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PHYSICO-CHEMICAL AND MICROBIOLOGICAL CONSTITUENTS OF HONEY OBTAINED FROM NIGER DELTA REGION OF NIGERIA

Adenekan, M.O[1], Onasanya, R.O[1], Ikuejewa, I[2], Olubode, T[3], Akinloye, J.O[1], Ala, A.O[1] and Akinboboye, O.A[4].

[1]Department of Agriculture, Federal College of Agriculture, Ibadan, Oyo State, Nigeria.

[2]Department of Agricultural Extension and Management, Federal College of Agriculture, Ibadan, Oyo State, Nigeria.

[3]Department of Microbiology, Southwestern University, Ijebu-Ode, Ogun State

Corresponding Author: *e-mail:* mopadenekan@yahoo.com

ABSTRACT

The physico-chemical and microbiological quality of honey samples obtained from the Niger Delta region of Nigeria were investigated. There is paucity of information on the physico-chemical characteristics of honey produced from the Niger Delta region of Nigeria; hence, the need to provide a database on the characterization of honey produced in this zone. A total of eighteen honey samples collected from this zone were analyzed for their chemical properties including moisture, ash, pH, glucose and fructose content, while the microbial population including total plate count (TPC), total coliform and the levels of spore-forming bacteria and fungi were determined. Data obtained were analyzed statistically by Analysis of Variance (ANOVA), while sample means were separated with the aid of Least Significant Difference (LSD) at $P \leq 0.05$ using Statistical Analysis System (SAS) package. The mean moisture content values ranged from 12.32 – 34.02 %, while the mean ash content ranged from 0.74 – 1.22 g 100 g^{-1}. The mean pH value of 2.6 obtained from honey samples collected in Akwa Ibom was significantly very low when compared with that of 4.3 and 3.2 obtained from Edo and Bayelsa States honey samples respectively. Glucose and fructose mean values detected in honey samples also showed significant values. Four mineral elements: Potassium (K), Magnesium (Mg), Calcium (Ca) and Iron (Fe) were detected, but with levels varying from one place to another. Potassium (K) was the most abundant element with a mean value of 8.06 ppm obtained from Akwa Ibom honey samples. Results of microbiological characteristics showed that microbial profile were low for all microorganisms detected in honey samples produced from the tropical Niger Delta region of Nigeria.

Key words: Honey, Bacterium, Spore, Fungi, *Bacillus*, Niger Delta

INTRODUCTION

Honey is the natural sweet substance produced by honeybees from the nectar of blossoms or from the secretion of living parts of plants or excretion of plant-sucking insects on the living parts of plants which honeybees collect, transform and combine with specific substances of their own, store and leave in the honeycomb to ripen and mature (Codex Standard for Honey, 2001). The quality of honey is mainly determined by its sensorial, chemical, physical and microbiological characteristics. Internationally, honey quality criteria are specified in regulatory standard, compiled in a Codex Alimentarius standard, which at present is under revision (Bagdanov, 2004). Honey has several sources of microbial contaminations. Primary source of which include pollen, the digestive tracts of honeybees, dust, air, soil and nectar which are somewhat difficult to eliminate. On the other hand, secondary sources due to honey handlers and processing are easier to control by the application of good manufacturing practices (Snowdon and Cliver, 1995). The major microbial contaminants of honey include moulds and yeast as well as the spores of *Bacillus* spp. and *Clostridium* spp. (Snowdon and Cliver, 1995), being their counts indicative of honeys' commercial quality and safety.

The present study aimed at investigating the biochemical and microbiological component of honey samples obtained from the tropical Niger Delta region of Nigeria.

MATERIALS AND METHODS

In this study, three samples of honey were randomly collected from each of the identified tropical rainforest ecological zone of Nigeria (Benin, Delta, Bayelsa, Cross River, Rivers and Akwa-Ibom States). A total of 18 samples of honey were collected in this zone and were stored in a black cupboard at room temperature until needed for analysis. The analyses were carried out in the Biochemistry and Microbiology laboratories of the International Institute for Tropical Agriculture (IITA) Ibadan in 2013.

