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HEAVY METALS IN HONEY CONSUMED IN SOUTHWEST, NIGERIA AND ITS HUMAN HEALTH RISK

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ABSTRACT

Honey is an agricultural product known for its nutritional and various medicinal values. There is a global concern about the challenges posed to human health by the toxicity of heavy metals from consumer goods, including agricultural products. Levels of heavy metals (lead, cadmium, copper, iron, zinc, and nickel) in some selected honey samples that were randomly collected from the Southwest geopolitical zone of Nigeria were assessed using Atomic Absorption spectroscopy. The concentrations (mg/kg) of lead, cadmium, copper, iron, zinc, and nickel ranged from 0.31±0.2 to 0.94±0.4 Pb, not detectable to 0.16±0.03 Cd, 0.42±0.1 to 6.00±9.6 Cu, 0.75±0.0 to 7.91±4.8 Fe, 1.64±0.1 to 2.64±0.5 Zn, and not detectable to 0.91±0.5 Ni, respectively. The estimated daily intake of metals and the health risk index were calculated to assess human health risk. The health risk assessment showed that there was no potential risk in the consumption of honey from this region. Evaluation of green analytical methods using analytical eco-scale approach ranked the results as "excellent green analysis". Across the region under consideration, the general characteristics of the honey studied ranged as follows: ash content (g/100g): 0.17-0.67, pH: 5.27-5.45, acidity (g/100g): 0.12-0.56, electrical conductivity (mS/cm): 4.91-14.9, refractive index: 1.48-1.48, moisture (g/100 g): 21.8-23.1, and total solid content (g/100 g): 98.0-98.9. The results indicated that honey consumed in the southwest states contain heavy metals at satisfactory levels and the general characteristics were also within the tolerable limits. However, there is a need for regular assessment of agricultural products to safeguard human health

Keywords: Honey, Heavy metals, Health risk, Sweetener, agricultural product, green analytical method.

INTRODUCTION

Honey is a natural sweetener and viscous fluid produced by honey bees from the nectar of flowers. It contains a mixture of fructose, glucose, maltose and sucrose with traces of sugars depending on the floral source, proteins, vitamins, minerals, enzymes, polyphenols, flavonoids and water (Ejikeme and Ugwu, 2023; Mukaila et al., 2023; Mulugeta and Belay, 2022). Honey has nutritional (Ejikeme and Ugwu, 2023; Ukomet al., 2019) and medicinal value in the treatment of various ailments such as burns, wounds, skin ulcers, eye diseases, cough and as an antimicrobial and antioxidant agent (Asari et al. 2022; Cırık and Aksoy, 2020; Demirkaya and Celep 2022; Ejikeme and Ugwu, 2023; Ranneh et al. 2021; Ukom et al., 2019). Nectar, honeydew, pollen, and plant exudates can be contaminated by foreign substances including heavy metals, radioactive and organic contaminants-which can be transported into the beehive by the bees, therefore honey and the bees can be used as biological monitor for the determination of environmental pollution (Adeoye et al., 2021; Mair et al., 2023). Heavy

metals are potential harmful environmental pollutants that are threats to human health when present above the standard maximum tolerable limit. They are one of the most serious pollutants in the natural environment due to their toxicity, persistence and bioaccumulation (Jadaa and Mohammed, 2023; Mitra et al., 2022). It is therefore necessary to understand the nature and magnitude of these pollutants in order to combat these threats. Furthermore, general characteristics of honey, such as ash content, pH, acidity, electrical conductivity, refractive index, moisture, and total solid content can be assessed to determine the quality and safety of honey for consumption by comparing the results with the available standards. Ash content of honey determines the inorganic minerals; pH being between 3.2 and 4.5 as standard, making honey characteristically acidic (Abdi et al., 2024; Chettoum et al., 2023), which can also help in prolonging its shelf life. Measurements of electrical conductivity, which result from electrolytes such as acids, minerals, and proteins, have a suggested standard value of 0.8 mS/cm

