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acta SATECH 11 (1): 24 – 34 (2019)

Journal of Life &Physical Sciences

Nutritional comparison of honey samples from four States in Nigeria

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Abstract

Nutritional assessment of locally harvested honey samples from four Nigerian States was undertaken to determine their quality and extent of adulteration. Proximate composition was determined using standard methods of AOAC while mineral elements were quantified using Flame photometry and Atomic Absorption Spectrometry. Data obtained were analyzed statistically using Analysis of Variance (ANOVA), while Fischer's Least Significance Difference (LSD) was used to separate the sample means at $P<0.05$. Results of proximate analysis showed significant differences in all their compositions. Moisture ranged between 9.52-19.57% ($F_{1,8}$=259.2), ash ranged between 0.49-1.10% ($F_{1,8}$=15.1), protein ranged between 1.67-4.57% ($F_{1,8}$=74.6), oil content ranged between 0.50-1.10% ($F_{1,8}$=12.9), total carbohydrate ranged between 72.94-84.66% ($F_{1,8}$=32.3) and energy ranged between 328.20-364.44Kcal/100g ($F_{1,8}$=8.8). All minerals tested and compared were significantly different with Potassium being the dominant mineral in all samples with values ranging between 35.1-340.0mg/100g, Sodium ranged between 9.50-36.0mg/100g, Calcium ranged between 15.40-25.0mg/100g, Phosphorus ranged 14.90-18.57mg/100g and Iron ranged from 12.88-21.30mg/100g. The results of this study indicate that the honey samples compared favourably with other reported samples from many parts of Nigeria because many of the nutrients in this investigation with the exception of Ash and Protein fell within the limit of international standard.

Keywords: Honey, Proximate Analysis, Nutrients, Adulteration, Economy

Introduction

Honey is one of the most important insect food products highly sought after worldwide because of its nutritional and health benefits. It is a complex viscous mixture composed of carbohydrates, primarily fructose and glucose, water, amino acids, minerals, vitamins, enzymes as well as several other minor phytochemicals (Bodnanov *et al.,* 2008; Kaskoniene *and* Venskutonis 2010; Ajibola e*t al.*, 2012; Špánik *et al.,* 2014). Although the major components of honey remains relatively similar, their quantity and quality are influenced by the foraged plants species (Cantarelli *et al.,* 2008; Ebenezer and Olubenga, 2010; James *et al.,* 2013), climatic conditions and vegetation of the forage area (Adeniyi *et al.,* 2014), Agricultural practices, handling procedures and storage (Marchini *et al.*, 2006; Iglesia *et al.,* 2012) as well as the species of the honey bees involved and their pollen collection preferences (Busuguma *et al.,* 2016).

Honey attracts enormous commerce because of its unique nutritional and chemical compositions. It has contributed significantly to the economy of individuals and Nations around the world including Nigeria (Tijiani *et al.,* 2011; Masuku, 2013). In Nigeria, a local honey farmer could realize as much as ₦151440.00 annually an equivalent of about 50% net profit on every ₦1.00 invested into the business (Mbah, 2012). Honey could contribute as much as ₦240million per annum to the Nigeria economy (Famuyide *et al.,* 2014). In fact honey could significantly assure food security and economic diversification (Oriolowo and John, 2016). However, honey has the potential of being faked or adulterated because it is expensive and is being produced under wide fluctuations of weather and harvesting conditions (Gallardo-Velazquez *et al.,* 2009). Honey adulteration was first reported in the World market in the 1970s when high-fructose syrup was fraudulently added by industries (Plizota and Nedic-Tiban, 2009). It is an example of Economically Motivated Adulterated products (EMA) (Spink and Moyer, 2011; Everstine *et al.,* 2013). In the United State, it is one of the six most adulterated foods, indeed it accounted for about 7% of all food fraud (Alnold, 2013; Olmsted, 2016). In Nigeria, there is a growing concern and apparent lack of consumer confidence due to suspected adulteration and hence less patronage of this highly valued commodity (Omode and Ademukola, 2008; Igwe *et al.,* 2012)

In the absence of laboratory authentication, many believed that one reliable way of avoiding the purchase and use of adulterated honey is to buy from respected local honey harvesters instead of obtaining it from stores or supermarkets. This according to Olmsted (2016) is the best bet out of the dilemma of being victim of honey adulteration. This study is therefore aimed at comparing the nutritional compositions of honey samples obtained from local honey farmers from four Nigerian State with the established standard.

Materials and methods

Sample collection

Honey samples used for this study were purchased from local harvesters from four different towns in four States in Nigeria; Lafiagi in Kwara State, Dongu-Daji in Kaduna State, Warra in Kebbi State and Nasko in Niger. They were separately collected in sterilized airtight glass bottles and kept in dark cupboard and separately analyzed at The Center for Genetic Engineering and Biotechnology, Federal

University of Technology Minna, Niger State, Nigeria.

