

Physico-Chemical And Vitamin Constituents Of Honey Samples Obtained From Different Agro-Ecological Zones Of Nigeria

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Abstract—A total of thirty six honey samples collected from different agro-ecological zones of Nigeria were investigated for their physico-chemical and vitamin contents. The results revealed that honey had an average electrical conductivity (EC) of 1.27mS/cm, moisture content, 25.33%, ash content, 0.63 %, pH 3.82 and hygroscopicity, 1.43. The colour ranged from light green to dark amber. The value of hydroxymethylfural content ranged from 0.01 - 0.06 ppm while total acidity ranged from 13.60-41.60meq/kg. The value of 52.95% fructose sugar obtained from the Sudan Savanna was significantly different from the values of 27.76% and 28.90% obtained from Mangrove Forest and Tropical Rain Forest respectively. The average values of glucose and sucrose obtained from Nigerian honey were 26.12% and 1.10% respectively. The honey samples showed a very low average protein value of 0.038ppm. Potassium (K) had the highest value of 6.34ppm followed by iron (Fe), 6.34ppm while calcium (Ca) had the least value of 0.40 ppm in Nigerian honey. Results of the vitamin content showed that five vitamins were detected (Biotin, Riboflavin, Thiamine, Ascorbic acid and Folic acid) in honey obtained from different agro-ecological zones with ascorbic acid having the highest value of 0.31 ppm.

Keywords—Biotin, Hydroxymethylfurfural, Electrical Conductivity, Natural Honey, Acidity, Vitamins.

INTRODUCTION

Honey is a natural energetic food produced by honey bees (*Apis mellifera*) [32]. It is one of the most widely sought products because of its unique nutritional and medicinal properties [21]. Natural honey is a sweet substance produced by honey bees from sugary solution of nectar and flowers as their source of food in time of scarcity or during harsh weather conditions. Natural honey is composed of mainly sugar and water, other constituents include; proteins, minerals, organic acids, vitamins, lipids, esters and enzymes. Each of these constituents was known to have distinctive nutritional or medicinal

properties and the unique blend account for the varied different applications of natural honeys [21]. The precise composition of honey varies according to the plant species on which the bees forage. On the average, honey is composed of water (17.2%), glucose (32.16%), fructose (38.4%), sucrose (1.5%), ash (0.18%) and nitrogen (0.05%) [17]. Water is quantitatively the second most important component of honey, its content is critical since it affects the storage of honey. According to [14], only honeys with less than 18 % water can be stored with little to no risk of fermentation. He stated that the final water content of honey depends on a number of environmental factors during production such as weather and humidity inside the hive, but also on nectar condition and treatment of honey during extraction and storage.

Several investigations have shown that honey contains varying amount of mineral substances ranging from 0.02 – 1.03 g/100g, the main element found in honey is potassium with about one-third of the total mineral element [16][17][17].

The acid content of honey is relatively low, but it is important for the honey taste. [30] stated that most acids are added by the bees. The main acid in honey is gluconic acid, a product of glucose oxidation by glucose oxidase [22]. The pH of honey varies between 3.3 – 4.6 due to their higher mineral contents [31].

The amount of amino acids and protein constituents of honey are relatively small, at the most 0.7 per cent. Thus, having relatively small nutritive effect, however, these components can be important for judging the honey quality [12].

Honey contains some detectable quantities of vitamins. [13] reported that honey should not be considered as a good source of vitamins as their concentrations are best described in the part per million (ppm) ranges.

The characterization of honeys aids understanding of its properties and application hence its use as food ingredients in human diet. There is paucity of data on Nigerian honeys, whereas there are large volume of data on the characterization of honeys from North America, Europe, Australia, India and South Africa.

Therefore, the objectives of this research work were to determine the physicochemical and vitamin constituents of honeys produced from different agro-ecological zones of Nigeria.

MATERIALS AND METHODS

A total of thirty-six (36) honey samples from six (6) different agro-ecological zones in Nigeria namely (Mangrove Forest, Tropical Rainforest, Derived Savanna, Southern Guinea Savanna, Northern Guinea Savanna and Sudan Savanna) were collected in sterile bottles from identified beekeepers. These were kept in the dark cupboard to prevent photodecomposition at ambient temperature condition until they were needed for laboratory analyses. The honey samples collection were made in the year 2008 and respectively analyzed for physical properties, chemical and vitamin constituents on the biochemistry laboratory of the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

The colour of the honey samples were tested using the fund scale (an official density reading generally used in international honey trade). Fifty milliliters (50 ml) of each honey samples was measured into a beaker and thoroughly stirred. Twenty milliliters (20 ml) was measured and placed on a profound scale in the laboratory. The readings obtained were compared and the scale tabulated for each of the honey sample.

