-1}), further dilutions were made to 10^{-9}.

2.6.8 Inoculation: A pour plate technique was used in the plating of the samples. One ml from dilution 10-3 was dropped in sterile petridish with the aid of a sterile pipette. Molten nutrient and potato dextrose agars were poured into the petridishes (about 10ml). The plates were rotated clockwise for easy mixing of the samples and the media. All the plates were allowed to solidify on the bench and duplicated.

2.6.9 Incubation: The potato dextrose agar plates were transferred to another incubator at 37^{o}C for 18-24 hours. Plates were examined daily for microbial growth.

2.6.10 Counting of colonies: After incubation of all the plates, counts of the number of colonies in each plate were done with a hand tally counter[22]. The mean of the counts were obtained and multiplied by the appropriate dilution factor. The mean count was calculated as:

$$\text{Mean} = \frac{\text{Total viable count}}{\text{Number of plates}}$$

The estimation of the viable counts in each sample was made in colony forming unit (CFU) and Total viable count = 1/weight of sample x N x D

Where N = Average number of colonies per plate; D = Dilution factor

2.7 Statistical analysis

Data was subjected to analysis using the statistical package for social sciences (SPSS), version 15.0. Results are presented as mean ± standard deviations. One way analysis of variance (ANOVA) was used for comparison of the means. Differences between means were considered to be significant at $P < 0.05$ using the Duncan Multiple Range Test.

3. Results

The colour of the honey samples decreased in the order: E >A >B > C > D (Table 1).

The ash contents of the honey samples ranged from 0.27 to 1.00(%) with honey A having the highest ash content (0.86±0.14%) while honey C had the least (0.30±0.03%) (Table 1).

The moisture contents of the five honey samples ranged from 15.66 to 19.35 (%) with honey E having the highest moisture content (19.32±0.03%) while honey C had the least (15.67±0.01%) (Table 1).

The pH values of the samples ranged from 4.32 to 4.85. Honey E was observed to have the highest pH (4.85) while honey C had the least (4.32) (Table 1).

Table 1. Physical properties of different honey samples

Sample	Colour (absorbance at 560nm)	Ash content (%)	Moisture content (%)	pH
A	1.986	0.86±0.14e	16.40±0.02b	4.83
B	1.781	0.67±0.02c	17.59±0.02d	4.52
C	1.637	0.30±0.03a	15.67±0.01a	4.32
D	1.542	0.60±0.03b	16.63±0.04c	4.34
E	2.294	0.74±0.03d	19.32±0.03e	4.85

Values with the same superscript along each vertical column are significantly different ($P < 0.05$)

In line with the standard given by the Biochrom Partners in Science and USDA (1985) (Table 2), the classification of honey colour using the pfund scale or absorbance reading at 560nm is as follows: Pfund scale of <8.0mm or absorbance reading of 0.0945nm = water white; 9-17mm or 0.189nm = Extra white; 18-34mm or 0.378nm = White; 35-50mm or 0.595nm = Extra light amber; 51-85mm or 1.389nm = Light amber; 86-114mm or 1.39 to 3nm = Amber; 114mm or >3.1nm = Dark amber (Table 2).

Table 2. Classification of honey samples based on colour absorbance at 560nm

Colour	Colour range	Pfund scale (mm)	Mid range absorbance at 560 nm
Water White	Very light colour	8.0 or less	0.0945
Extra White	Darker than water white	9-17	0.189
White	Darker than extra white	18-34	0.378
Extra Light Amber	Darker than white	35-50	0.595
Light Amber	Darker than extra light amber	51-85	1.389
Amber	Darker than light amber	86-114	1.39-3.008
Dark Amber	Darker than amber in colour	114	>3.1

Source: Biochrom Partners in Science., USDA (1985).

The USDA (2009) and Codex (1994) gave the standard for water in honey as ≤ 18.6 (good), ≤ 20 as reasonably good and >20 as bad (Table 3). The standard for reducing sugars in honey as given by the USDA (2009) and Codex (1994) is a percentage reducing sugar content of not less than 65% (Table 3).

Table 3. Standard for water in honey (%).

Grade	Water content %	Reducing sugar
Good	≤ 18.6	Not less than 65%
Reasonably good	≤ 20.0	
Low	> 20	

Adapted from USDA [46] and Codex [15]

The honey samples that were studied contained large amount of phenols ranging from 0.846 to 1.087 (g/100g), significant quantities of flavonoids ranging from 10.154 to 10.252mg/100g, significant quantities of reducing sugars except honey E as well as higher scavenging activities on DPPH free radical compared with standard quercetin (Table 4). In addition, the scavenging activities of the five honey samples analyzed and standard quercetin decreased in the following order: Honey E > Honey A > Honey B > Honey C > Honey D > Quercetin.