Moisture content analysis

One-hundred grams of honey sample each was weighed accurately in a pre-weighed platinum dish and gently heated in a muffle furnace at 105^0C until the sample turned black and dried. This was allowed to cool in a desiccator and re-weighed again until a constant weight was obtained. The weight loss in respect of 100g represented the moisture contents of the honey sample. The percentage moisture content (MC) was calculated for all samples using the formula below: $\%MC = \frac{M_1 - M_2}{M_1 - M_0} \times 100$

Where M_0 = wt (g) of dish

M_1 = wt (g) of dish of honey sample before drying

M_2 = wt (g) of dish of honey sample after drying

Determination of Ash content

Honey sample (2g) was ashed in a furnace at 600 ^{0}C to a constant weight. Percentage ash was calculated for all honey samples as: $\%Ash = \frac{(Wt.\ of\ crucible + ash) - Wt.of\ empty\ crucible}{Wt.\ of honey\ sample} \times 100$

Determination of crude Protein (CP)

Honey samples were analyzed for crude Protein using the routine Kjeldahl Nitrogen method (Joslyn, 1970). 10g of the homogenous honey sample was weighed into digestion flask and dissolved with 10ml of ultra-pure water. The diluent was transferred into the volumetric flask, while Kjeldahl catalyst tablet (Potassium Sulphate) was added and thoroughly shaken. 20ml of concentrated H_2SO_4 was added and fixed into the digester. The flask was cooled and the digest transferred into 100 ml volumetric flask. 5 drops of Bromocresol (indicator) and 75 ml of ultra-pure water were added. 10 ml of the digest was pipetted into the Kjeldahl distillation flask and titrated with 0.05N of Hydrochloric acid, while the percentage total nitrogen was calculated according to Joslyn (1970).

$\%Total\ nitrogen = \frac{14.0(Sample\ titre - blank\ titre)}{10 \times wt.of\ sample} \times N$

Where N = normality of acid

% CP was obtained for all the honey samples by:

% CP = % total nitrogen x 6.25

Determination of crude fat content

This was determined by extraction, using Majonnier fat extraction apparatus (AOAC, 2002). 10g of honey sample was weighed into a porous thimble. 200ml of petroleum ether was added into 250ml extraction flask. The covered porous thimble was placed into the condenser and the apparatus was assembled for extraction, which continues for 6hrs. The porous thimble was removed and the extraction flask was placed on the water bath to make it free from petroleum ether. Then the weight was taken as (W3).

Percentage fat was calculated as follows:

$$\%Fat = \frac{W_3 - W_2}{W_1 - W_0} \times 100$$

W_0 = Weight of empty porous Thimble, W_1 = Weight of Thimble + honey Sample, W_1 - W_0 = Weight of honey Sample, W_2 = Weight of empty extraction flask, W_3 = Weight of extraction flask + oil

Determination carbohydrate and energy

Carbohydrate contents of the honey samples were determined by calculation (by difference) as follows:

$$\%\text{Carbohydrate} = 100\% - (\%\ \text{Moisture} + \%\text{Crude Fat} + \%\text{Crude Protein} + \%\text{Ash})$$

The energy values of the samples were determined by calculation as follows:

Energy (KJ/100 g) = 4.186 [(%Crude Protein x 4) + (%Crude Fat x 9) + (%Carbohydrate x 4)]

Determination of crude fiber

2g of honey sample was weighed into 1liter conical flask as (W1). 200ml of boiling 1.25% H_2SO_4 and 200ml of water were added gently and boiled for 30mins using cooling finger to maintain a constant volume. A muslin cloth was used to filter and distilled water was used in rinsing. Spatula was used to scrape the material back into the flask. 200ml of boiling 1.25% NaOH was added and boiled for 30mins using a cooling finger to maintained a constant temperature. A poplin cloth was used in filtering; the residue was rinsed thoroughly with hot distilled water, and was also rinsed once with 10% HCl. It was then allowed to dry overnight in the oven at 105^0C, then cooled in a desicator and weighed as (W2); it was then kept in the muffle furnace to ash at 550^0C for 90mins. There after it was cooled and weighed as (W3). Percentage fiber was obtained by the formula:

$$\%\text{Fibre} = \frac{W_2 - W_3}{W_1} \times 100$$

Mineral Element Analysis

The honey samples were analyzed for mineral elemental determination using Atomic Absorption Spectrophotometer (AAS) and Flame Photometer according to AOAC (2005). 10g of honey sample was weighed and dissolved in ultra-pure water after thorough mixing; the solution was stirred for 15 min on a mechanical stirrer at 1550 rpm. A solution of Perchloric acid and Nitric acid were added and mixed thoroughly. This homogenous solution was dispensed into the AAS in order to determine the concentration of K, Ca, Na, Fe and P at different wavelengths. A standard was prepared for each of these elements.