Electrical conductivity was determined by measuring 20 g of dry matter of honey in 100 ml of ultra-pure water. This was thoroughly mixed to form a solution. The electrical conductivity cell was immersed at 20°C while the reading was expressed in Millisiemens per centimeter (mS/cm) [6]. The moisture content was determined by weighing 10g of honey samples in a pre-weighed crucible, which was then dried at 105°C until a constant weight was obtained [19]. Ash content was determined by igniting at 550°C in a furnace to constant mass. The pH was measured by pH meter in a solution containing 10g of honey sample in 75ml of distilled water according to Association of Official Analytical [6].

Hygroscopicity was determined by measuring 50 ml of honey samples and left open in the beaker for about three hours. The difference in volume of the honey sample was computed from which the hygroscopicity was determined. The composition of the hydroxymethylfurfural- dehyde (HMF) of the honey samples was determined by measuring ten grammes (10 g) of unheated honey sample and dissolved in 20 ml of ultra-pure water, which was then transferred into a 50 ml Volumetric flask. Two milliliter (2 ml) of each sample was introduced into 2 test tubes and 5.0 ml solution was added to each tube. The blank was prepared by adding 1 ml barbituric acid to a tube with 1 ml of ultra-pure water. The absorbance of the test sample was read against the blank at 550 nm wave length using the spectrophotometer (Spectronic 20D model). The HMF was calculated using the equation proposed by Winker (1995).

$$\text{HMF (mg/100g)} = \text{Absorbance} \times 100$$

Cell path Length

Honey acidity was determined by the trimetric method. The addition of 0.05N NaOH, was stopped at pH 8.50 (free acidity), immediately a volume of 10 ml of 0.05 m HCL from 10 ml to pH 8.30 (lactonic acid). Total acidity was obtained by adding free and lactonic acidity [8]. Results were expressed as milliequivalent of acid per kg of honey (meg/kg) [6].

Sugar constituent of the honey samples was obtained by using the phenol-sulphuric acid method of [2]. 10 ml of each honey sample was dissolved in 250 ml of distilled water in a calibrated flask. This was stirred thoroughly and centrifuged to obtain a supernatant solution for the analysis. One milliliter (1 ml) of the diluted solution was pipetted into test tube and 1 ml of 52% phenol was added to each test tube. Five millilitre (5 ml) of 96% H₂SO₄ was also added in drops. The test tube was then allowed to stand for 10 minutes before the content was transferred into clean green-free cubettes. Stock glucose was prepared as standard. The values of the reducing sugar (Fructose and Glucose) present in each honey samples were read on a spectrophotometer at a wave length of 490 nm.

Honey samples were analyzed for crude protein using the routine Kjeldahl nitrogen method [3]. Ten grammes (10 g) of the homogeneous honey sample was weighed into digestion flask and dissolved with 10 ml of ultra-pure water. The diluent was transferred into the volumetric flask, while Kjeldhal catalyst tablet (potassium sulphate) was added and thoroughly shaken. 20 ml of concentrated H₂SO₄ was added and fixed into the digester. The flask was cooled and the digest transferred into 100 ml volumetric flask. Five drops of bromocresol (indicator) and 75 cm of ultra-pure water were added. Ten milliliters (10 ml) of the digest was pipetted into the Kjeldahl distillation flask and titrated with 0.05 N of HCL, while percentage total nitrogen was calculated using the [3] equation. The percentage crude protein was obtained for all the honey samples by multiplying the total nitrogen by 6.25 for each of the honey sample.

The honey samples were analyzed for mineral elemental content by using the atomic spectrophotometer (AAS) and Flame Photometer according to [7]. Ten (10 g) of each of the samples was weighed and dissolved in ultra-pure water and mixed thoroughly. The solution was stirred for 15 minutes on a mechanical stirrer at 1550 rpm. A solution of parloric acid and nutric acid were added and mixed thoroughly. This homogenous solution was dispensed into the AAS in order to determine the concentration of Calcium (Ca), Iron (Fe) and Potassium (K) at different wave lengths. A standard was prepared for each of these elements.