The percentage reducing sugar content of honey B (76.34±0.11%) was the highest among the samples that were studied while that of honey E (49.14±1.14%) was the least.

Table 4. Assay of chemical composition of different honey samples

Sample	Phenols(g/100g)	Flavonoids (mg/100g)	Scavenging activity (%)	Reducing sugars (%)
A	1.084±0.03e	10.196±0.00d	98.63±1.36c	65.79±1.14c
B	0.954±0.00c	10.250±0.02e	98.20±1.20c	76.34±0.11e
C	0.898±0.01b	10.166±0.01c	95.93±1.36c	65.36±1.10b
D	0.848±0.02a	10.154±0.01b	91.95±1.95b	66.67±1.25d
E	1.069±0.01d	10.178±0.05a	98.64±1.07c	49.14±1.14a
Quercetin			83.64±9.65a	

[a-e]Values with different superscript along each vertical column are significantly different ($P < 0.05$). N = 5 honey samples.

The microbial load of the samples ranged from 0 to 5.0 x 10^3cfu/ml for bacteria and 0 to 1.5 x 10^3cfu/ml for fungi. Sample D was observed to have the highest bacterial count (5.0 x 10^3cfu/ml) while sample A had none (0cfu/ml) whereas for fungi counts, Sample E was observed to have the highest fungi count (1.5 x 10^3cfu/ml) while samples A and B had none (0cfu/ml). The microorganisms isolated include: *Staphylococcus*, *Fusarium* and *Penicillum* (Tables 5 to 6).

Table 5. Microbial load of different honey samples and microorganisms isolated (Bacteria)

Sample	Replicate I	Replicate 2	Mean	Isolated microorganisms
A	-	-	-	NIL
B	1	-	0.5	*Staphylococcus* species
C	3	3	3	*Staphylococcus* species
D	4	6	5	*Staphylococcus*
E	5	2	3.5	*Staphylococcus*

Table 6. Microbial load of different honey samples and microorganisms isolated (Fungi)

Sample	Replicate I	Replicate 2	Mean	Isolated microorganisms
A	-	-	-	NIL
B	-	-	-	NIL
C	1	1	1	*Fusarium* species
D	1	1	1	*Fusarium* species
E	2	1	1.5	*Fusarium, Pennicillum*

Correlation analysis carried out revealed that there was a positive correlation between the colour of these samples of honey versus their pH (0.879), Phenols (0.850), percentage inhibition of DPPH radical (0.750), ash (0.622) and flavonoids (0.157) but a negative correlation between colour versus reducing sugars (-0.707), bacterial counts (0.-252) and fungi counts (-0.170) (Table 7). We obtained a positive correlation between the percentage inhibition of DPPH free radical versus phenol (0.859) and flavonoids (0.621) while we obtained a negative correlation between bacterial load of the honey samples versus pH (-0.448), ash (-0.443), phenol (-0.566) and flavonoid contents (-0.79). Similarly, we obtained a negative correlation between the fungi loads of the samples versus pH (-0.097), ash (-0.371), phenol (-0.171) and flavonoids (-0.732) (Table 7).

Table 7. Pearson correlation between the colour absorbance of honey versus its physico-chemical composition and microbial quality.

	pH	Phenol	Inhibition	Flavonoids	Reducing sugars	Ash	Bacterial count	Fungi count
Colour	0.879**	0.850**	0.769**	0.157	-0.707*	0.622	-0.252	-0.170
Inh		0.859**		0.621				
BC	-0.448	-0.566		-0.790**	-0.441	-0.443		
FC	-0.097	-0.171		-0.732*	-0.783**	-0.371		

**Correlation is significant at 0.01 level; *Correlation is significant at 0.05 level: Inh = Percentage inhibition of DPPH free radical. BC = Bacterial count; FC = Fungi count.

4. Discussion

The colour of the honey samples decreased in the order: E >A >B > C > D. Based on their absorbance at 560nm and visual observation with a colour chart, samples E and A which had the darkest colours among the samples investigated, could be classified under Amber while the rest of the honey samples could be classified under Light Amber.

Ash content is a reflection of the total inorganic minerals that are present in the sample. The ash contents of the honey samples ranged from 0.27 to 1.00(%) with honey A having the highest ash content (0.86±0.14%) while honey C had the least (0.30±0.03%) (Table 1). The percentage ash contents of all the studied honey samples were within the range of 0.02 to 1.03 that were reported by Anonn[3]) and Crane[18] as the ash contents of honey.