Results

Proximate composition

The proximate compositions of honey sample from these four Nigerian States are presented in table 1. The results showed significant differences ($P < 0.05$) between honeys for moisture, ash, protein, fat, carbohydrate and energy. Sample D showed significant ($P < 0.05$) lower moisture content (9.52 ± 0.79%) when compared to samples A (13.72 ± 0.59%), B (18.05 ± 0.82%) and C (19.57 ± 0.76%). The ash content of sample C was significantly higher than samples A and B, but does differ significantly ($P > 0.05$) from sample D. The fat content of sample C is significantly higher than any of the other samples. Sample B has a significantly ($P<0.05$) lower protein content of (1.67 ± 0.07%) than (6.56 ± 0.82%) and (4.57 ± 0.91%) obtained for samples C and D respectively. Sample D has Carbohydrate content of (84.66 ± 1.95%) which is significantly higher than those obtained for samples B and C. the energy value of (364 ± 12.23%) obtained for sample D was significantly ($P<0.05$) higher than those obtained for other three samples.

Table 1: Proximate compositions of honey samples (%)

	Moisture	Ash	Protein	Fiber	Oil Extract	Carbohydrate	Energy (Kcal)
Sample A	13.72 ± 0.59[a]	0.62 ± 0.04[a]	2.59 ± 0.08[a]	ND	0.76 ± 0.26[a]	82.31 ± 1.07[a]	346.44 ± 8.16[a]
Sample B	18.05 ± 0.82[b]	0.49 ± 0.07[a]	1.67 ± 0.07[a]	ND	0.50 ± 0.02[b]	79.29 ± 0.82[b]	328.34 ± 6.81[b]
Sample C	19.57 ± 0.76[c]	1.10 ± 0.29[b]	6.56 ± 0.82[b]	ND	1.10 ± 0.15[c]	72.94 ± 0.89[c]	328.20 ± 3.76[b]
Sample D	9.52 ± 0.79[d]	0.80 ± 0.08[ab]	4.57 ± 0.91[c]	ND	0.81 ± 0.09[d]	84.66 ± 1.95[a]	364.21 ± 12.23[c]
LSD $_{0.05}$	*1.46*	*0.35*	*1.35*		*0.20*	*2.28*	*5.80*

The values given in the table above are mean and standard deviation (means ± SD) of triplicate analysis
*Mean values in the same **column** with the same superscript letters are not significantly different (ANOVA, LSD, P < 0.05)*
ND=Not detected

Mineral elements

The result of mineral contents of the four honey samples analyzed is presented in Table2. Honey sample A has an Iron value of (18.85 ± 1.22 mg/100g), a value which is significantly ($P < 0.05$) higher than those obtained for other three samples. Phosphorus contents ranged between (14.90 - 18.57 mg/100g). Sample A has significantly higher Calcium content of (25.00 ± 1.72 mg/100g) than either of samples B, C and D. Honey samples C and D do not differ significantly in their Sodium contents but they both have higher contents of Sodium compared to samples A and B. Sample A has the lowest content of Potassium of (35.10 ± 0.82).

Table 2: Elemental concentrations in the honey samples (mg/100g)

	Fe	P	Ca	Na	K
Sample A	18.85 ± 1.22^{a}	17.25 ± 0.82^{a}	25.00 ± 1.72^{a}	9.50 ± 0.73^{a}	35.10 ± 0.82^{a}
Sample B	21.30 ± 1.47^{b}	14.90 ± 0.92^{b}	16.00 ± 1.22^{b}	8.30 ± 0.57^{a}	51.10 ± 3.63^{b}
Sample C	15.43 ± 0.55^{c}	18.57 ± 0.21^{ac}	15.40 ± 0.08^{b}	36.00 ± 0.02^{b}	335.33 ± 2.05^{c}
Sample D	12.58 ± 0.62^{d}	16.90 ± 0.33^{a}	17.20 ± 0.25^{b}	34.00 ± 1.63^{b}	340.00 ± 2.94^{c}
$LSD_{0.05}$	*1.74*	*1.36*	*2.25*	*2.17*	*5.13*

The values given in the table above are mean and standard deviation (means ± SD) of triplicate analysis
*Mean values in the same **column** with the same superscript letters are not significantly different (ANOVA, LSD, $P < 0.05$)*

Discussion

The moisture contents of the honey samples under investigation were found to be within the limit of not more than 20% prescribed by Codex Alimentarus Commission (1998, 2001). All samples satisfied the international standard for moisture content. The moisture contents were similar to those earlier reported (Ajao *et al*., 2013; Adeniyi *et al.*, 2014; Oyeyemi *et al*., 2015). Moisture content is an important index for certifying the quality of honey because it determined the shelf life of honey (Bognadov, 2009). Moisture content also influence honey viscosity and savour (Kayode and Oyeyemi, 2014). Moisture content also determines the yeast count and subsequently the tendency for fermentation. Thus at lower moisture level of 20% and below there is minimal fermentation (Bognadov, 1999).