(Abdi et al., 2024; Chettoumet al., 2023; Codex, 2001). The type and quality of honey can be known by the effect it has on light (Mahmoud et al., 2023). Honey is hygroscopic and has the ability to absorb moisture directly from the air. The refractive index of honey is altered by variations in water content, which can be measured with a refractometer. The refractive index of honey ranges from 1.504 at 13% humidity to 1.474 at 25%. It is a saturated or super-saturated solution of sugars containing 84% mixture of fructose and glucose. The water content is 15-21% by weight, and the sugar molecules interact with water molecules, making very few of the water molecules to be available for microorganisms. Food safety of honey must be taken into consideration when preparing and storing it and retain enough nutrients essential for a healthy diet, as different environmental conditions can affect the quality of honey (Szilárd et al., 2020). In order to combat the threats through consumption of honey, this study therefore aimed at determining the quality of honey consumed in the Southwest, Nigeria by determining the levels of heavy metals (Pb, Cd, Cu, Fe, Zn, and Ni) and its risk to human health. The general characteristics such as ash content, pH, acidity, electrical conductivity, refractive index, moisture and total solid content were also assessed using standard analytical procedures. The greenness of the analytical procedures was assessed using the analytical ecoscale approach.

MATERIALS AND METHODS Sample collection and preservation

In this research work, twenty bottled honey samples were randomly collected by purchasing from selected local markets and supermarkets in the Southwest, Nigeria (that is, Oyo, Ondo, Ogun, Osun, Lagos and Ekiti States). To attain the twenty representative samples, three honey samples were collected per state except Oyo state where five samples were collected. In terms of land mass, Oyo State is the largest of all the Southwestern states and was the capital of the old western region. Ibadan, the capital city of the state which is also the third largest city in Africa has large populace. It is to justify the five samples collected in Oyo state. The samples were kept in their original containers and well labelled at room temperature until analysis.

Sample pre-treatment and analysis

Approximately 2 g of each samples were measured into 100 mL quartz container, and 6 mL nitric acid (70%) and 3 mL hydrogen peroxide (30%) were added. The mixtures were placed on a heating source (digestion block) and heated for two hours in fume cabinet. Samples were filtered and transferred into 50 mL volumetric flasks and the flasks made up to mark with distilled water (Demaku et al., 2023). The digestates were subsequently analysed for lead, nickel, copper, cadmium, iron, and zinc using Atomic Absorption spectroscopy (AAS) (Perkin Elmer, Model A Analyst 200). To determine the ash content, 5 g of each honey samples were accurately weighed into crucibles. The crucibles were then placed in a muffle furnace and ignited for 6 hours until white ash was obtained. The crucibles were cooled in a desiccator and weighed until constant weight was obtained to determine the ash content. The pH of honey was determined using a pH meter (EDT Instrument, BA 350). Acidity was determined by weighing 10 g each of the honey samples into a conical flask, dissolved in 75 mL of distilled water, and then mixed thoroughly. The solution was titrated against 0.1 N NaOH solution using phenolphthalein indicator until the pink colour of the indicator persisted for at least 10 seconds. Volume of NaOH solution used was corrected by the determination of blank in water and indicator. Electrical conductivity in miliSeimens (mS) was determined by preparing 0.1M potassium chloride (KCl) solution to the determination of the cell constant, 40 mL of KCl solution was transferred into a beaker, and the conductivity cell was connected to the conductivity meter. The sample solutions were prepared by dissolving 20.0 g of honey samples in distilled water. The solutions were transferred quantitatively to a 100 mL volumetric flask, and made up to mark with distilled water. 40 mL of the sample solutions were poured into beakers, and placed in a thermostated water bath at 20 °C. Conductance in mS was read after the temperature equilibrium had been reached (Dobrinas et al., 2022). Refractive index and moisture content of honey samples were determined by homogenizing the samples in a water bath at 50 °C (± 0.2) until all the sugar crystals were dissolved, cooled to room temperature and then stirred. It was ensured that the flask was airtight. The prism of the cleaned dry