The vitamins present in honey samples being analyzed were determined by titrimetry method in accordance with the method of [28] and [7]. Biotin,

Riboflavin, Thiamine, Absorbic acid and Folic acid were determined from the honey samples obtained from different agro-ecological zones of Nigeria. Data generated were statistically analysed, using the Statistical Application System [29]. The data were subjected to Analysis of Variance (ANOVA) while the means were separated with the aid of Duncan Multiple Range Test (DMRT) at $p \leq 0.05$.

RESULTS AND DISCUSSION

The physical properties of the samples of honey obtained from different agro-ecological zones of Nigeria are summarized in Table 1. The colour ranged from light green to dark green and completely dark (Mangrove Forest – Sudan Savanna). The electrical conductivity (EC) values varied from 1.08 – 1.57 mS/cm. The lowest value was obtained from honey samples from Northern Guinea Savanna and was significantly different from the highest value of EC obtained from Mangrove forest of Nigeria. The EC is a good criterion related to botanical origin of honey and this is often used in routine honey control instead of the ash content. The electrical conductivity may also be explained by taking into account the ash and acid content of honey which reflects the presence of ions and organic acid, the higher their content, the higher the resulting conductivity [18]. The mean value of 1.27 mS/cm of EC obtained from Nigerian honey is closer to the minimum value of 1.95 mS/cm of EC reported for Liptov honey by [24].

The percentage water content is a quality parameter which is used to estimate shelf-life, staling rate and crystallization rate of honey samples. The moisture content of honey samples investigated varied from 21.13% in Sudan Savanna honeys to 30.28% in Mangrove Forest honeys (Table 1). There was a significant difference in the values of moisture content (28.98 %) of honey obtained from Tropical Rainforest and the values (23.02 %) of moisture content of the honey sample obtained from Northern Guinea Savanna and 26.47 % of moisture content obtained from Southern Guinea Savanna honeys. The moisture content variation can be explained by the composition and floral origin of honey samples. An increase in moisture content of honey is also indicative of adulteration. The values of ash content varied in the range of 0.54 – 0.79 g/100g. The highest value of ash content (0.79 g/100g) was obtained from honey samples from the Mangrove Forests and this was significantly different from the values of 0.64, 0.69 and 0.57 g/100g obtained from Tropical Rainforest, Southern Guinea Savanna and Northern Guinea Savanna respectively (Table 1). Ash represents the direct measure of inorganic residues after honey carbonization. The mean ash value of 0.63 g/100g obtained from Nigerian honey is different from the value of 0.50 g/100g obtained from honey from Ibadan as reported by [19]. This variability in ash content can be explained by the floral sources of the honey [28]. There was a significant difference in the pH values of honey samples obtained from different agro-ecological zones of Nigeria ($p \leq 0.05$). There was no significant

difference between the value of pH of 3.78 and 3.74 obtained from the Tropical Rainforest and Derived Savanna respectively but was significantly different when compared with the value of 3.65 and 4.27 obtained from Sudan Savanna and Northern Guinea Savanna respectively (Table 1).

The mean value of pH (3.87) obtained for Nigerian honey is closer to the mean value of 3.96 obtained by [15] for Nigerian honey. The hygroscopicity of honey obtained in Nigeria showed that it is a strong hygroscopic compound and that it is an important characteristic in the processing and storage of honey. The mean value of 1.43 of hygroscopicity obtained from honey samples collected, indicated that honey will absorb moisture from the atmosphere at relative humidity of about 60%. This is in agreement with the results obtained for U.S. honeys as reported by [14]. The result of hydroxymethylfurfuraldehyde (HMF) constituents of honey samples obtained from different agro-ecological zones of Nigeria revealed trace amounts. The mean HMF values varied from 0.01 – 0.06 ppm with an overall average of 0.03 ppm obtained from Tropical Rainforest and Southern Guinea Savanna and 0.02 ppm obtained from the Mangrove Forest and the Northern Guinea Savanna. However, these were significantly different from the mean values of 0.05 and 0.06 ppm obtained from the Derived Savanna and Sudan Savanna respectively, though not significantly different when compared with each other (Table 2).