The ash content of honey obtained in this study ranged between (0.49-1.10%), values higher than (0.44-0.58%) reported from Ado-Ekiti (Oyeyemi *et al.*, 2015) and (0.37-0.54%) reported from North East Nigeria (Buba *et al.*, 2013). However, the ash content is lower compared to an average of (4.16 ± 1.78%) reported from university of Ilorin Apiary (Ande *et al.*, 2010). One out of four honey samples in this study showed ash contents within the limit of <0.6% set internationally (Codex Alimentarus Commission, 2001). The ash content of honey is also a criterion for determining the flora origin of honeys (Buba *et al.*, 2013). The ash is a function of mineral and trace element contents. Blossom honeys have ash content mostly between 0.1-0.3% while that of Honeydew may be as high as 1% and above (Bognadov, 2009).

The crude fats of samples in the study ranged between 050-1.10%. This value was higher

than 0.23-0.33% reported for bitter and sweet honeys (Adeniyi *et al.*, 2014). However the value fall within the range of 080-1.23% reported for honey samples from Ado-Ekiti (Oyeyemi *et al.,* 2015). Generally honey contain little amount of lipids and therefore not considered as a good source of lipids. Crude protein content of investigated honey samples falls between 1.67-6.56%. This is similar to 5.65-6.25% reported by Oyeyemi *et al.* (2015) but higher than 0.50-1.04% reported by Buba *et al*. (2013) and 0.69-0.74% by Adeniyi *et al*. (2014). Honey contains little amount of protein and 37% of protein in it are enzymes originating from the honey bee and not from the nectar. Significant differences in the protein content of honeys from this study could be ascribed to differences in their botanical origin because enzymes diastase and invertase are known to vary depending on botanical origin of the foraged flora (Oddo *et al.*, 1999).

The carbohydrate content of the honey samples ranged between 72.94-84.66%. This value was similar to 81.10-83.20% reported by Buba *et al*. (2013). It was however higher than 62.27-66.04% reported earlier (Oyeyemi *et al*., 2015). Carbohydrates are the main constituent of honey comprising about 95% of dry weight (Buba *et al.*, 2013). Total carbohydrate in the sampled honeys was within the range of 45.3-86.0% recommended limit (Ajlouni and Sujirapinyokul, 2009; Saxena *et al.,* 2010). The energy values obtained in our study were in agreement with the report of Adeniyi *et al*. (2014) who recorded 329.12-333.64 Kcal/100g for Nigerian sweet and bitter honeys. The energy values of these sampled honeys make them good sources of dietary calories. Thus is regarded as a good food for infants and adults.

The honey samples investigated are rich in essential minerals. Potassium appears to be the dominant mineral from our study. This is in conformity with the report of Agbagwa *et al* (2011) and Ndife *et al* (2014) who separately found Potassium as the dominant mineral in their studies of honey samples. The mineral content of the honey is affected by the species of plants visited by the honey bees as well as the soil types on which the floras are found (Oyeyemi *et at*, 2015). Minerals are very important in physio-chemical functioning of human body. Calcium is needed for growth and maintenance of teeth, bones and muscles (Tural *et al.,* 2003). Potassium and Sodium are components of intracellular fluids where they maintain electrolyte balance and membrane fluidity (Ahmed and Chandhavy, 2009). Iron plays an important role in haemoglobin formation, normal functioning of the central nervous system and oxidation of carbohydrate, protein and fats (Adeyeye and Okotiti, 1999). Phosphorus is a component of bones and soft tissues as phosphorus ion. It is present in enzymes as a modulator of their activities (USFNB, 1989). Energy for metabolic processes is derived from the phosphate bonds of Adenosine Triphosphate (ATP) and Creatine phosphate (USFNB, 1989).

Conclusion

Our study revealed that honey samples from these four Nigerian States are significantly different in their nutritional parameters probably because of differences in their floral origins and geographical locations. However their nutritional compositions are within the limit set by the international honey regulations. Therefore despite worldwide concerns about the quality of honeys being sold, many of the

honey sold by local harvester in Nigeria could still be nutritionally dependable.

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