Abbé refractometer (Erma Hand Refractometer, Japan Model RHB-10 (ATC)) was evenly covered with the sample after homogenisation and the refractive index was measured. Refractive index values were used to determine the moisture content by converting these values to moisture content using the refractive index for water (nD) at 20°C as 1.3330 (Abera and Alemu, 2023; Achudume and Nwafor, 2010; Mahmoud *et al.*, 2023). The prepared samples were homogenized for the determination of total solid content using an Abbé refractometer (Bausch and Lomb, USA Model 09.45.58) (Abdi *et al.*, 2024; Dobrinas*et al.*, 2022).

Health risk assessment

The risk of heavy metals on human health from the consumption of honey considered in this study were estimated by calculating the estimated daily intake (EDI) using the formula:

EDI = CxDW

Where: EDI is the estimated daily intake, C is the mean concentration of the metals, D is the daily average consumption and W is the average human body weight (Hassan et al., 2022; Ogungbemi and Owoade, 2022). Human health risks were estimated using the Health Risk Index (HRI) which was calculated by dividing the estimated daily intake by the reference doses of the metals. Reference dose for lead, cadmium, copper, iron, zinc, nickel is 0.5, 0.1, 10, 15, 40, 1.2 mg/kg/day, respectively. The value of hazard indices (HI) must be less than 1 to not have health hazards but when the hazard index values exceed one, there may be health risks associated with over consumption of the product (Hassan et al., 2022; Ogungbemi and Owoade, 2022).

Greenness of methods

Evaluation of green analytical methods was carried out to know the environmental impact of the analytical methods by using the analytical ecoscale approach. Penalty points were assigned to parameters such as reagents, instruments by the energy consumed, waste generated and occupational hazard (Gałuszka *et al.*, 2012). Ideal value for green analysis in the eco-scale approach is 100 and penalty points are deducted from ideal figure lowering the total score. The higher the analytical eco-scale score, the greener and economical the method is.

Statistical analysis

Data obtained were subjected to descriptive statistics (mean and standard deviation). A bar chart was used to display the mean concentrations of heavy metals in honey samples collected.

RESULTS AND DISCUSSION

Total concentrations of heavy metals in honey Heavy metals in honey samples collected from different locations in the Southwest, Nigeria showed varied concentrations as presented in Table 1. All samples contained Cu and Zn in varying concentrations while in some of the samples, Pb, Cd, Fe and Ni were below the detection limit of the instrument. The mean concentrations of heavy metals in the samples are presented in Fig. 1. The highest mean concentration of Pb was 0.94±0.4 mg/kg in samples collected in Ekiti State. This was followed by Lagos State with 0.72±0.3 mg/kg, closely trailed by Oyo State with 0.70 ± 0.2 mg/kg. The lowest concentration was 0.31±0.2 mg/kg in samples collected in Osun State. The established concentration of Pb in food permitted by Health Ministry of Brazil (HMB) was 0.5 mg/kg and 0.3 mg/kgw/w by the European Commission (EC) (Adedokun et al., 2016; EC, 2006; Silva et al., 2017). Achudume and Nwafor, (2010) reported the highest mean concentration of Pb as 0.23 mg/kg in Ondo State, closely followed by Ekiti State with 0.19 mg/kg while the least was 0.04 mg/kg in Ogun State among the honey samples collected in the Southwest, Nigeria (Table 2). Lead in blood can cause nerve damage, loss of appetite, metallic taste and constipation, weakness, insomnia, headache and muscles pain, effects on haemoglobin synthesis, kidney, lung, liver, gastrointestinal tract, joints, reproductive and nervous system (Begum et al., 2023; Hassan et al., 2022; Raj and Das, 2023).