The moisture content of honey is one of the criteria that determines its shelf life and ability to resist spoilage by microbial fermentation. Thus the higher the moisture, the higher the probability that honey will ferment upon storage as it may serve as a substrate for the growth of microorganisms. The United States gave the standard for water in natural honeys (Table 3).The moisture contents of the five honey samples ranged from 15.66 to 19.35 (%) with honey E having the highest moisture content (19.32±0.03%) while honey C had the least (15.67±0.01%) (Table 1). The values that were obtained for moisture in these honey samples were within the range that was reported by USDA[46] (Table 3) and Codex Alimentarius[14,,15,16] which is a good attribute for these honey samples.

The acidic pH of honey is a good attribute as it promotes healing by causing oxygen release from hemoglobin[33]. In addition, this acidic pH of honey also prevents the growth of many species of bacteria. The pH of blossom honeys varies between 3.3 to 4.6. An exception is the chestnut honey with a relatively high pH value of 5 to 6. Honeydew honeys tend to have higher pH value because of their higher minerals, ranging from 4.5 to 6.5 [9]. Honey is a buffer, thus its pH does not change by the addition of an acid or base. The values we obtained for pH in the studied honey samples (4.32 to 4.85) as shown in Table 1, were consistent with the range of 4.31 - 6.0 that was reported for Nigerian honeys from other locations[1], but higher than the range of 3.2 and 4.5 that was reported by White[47]. In addition, on the basis of their pH, the honey samples could be classified as A = Honey dew; B = Honey dew; C = Blossom honey; D = Blossom Honey; E = Honey dew.

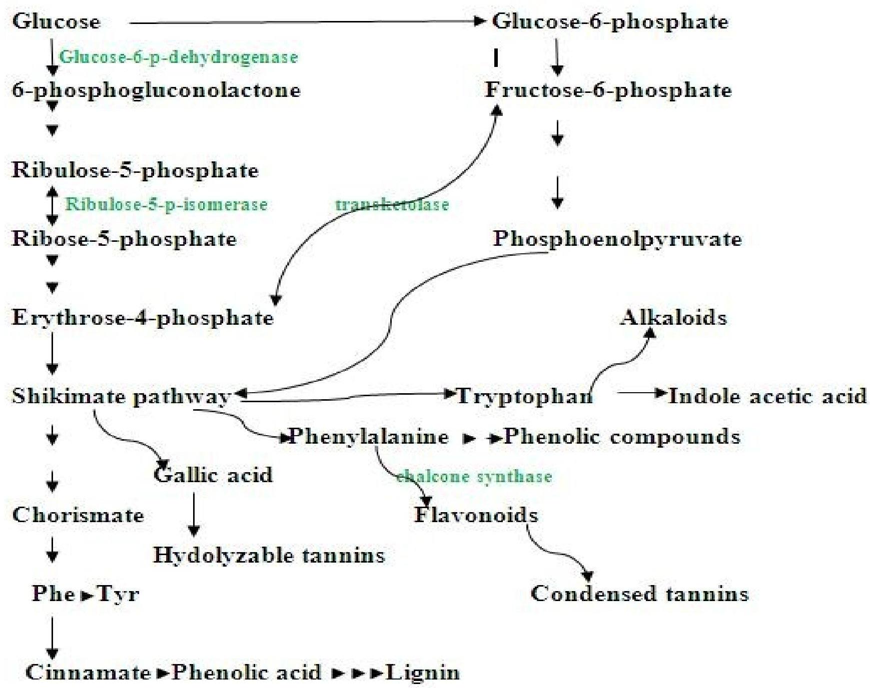
Phenolics are one of the most numerous group of compounds in plants ranging from simple molecules, such as phenolic acids, to complex compounds, such as flavonoids, flavonols, proanthocyanidins. These phenolic compounds which are derived from secondary metabolism of plants (Figure 1), have been reported to possess considerable antioxidant and antibacterial activities[21]. These activities are believed to be mainly due to their redox properties[49], which play important roles in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. The presence of these phenolic compounds is also believed to be responsible for the free radical scavenging activities of many medicinal plants. Results obtained in Table 4 indicate that all the five samples of honey that were studied, contained significant quantities of phenolic compounds, indicating possibilities of antioxidant potentials for the samples. In addition, the values we obtained for these honey samples were higher than our previous reports in some honey samples from a different location in South Eastern Nigeria[20] and this shows that honey samples from different locations tend to have different composition.