Biochemical Analysis

Moisture content was determined by weighing 10 g of honey samples in pre-weighed crucible which was then dried at 105°C until a constant weight was obtained. Ash content was determined by igniting at 550°C in a furnace to constant mass (Cavia *et al.,* 2004). The phenol-sulphuric acid method of Maynard (1970) was used to determine the reducing sugar (glucose and fructose) content of the honey samples. 10 ml of honey sample was dissolved in 250ml of distilled water in a calibrated flask. This was stirred thoroughly and centrifuged to get the supernatant solution for the analysis. 1 ml of the diluted solution was pipetted into test tubes and 1 ml of 52% phenol was added to each test tube. 5ml of 96 % H_2SO_4 was also added drop by drop and the test tube was allowed to stand for 10 min before the content was transferred into a clean, grease-free bottles and read with a spectrophotometer at a wavelength of 490nm. A blank was prepared and used to set the equipment to the zero mark. Glucose was used as a standard. The values of the reducing sugars present in all honey samples were read from the spectrometer and recorded.

Honey samples were analyzed for crude protein using the routine Kjeldahl nitrogen method (Joslyn, 1970). 10g of homogenous honey samples was weighed into digestion flask and dissolved with 10ml of ultra-pure water. The diluent was transferred into the volumetric flask, while Kjedahal catalyst tablet (potassium sulphate) was added and thoroughly shaken. 20 ml 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 0.05 N of HCl, while the percentage total nitrogen was calculated using the Joslyn (1970) equation:

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

Where N = normality of acid

% crude protein (CP) was obtained for all the honey samples using

% CP = % Total nitrogen x 6.25

The honey samples were analyzed for mineral elemental determination using Atomic Absorption Spectrophotometer (AAS) and flame photometer according to AOAC (2005). 10g of honey samples 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 Parkloric 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, Mg, Ca and Fe at different wavelengths. A standard was prepared for each of these elements.

Honey samples were analyzed using basic and additional microbiological analysis. The basic microbiological analysis consisted of total plate counts and coliform bacteria counts. The number of these microbial groups in the honey samples tested was identified and confirmed using the manual of determinative bacteriology (Beckatt and Stalanke, 1986). Data generated from the laboratory analyses were analyzed statistically using Analysis of Variance (ANOVA), while the sample means were separated using Least Significant Difference (LSD) at $P \leq 0.05$.

RESULTS AND DISCUSSION

The results of the biochemical characteristics of honey samples obtained from Niger Delta region of Nigeria are summarized in Table 1.

Honey moisture content depends on environmental conditions and the manipulation from beekeepers at the harvest period and can vary from season to season (Acquanone *et al.*, 2007). Moisture contents of honey samples from different parts of the Niger Delta region ranged from 12.32 – 34.02%. There was significant difference between the mean moisture content of 32.8% obtained from Edo State honey and 12.32% obtained from Delta State honey samples. The different moisture content of honey depends on harvest season, the degree of maturity reached in the hive and the moisture content of original plant (Finola *et al.*, 2007).

There were no significant differences in the values of ash content of the honey samples obtained from different Niger Delta region of Nigeria. The highest (1.22g 100 g^{-1}) ash content was obtained from Akwa Ibom honey sample, while the lowest (0.74g 100 g^{-1}) was obtained from Cross River State honey sample (Table 1). Ash represents the direct measure of inorganic residues after honey carbonization. The variability in ash content may be explained by the floral source of the honey (Adenekan *et al.*, 2010). pH values were in the range of 4.3 – 2.6. There was a significant difference in the pH values in honey samples obtained from Edo and Cross River states which was not significantly different ($P \leq 0.05$) when compared with the pH value of honey samples obtained from Rivers, Akwa-Ibom and Bayelsa states respectively. These results are in agreement with White (1975) who reported that honey was characteristically quite acidic, its pH being between 3.2 and 4.5.

The mean glucose value obtained from the tropical Niger Delta region of Nigeria showed a range of 30.14 – 43.12 % indicating that after fructose, glucose sugar is the main constituent of honey. This result is in conformity with the research work reported by Krell (1996) that the majority of sugars in honey are the simple sugars, fructose and glucose, which represent 85 – 95 % of total sugar found in honey.