The highest mean concentration of Cd was 0.16 ± 0.03 mg/kg in the samples collected in Ogun State, followed closely by Ondo State, 0.14 ± 0.1 mg/kg. The least detectable mean concentration was 0.05 ± 0.0 mg/kg in samples collected in Lagos State and it was non detectable in samples collected in Ekiti State. The maximum tolerable intake of Cd in food set by the European

Commission was 0.1 mg/kg and 0.2 mg/kg w/w (Adedokun *et al.*, 2016; Al-Eed *et al.*, 2002; EC, 2006). Comparison of data obtained from this study with previous studies were presented in Table 2. Long-time exposure to cadmium can cause headache, chest pain, cough and bloody sputum, muscular weakness, lung disease, lung and kidney cancer, bone defect, and death (EC, 2006; Hassan *et al.*, 2022).

6.00±9.6 mg/kg in samples collected in Ogun State and the least, 0.42±0.1 mg/kg, was found in samples collected in Osun State. The established concentration limit for copper in food was 10 mg/kg (Institute of Medicine, 2001; VKM, 2017) while the maximum tolerable intake in food set by World Health Organization and Food and Agriculture Organization (WHO/FAO) was 73 mg/kg (Al-khatib *et al.*, 2022).

The highest mean concentration of Cu was

State	Sample code	Pb	Cd	Cu	Fe	Zn	Ni	
Oyo	Oy1	0.60 ± 0.01	0.08 ± 0.001	0.58 ± 0.001	11.6 ± 0.001	2.45 ± 0.0	0.75 ± 0.002	
	Oy2	0.80 ± 0.02	0.05 ± 0.002	0.65 ± 0.001	11.7 ± 0.01	2.50 ± 0.003	1.45 ± 0.01	
	Ov3	0.43 ± 0.02	0.05 ± 0.002	0.70 ± 0.0	3.95 ± 0.02	2.60 ± 0.003	0.53 ± 0.0	
	Ov4	0.98 ± 0.01	ND	0.58 ± 0.0	1.65 ± 0.002	2.13 ± 0.001	ND	
	Ov5	ND	ND	0.73 ± 0.0	10.7 ± 0.02	354 ± 0.002	ND	
	Mean+SD	0.70 ± 0.2	0.06 ± 0.01	0.65 ± 0.1	791 ± 48	2.64 ± 0.5	0.91 ± 0.5	
	FDI	0.5	0.043	0.46	5.65	1.89	0.65	
	HRI	1	0.43	0.46	0.38	0.047	0.54	
	TIKI	1	0.45	0.040	0.50	0.047	0.54	
Ondo	On1	0.58 ± 0.04	0.25 ± 0.01	0.50 ± 0.001	3.50 ± 0.01	1.85 ± 0.01	0.43 ± 0.003	
	On2	0.73 ± 0.02	0.15 ± 0.001	0.53 ± 0.0	3.13±0.02	2.03 ± 0.002	0.58 ± 0.001	
	On3	0.38 ± 0.01	0.03 ± 0.001	0.53 ± 0.0	11.1 ± 0.002	1.63 ± 0.002	0.25 ± 0.002	
	Mean+SD	0.56 ± 0.2	0.14 ± 0.1	0.52 ± 0.01	2.58 ± 1.3	1.83 ± 0.2	0.42 ± 0.2	
	EDI	0.4	0.1	0.37	1.84	1.31	0.3	
	HRI	0.8	1	0.037	0.12	0.03	0.25	
			-					