Flavonoids are the largest group of polyphenolic compounds in plants which are synthesized from the Shikimic acid pathway[39] (Figure 1). It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process[17,31, 37]. Flavonoids possess anti-inflammatory, anticancer and anti-carcinogenic activities. Honey has been reported to contain flavonoids of approximately 2 mg/100g.[23] The larger amounts of flavonoids that we obtained for the five samples investigated as shown in Table 4 compared with previous reports of Ferreres *et al*.[23] could be attributed to their floral source, environmental factors and method of processing as these have been reported to affect the composition of honey[24]. The amount of flavonoids that were obtained for the samples investigated indicate that they could possess some considerable levels of biological properties against allergies, inflammation, cancer, etc.

The DPPH assay is a widely accepted method for the determination of the antioxidant activities of various food substances. This is because, DPPH is a stable free radical in methanol or aqueous solution and accepts an electron or hydrogen radical to turn into stable diamagnetic molecule, in addition to producing a strong absorption band at 517 nm in the visible region of the electromagnetic radiation. As observed in Table 4, the scavenging activities of the five honey samples analyzed and standard quercetin decreased in the following order: Honey E > Honey A > Honey B > Honey C > Honey D > Quercetin. All the honey samples that were studied were observed to possess higher scavenging activities on DPPH free radical than standard quercetin and this is a significant finding in this present study as it shows that these honey samples could be utilized in the treatment of diseases that have free radical origin.

The main reducing sugars in honey are the monosaccharides-hexoses fructose and glucose, which are products of the hydrolysis of the disaccharide sucrose[19]. The reducing sugar contents of most of the honey samples that were studied fell within the range that was reported by Codex (1994) except honey E.

Figure 1. Biosynthesis of phenolic phytochemicals. Green colour = enzymes.



The antibacterial property of honey A could be attributed to its low pH, large amounts of polyphenolic compounds, reducing sugars as well as floral source. We assume that by synergistic interactions, they could confer high antibacterial properties on this honey. Its worth noting that honey D that had lower pH than most of the honey samples studied as well as high reducing sugar content, contained the largest amount of bacterial load. This could be attributed to its to its lower amounts of polyphenols compared with other honey samples investigated. Moreover, its possible that the honey from this source contained large amounts of catalase. Catalases act by breaking down the hydrogen peroxide (a high antibacterial agent) that is produced by glucose oxidase in honey, thereby decreasing the antibacterial action of honey [12, 13]. Thus honey samples with high catalase activities tend to have low antibacterial properties. Moreover, the species of bacteria that were isolated from this honey sample could withstand the concentrated sugar and acidity of honey[42].

The near or absolute sterile quality of samples A and B could be attributed to the possession of large amounts of polyphenolic compounds, reducing sugars as well as their floral source. The microorganisms that were isolated in the honey samples include some species of bacteria (*Staphylococcus s*pecies) and fungi (*Fusarium* and *Pennicilum* species). Although these organisms are potentially pathogenic, their counts (< 10cfu/ml) are quite too low to cause any deleterious effects. These species of micro-organisms could withstand the concentrated sugar and acidity of honey[42].

The significant correlation we obtained between the colour of honey samples versus their pH, phenols, sugars and percentage inhibition of DPPH radical suggest to us that darker honeys may have higher pH, phenols, antioxidant activities but lower amount of sugars than lighter honeys. This result could be valuable to those that utilize honey for various therapeutic purposes as it could guide them in the classification of different samples of honey on the basis of their pH, phenols, sugars and antioxidant activities.

Similarly, the significant correlation that was obtained between the percentage inhibition of DPPH free radical versus the phenolic content shows that either could used in determining the antioxidant activities of different samples of honey. Similar results of positive correlation between DPPH antioxidant activity and phenols have been previously reported[34].

Lastly, the significant correlation that we obtained between the flavonoid contents of the honey samples versus microbial load and the reducing sugar contents of the honey samples versus fungi load suggest that the flavonoid contents of honey may have more antimicrobial action than either the pH or phenolic contents of honey while the reducing sugar contents of honey may have higher anti-fungal action than either the flavonoid, pH or phenolic contents of honey. This however is subject to further investigation and confirmation.

5. Conclusion

This study shows that the colour of honey could serve as a useful tool in classifying different honey samples on the basis of their phenolic contents, reducing sugars, antioxidant activities and pH. In addition, the sterile quality of honey A confers very high therapeutic/biological properties to it. Finally, these honey samples investigated could serve as natural sources of antioxidants and could be utilized in the treatment of diseases that have free radical origin.

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