The HMF measures the quality of honey via the formation of 5-hydroxymethylfurfuraldehyde by acid hydrolysis of its sucrose with the formation of red colour. The mean HMF obtained from honeys from the Sudan Savanna varied widely from that collected from the tropical rainforest [25]. This variation could be as a result of the type of the floral source and level of deterioration (Crane, 1990). The results of lactic acid free and total acidity showed that honey is very acidic. The mean values of 0.22, 18.85 and 24.28 meq/kg were obtained for the lactic, free and total acidity respectively (Table 2). The values obtained for total acidity for Nigerian honeys in this study fell within the range for Moroccan honey [20]. The acidity of honey contributes to its stability against micro-organisms. The sugar found in honey is the source of energy needed for life physiological activities. Fructose and glucose sugar are the major sugar found in the honeys collected from different agro-ecological zones of Nigeria (Table 3). The mean value of fructose of 52.95% obtained from honey samples collected from the Sudan Savanna was significantly different from 27.76, 28.90 and 25.30 % obtained from honey samples collected from Mangrove Forest, Tropical Rainforest and Northern Guinea Savanna respectively (Table 3). The mean glucose value of honey collected from Nigeria showed a range of 14.87 – 42.06 % indicating that after fructose, glucose sugar is the main constituent of honey. The mean value of 32.38 % of glucose obtained from the Mangrove Forest was significantly different from the mean value of 14.87 %

and 23.18 % obtained from Tropical Rainforest and Derived Savanna honeys respectively (Table 3). This result is in conformity with the research reported by [26] that the majority of sugar in honey are the simple sugars, fructose and glucose which represents 85 – 95 % of total sugars found in honey. Generally, fructose sugar is greater than glucose sugar for honeys collected from various agro-ecological zones of Nigeria. This is in agreement with the report of Crane (1990) who reported that fructose is more abundant than glucose sugar constituents in U.S. honeys.

The protein content detected in Nigerian honeys is in small quantities with the highest value of 0.05 ppm obtained from the Tropical Rainforest and was significantly different from the mean values of protein of 0.03 and 0.04 ppm obtained from Southern Guinea Savanna and Sudan Savanna respectively (Table 3). This result is in agreement with the report of [4] who stated that honey is not intended as a proteinaceous food, but contains a series of free amino acids necessary for health-promoting effects in human being.

The mineral elements detected in honeys collected from different agro-ecological zones of Nigeria showed that minerals are in trace quantities, except Potassium (K) and iron (Fe) which were found to occur in significant quantities in honeys investigated. The mean values of 0.40, 5.23 and 6.34 ppm of Calcium (Ca), iron (Fe) and Potassium (K) respectively obtained from honey samples obtained from Nigeria showed that these mineral elements are found in trace quantities, but significantly different from one agro ecological zone to another. Potassium regulates acid-alkaline balance in the blood and is involved in the transmission of nerve impulses, activates the functions of several enzymes and the muscular function of the heart. It also has a positive effect on the function of skin and kidney. It is the most abundant trace element followed by Fe and Ca in the honey samples investigated. This finding is in accord with many other studies on honey [14] [16] [17] reported that the main element found in honey is potassium (K) with an average of about one-third of the total composition of mineral elements in honey. Several investigations have shown that trace elements content of honey depends mainly on the botanical origin of the honey; hence, the different values of mineral elements varied from one zone to the other in Nigeria [11] [5].

Honeys obtained from Nigeria contain small, but detectable quantities of vitamin. The vitamins detected include Biotin, Riboflavin, Thiamine, Ascorbic acid and Folic acid with Ascorbic acid (vitamin C) being the dominant, followed by Folic acid in honeys investigated. The highest mean value of biotin of 0.34 ppm obtained from Guinea Savanna was significantly different from the lowest value of 0.16 ppm and 0.20ppm obtained from the Sudan Savanna and Mangrove forest honeys respectively. Biotin has a positive effect on these with seborrheic dermatitis. It is

very important for growth and developmental disorders, fragile or splitting finger nails or premature ageing of the skin. Riboflavin contributes to metabolism and helps the uptake of fat, normalizes the functions of the nervous, digestive and cardiovascular system. It also supports the function of the lower gastrointestinal tract (GIT) while Thiamine contributes to carbohydrate metabolism, supports the functions of the nervous system, heart and liver. Ascorbic acid (Vitamin C) contributes to all metabolic activities in animals. Folic acid also contributes to the production of red-blood cells and nucleic acid. It is also essential as it supports healthy immune system. However, honey should not be considered as a good source of vitamins as their concentration is best described in parts per million (ppm) range. This result agrees with the report of Baker and [10] who submitted that honey contains trace elements of several vitamins. The mean value for riboflavin detected in Nigerian honey is closer to that of the U.S. as reported by [13].