The mean values of crude protein detected in honey samples collected from different areas of Niger Delta regions of Nigeria were in trace amount with a range value of 0.022 – 0.052%. The mean value of 0.052% obtained from honey samples collected from Cross River was significantly different from 0.022% obtained from Edo and Akwa-Ibom honey samples respectively (Table 1). The protein content detected is in small quantities and was in agreement with the report of Terrab *et al.* (2003) who stated that honey is not intended as protenaceous food, but contains a series of free amino acids necessary for health promoting effects in human being.

The mineral elements detected in honey samples collected from the Niger Delta region showed that minerals are in trace quantities. Potassium, magnesium, calcium and iron were the mineral elements

detected in trace quantities, except K and Fe, which were found to occur in significant quantities. K 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 kidneys. It is the most abundant trace elements followed by Fe and Mg found in the honey samples produced in the tropical Niger Delta region of Nigeria. This finding is in accord with Feller *et al.* (1989) and Gonzales *et al.* (2005) who reported that the main elements found in honey is K with an average of about one-third of the total composition of mineral elements in honey. Several investigators have shown that trace element contents of honey depends mainly on the botanical origin of the honey (Anon,2001 ; Adenekan *et al.*,2010), hence the different values of mineral elements obtained from honeys form rainforest zone of Nigeria.

The microbiological characteristics of honey samples obtained from the Niger Delta region of Nigeria are summarized in Table 3. The mean total bacteria count obtained from honey samples collected from Edo State was 1.3 x 10^3cfu g^{-1}, while the mean value of 4.8 x 10^3cfu g^{-1} was obtained from honey samples collected from Akwa-Ibom State. The lowest (0.72cfu g^{-1}) of TBC was obtained from Rivers State honey sample. The results of the microbial counts in the different samples of honey obtained, showed that rainforest honeys have low levels of microbes. This is in agreement with Malika *et al.* (2005) who reported that honey collected from Morocco contained low level of microbial cells.

Total coliform count (TCC) was not detected in honey samples obtained from Edo and Delta states, while TCC was very low and ranged from 0.5 – 2.0 x 10^3cfu g^{-1} in honey samples collected from Akwa-Ibom, Cross River and Rivers states, respectively (Table 3). There were no fungal spore observed in most honey samples investigated, but a very low count ranging from 0.4 – 0.3 x 10^2cfu g^{-1} was obtained from honey samples collected from Delta and Bayelsa states respectively (Table 3).

The *Bacillus* spp. bacterium detected in honey samples in this study was also low with 0.1 x 10^3 cfu g^{-1} in Delta and Akwa-Ibom states. There were no *Baccilus* cells in honey samples obtained from Edo, Bayelsa, Rivers and Cross River states. This is not surprising since Malika *et al.* (2005) reported counts of less than 10 cfu g^{-1} in Moroccan honey. The results of the selective media confirmed that the bacteria isolated from the honey samples belong to the species *Pseudomonas syringae* and *Xanthomonas axonopode*, while the *Bacillus* spp found were *Bacillus subtilis* and *B. Mycoides*. Coliform bacteria spores were not detected in most of the honey samples, except those obtained from Bayelsa and Akwa-Ibom states (Table 4).

The low levels of microbes obtained for tropical Niger Delta region honey samples are indications that honey has high anti-microbial activity. However, microbial contamination during and / or post processing rather than the indigenous microflora of honey itself can result in spoilage or persistence of some bacteria (Anon, 2001). In this investigation, the low level of total coliform count (TCC) could be explained by the evidence that honey is well preserved against bacteria so that these microorganisms would not survive unfavourable conditions. The detected microbes are plant pathogens, except *Baccilus* spp which is the main spoilage organism in food due to its versatile metabolism and heat resistant spores; hence, might have originated from the plant host containing the nectars where the bees visited.

The absence and low level of fungal spores and coliform bacteria means that the sanitary conditions of honeys produced in the Niger Delta region of Nigeria were quite efficiently handled in the apiaries and post-harvesting processes are also very efficient.