CONCLUSION

The results obtained in this study showed that physiochemical properties of Nigerian honey compared favourably with honey from other countries for the period of investigation. The colour and other physical characteristics tested varied slightly from one floral source location to the other and from one phytogeographical zone to another. Dark honey obtained from most of the honeys analyzed showed that the honey is rich in mineral constituents as reported by Aubert and [29]. The most important aspect of honey colour is in its market value and the determination of its end use. Dark honeys are most often used for industrial purposes, while light honey is market for direct consumption. Honey is deceptively acidic as its acidity contributes to its stability against micro-organisms and flavor. The results showed that Nigerian honey is very acidic with the values for total acidity falling within the range reported by [20] for Moroccan honey. In this study, honeys obtained from different agro-ecological zones in Nigeria were found to have various nutritional compositions. The fructose and glucose sugars were found to be the predominant sugars in all the honeys investigated. These results were in conformity with the range value requirement of the USDA standard for honey. The differences and other constituents obtained from different agro-ecological zones may be due to the composition and floral origin of the honey.

Table 1: Mean values (\pm SE) of physical properties of honey samples obtained from different agro-ecological zones of Nigeria

Agro-ecological zone	Colour	EC (mS cm ⁻¹)	MC (%)	Ash (%)	pH	Hygroscopicity
		1.57	30.28		3.33	
		\pm	\pm		\pm	
Mangrove forest		0.03 ^a	4.11 ^a	0.039	0.02 ^d	
		1.27	28.98		3.78	
		\pm	\pm	\pm	\pm	
Tropical rainforest	Light green	0.12 ^c	3.10 ^b	0.03 ^a	0.01 ^c	
Derived Savanna	Dark amber	1.23	22.10	0.64 \pm	3.74	1.27 \pm 0.12
		\pm	\pm	0.06 ^c	\pm	1.10 \pm 0.03
Southern Guinea Savanna	Light green	0.01 ^c	2.11 ^e	0.64 \pm	0.02 ^c	1.38 \pm 0.14
		1.35	26.47	0.04 ^c	4.17	1.18 \pm 0.031
		\pm	\pm	0.69 \pm	\pm	1.86 \pm 0.05
Northern Guinea Savanna	Light green	0.04 ^b	3.04 ^c	0.036 ^b	0.03 ^b	1.77 \pm 0.03
		1.08	23.02	0.57 \pm	4.27	1.43
		\pm	\pm	0.13 ^d	\pm	
Savanna Sudan	Dark	0.01 ^e	3.11 ^d	0.54 \pm	0.03 ^a	
		1.12	21.13	0.06 ^d	3.65	
		\pm	\pm	0.63	\pm	
Mean		0.02 ^d	2.12 ^f		0.02 ^d	
		1.27	25.33		3.82	

Mean with the same letters in the same column are not significantly different at $P \leq 0.05$ according to Duncan Multiple Range Test

EC – Electrical conductivity

MC - Moisture content

Table 2: Mean values (\pm SE) of chemical properties of honey samples obtained from different agro-ecological zones of Nigeria

Agro-ecological zone	Hydroxymethyl furfural (ppm)	Lactose acidity (m _{eq} kg ⁻¹)	Free acidity (m _{eq} kg ⁻¹)	Total acidity (m _{eq} kg ⁻¹)
Mangrove forest		3.20 \pm	10.40 \pm	13.60 \pm
Tropical rainforest		0.13 ^c	3.11 ^e	2.13 ^e
Derived Savanna	0.02 ^b	0.80 \pm	17.50 \pm	18.30 \pm
		0.12 ^f	3.01 ^c	2.14 ^d
Southern Guinea Savanna	0.01 ^b	9.00 \pm	13.50 \pm	22.50 \pm
		0.05 ^a	2.11 ^d	1.67 ^c
Northern Guinea Savanna	0.01 ^b	2.70 \pm	12.00 \pm	14.70 \pm
		0.02 ^b	2.05 ^d	2.11 ^e
Savanna Sudan	0.03	7.50 \pm	34.10 \pm	41.60 \pm
		1.21 ^b	3.11 ^a	2.31 ^a
Mean		2.10 \pm	26.00 \pm	35.00 \pm
		0.41 ^a	2.61 ^b	2.11 ^b
		4.22	18.85	24.28

Mean with the same letters in the same column are not significantly different at $P \leq 0.05$ according to Duncan Multiple Range Test

Table 3: Mean values (\pm SE) of sugar, protein and mineral constituents of honey samples obtained from different agro-ecological zones of Nigeria

Agro-ecological zone	Fructose (%)	Glucose (%)	Sucrose (%)	Protein (ppm)	Calcium (ppm)	Iron (ppm)	Potassium (ppm)
Mangrove forest						5.09	
						\pm	
Tropical rainforest		32.38		0.03		1.03 ^c	
Derived Savanna	27.76	2.40 ^b	1.02 \pm	0.05		4.18	
	\pm 2.58 ^b	14.87	0.01	\pm	0.45 \pm 0.18	4.78 \pm	
Southern Guinea Savanna	28.90	1.57 ^d	1.13 \pm	0.02	0.06 ^a	1.02 ^f	
	\pm 2.01 ^b	23.18	0.01	0.04	0.41 \pm 6.18	6.54 \pm	
Northern Guinea Savanna	28.68		1.12 \pm	\pm	0.11 ^c	1.04 ^c	
	\pm 2.95 ^b		0.01	0.01	0.43 \pm 1.02	7.42 \pm	
Savanna Sudan	25.30	1.67 ^c	1.04 \pm	0.04	0.10 ^b	1.52 ^a	
	\pm 2.07 ^b	25.26	0.12	\pm	0.42 \pm 5.06	6.76 \pm	
Mean	25.30		1.12 \pm	0.01	0.12 ^b	1.03 ^b	
	\pm 2.18 ^b	1.82 ^c	0.03	0.03	0.33 \pm 1.01	6.20 \pm	
	52.95	18.99	1.17 \pm	\pm	0.01 ^e	1.01 ^e	
	\pm 4.16 ^a	2.14 ^d	0.10	0.01	0.35 \pm 5.08	6.37 \pm	
	27.57		1.10	0.04	0.03 ^d	1.70 ^d	
	31.48	42.06	Ns	\pm	0.40	1.34 ^d	6.34
Savanna Sudan		2.89 ^a		0.01		5.77	
		26.12		0.038		\pm	
				Ns		0.95 ^b	
Mean						5.23	

Mean with the same letters in the same column are not significantly different at $P \leq 0.05$ according to Duncan Multiple Range Test

Table 4: Mean values of vitamins (\pm SE) obtained in honey samples obtained from different agro-ecological zones of Nigeria

Agro-ecological zone	Biotin (ppm)	Riboflavin (ppm)	Thiamine (ppm)	Ascorbic acid (ppm)	Folic acid (ppm)
Mangrove forest	0.20 \pm	0.22 \pm	0.21 \pm	0.24 \pm	0.12 \pm
Tropical rainforest	0.01 ^c	0.01 ^c	0.01 ^c	0.01 ^b	0.01 ^c
Derived Savanna	0.25 \pm	0.30 \pm	0.23 \pm	0.85 \pm	0.28 \pm
	0.01 ^d	0.02 ^a	0.01 ^c	0.02 ^a	0.02 ^a
Southern Guinea Savanna	0.12 \pm	0.26 \pm	0.12 \pm	0.25 \pm	0.21 \pm
	0.01 ^d	0.02 ^b	0.01 ^d	0.01 ^b	0.01 ^b
Northern Guinea Savanna	0.34 \pm	0.31 \pm	0.32 \pm	0.18 \pm	0.28 \pm
	0.02 ^a	0.02 ^a	0.02 ^a	0.01 ^d	0.02 ^a
Savanna Sudan	0.18 \pm	0.23 \pm	0.26 \pm	0.14 \pm	0.22 \pm
	0.01 ^c	0.02 ^c	0.02 ^b	0.01 ^c	0.01 ^b
Mean	0.16 \pm	0.28 \pm	0.27 \pm	0.21 \pm	0.25 \pm
	0.01 ^c	0.02 ^b	0.02 ^b	0.01	0.02 ^a
	0.21	0.22	0.26	0.31	0.23

Mean with the same letters in the same column are not significantly different at $P \leq 0.05$ according to Duncan Multiple Range Test

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