Table 1: Biochemical characteristics of honey samples obtained from Niger Delta region of Nigeria

*Source	Moisture (%)	Ash (g 100 g^{-1})	pH	Glucose (%)	Fructose (%)	Crude protein (%)
Edo (Benin)	32.81 ± 0.02	0.89 ± 0.15	4.3 ± 0.84	43.12 ± 0.15	28.16 ± 4.21	0.022 ± 0.01
Delta (Warri)	12.32 ± 11.34	0.86 ± 0.18	3.9 ± 0.64	43.01 ± 15.79	39.11 ± 4.32	0.032 ± 0.01
Bayelsa (Yenagoa)	29.77 ± 4.32	0.81 ± 0.31	3.2 ± 0.41	39.22 ± 3.49	30.41 ± 2.36	0.029 ± 0.01
Rivers (Port-	33.69 ± 5.23	0.89 ± 0.13	3.1 ± 0.04	30.14 ± 6.21	19.31 ± 4.01	0.028 ± 0.00
Harcourt)	34.02 ± 8.03	0.74 ± 0.17	2.6 ± 0.41	36.90 ± 3.21	25.11 ± 6.20	0.052 ± 0.02
Cross River	28.81 ± 4.31	1.22 ± 0.31	2.9 ± 1.02	41.16 ± 9.12	26.11 ± 17.14	0.022 ± 0.00
(Calabar)	6.53	0.25	1.39	8.35	11.12	0.02
Akwa-Ibom (Uyo)						
$LSD_{0.05}$						

*Values are mean of 3 samples.
Words in parenthesis are collection points.
± Standard deviation.

Table 2: Mean mineral constituents of honey samples obtained from Niger Delta region of Nigeria

Source	K (ppm)	Mg (ppm)	Ca (ppm)	Fe (ppm)
Edo (Benin)	4.78 ± 1.02	1.74 ± 0.01	0.46 ±	5.49 ± 1.22
Delta (Warri)	5.61 ± 1.13	1.98 ± 0.02	0.12	7.16 ± 0.12
Bayelsa (Yenagoa)	0.02 ± 0.00	1.21 ± 0.01	0.48 ±	5.39 ± 1.11
Rivers (Port-Harcourt)	0.43 ± 0.02	1.03 ± 0.01	0.12	2.49 ± 0.14
Cross River (Calabar)	5.16 ± 1.02	0.97 ± 0.11	0.43 ±	6.95 ± 1.10
Akwa-Ibom (Uyo)	8.06 ± 1.25	1.62 ± 0.01	0.14	7.09 ± 1.14
LSD $_{0.05}$	1.37	0.41	0.48 ± 0.12	0.76
			0.48 ± 0.16	
			0.51 ± 0.12	
			0.11	

Table 3: Microbial characteristics of honey samples obtained from Niger Delta region of Nigeria

Source	Total bacterial count ($x\ 10^3$ cfu g^{-1})	Total coliform count ($x\ 10^3$ cfu g^{-1})	Total fungi count ($x\ 10^3$ cfu g^{-1})	*Bacillus* spp. ($x\ 10^3$)
Edo (Benin)	1.3	-	-	-
Delta (Warri)	2.8	-	0.4	0.1
Bayelsa (Yenagoa)	1.4	1.5	0.3	-
Rivers (Port-Harcourt)	0.72	2.0	-	-
Cross River (Calabar)	2.6	0.5	-	-
Akwa-Ibom (Uyo)	4.8	0.5	-	0.1

Table 4: Occurrence of bacteria detected in honey samples obtained from Niger Delta region of Nigeria

Source	*Pseudomonas* spp.	*Xanthomonas* spp.	*Bacillus* spp.	Coliform bacteria
Edo (Benin)	++	++	--	--
Delta (Warri)	++	--	--	--
Bayelsa	++	++	++	++
(Yenagoa)	++	++	++	--
Rivers (Port-	++	--	--	--
Harcourt)	++	++	++	++
Cross River (Calabar)				
Akwa-Ibom (Uyo)				

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