Osun	Os1	0.18 ± 0.1	0.05 ± 0.003	0.35 ± 0.001	1.15 ± 0.02	1.55 ± 0.001	0.45 ± 0.003	
	Os2	ND	ND	0.48 ± 0.001	2.88 ± 0.01	1.65 ± 0.002	0.23 ± 0.01	
	Os3	0.48 ± 0.01	0.08 ± 0.002	0.43 ± 0.001	3.30 ± 0.02	1.73 ± 0.002	0.45 ± 0.004	
	Mean±SD	0.31 ± 0.2	0.06 ± 0.02	0.42 ± 0.1	2.44±1.1	1.64 ± 0.1	0.38 ± 0.1	
	EDI	0.22	0.04	0.3	1.74	1.17	0.27	
	HRI	0.44	0.40	0.03	0.12	0.029	0.23	
Ogun	O_{c1}	1 10+0 002	0.18 ± 0.001	0.43 ± 0.001	ND	1.38 ± 0.01	0.50 ± 0.001	
Ogun	Og1	0.10 ± 0.002	0.18 ± 0.001	17.1 ± 0.001	0.75 ± 0.01	1.30 ± 0.01 1.73 ± 0.003	0.35 ± 0.001	
	Og2	0.10 ± 0.01	0.13 ± 0.004 0.12 \pm 0.02	0.52 ± 0.001	0.75±0.01	1.75 ± 0.005	0.33 ± 0.004	
	Og5 Maar±€D	0.50 ± 0.02	0.15 ± 0.005	0.33 ± 0.001	0.75 ± 0.0	2.20 ± 0.003	0.25 ± 0.005	
	mean±5D	0.50 ± 0.5	0.10 ± 0.03	6.00±9.6	0.75 ± 0.0	1.79±0.5	0.36±0.1	
		0.50	0.11	4.29	0.026	1.20	0.20	
	HKI	0.72	1.10	0.45	0.036	0.032	0.22	
Lagos	La1	0.83 ± 0.01	0.05 ± 0.002	0.73±0.0	3.03 ± 0.004	3.35 ± 0.01	ND	
	La2	0.38 ± 0.004	ND	0.95 ± 0.0	14.8 ± 0.01	2.60 ± 0.01	ND	
	La3	0.95 ± 0.004	ND	0.35 ± 0.001	ND	0.55 ± 0.003	ND	
	Mean±SD	0.72 ± 0.3	0.05 ± 0.0	0.68 ± 0.3	3.90 ± 1.2	2.17±1.5	ND	
	EDI	0.51	0.036	0.49	2.79	1.55	ND	
	HRI	1.02	0.36	0.049	0.19	0.039	ND	
Ekiti	Ek1	0.68+0.001	ND	0.58 ± 0.001	ND	2.38+0.01	ND	
Linu	Ek2	ND	ND	0.48 ± 0.001	545 ± 0.01	2.00 ± 0.01 2.10±0.0	ND	
	Ek3	1.20 ± 0.003	ND	0.78 ± 0.001	6.93 ± 0.01	335 ± 0.01	ND	
	Mean+SD	0.94 ± 0.003	ND	0.61 ± 0.001	6.19 ± 1.01	2.53 ± 0.01	ND	
	FDI	0.67	ND	0.01 ± 0.2 0.44	4.42	1.86	ND	
	LDI	1.34	ND	0.44	T.T2	0.047	ND	
	TIN	1.34	ND	0.044	0.29	0.047	nD	
	Range across States	0.31- 0.94	ND- 0.16	0.42- 6.00	0.75- 7.91	1.64- 2.64	ND- 0.91	

Note: ND - Not Detected; EDI - Estimated Daily Intake; HRI - Health Risk Index



Figure 1: Mean concentrations of heavy metals in honey samples collected from States in the Southwest.

S/N	Pb	Cd	Cu	Fe	Zn	Ni
Southwest Nigeria (This	0.31-0.94	ND-0.16	0.42-6.00	0.75-7.91	1.64-2.64	ND-0.91
present study)						
Southwest Nigeria	0.04-0.23	-	21.56-32.73	19.40-43.20	0.06-0.22	0.85-2.60
(Achudume and						
Nwafor, 2010)						
Southern Nigeria	-	-	-	1.03- 3.02	0.43- 1.15	-
(Ukom et al., 2019)						
Ethiopia	ND	0.025-0.031	1.93-2.0	-	1.97-2.04	-
(Tibebe et al, 2022)						
Kosovo	0.235-0.268	0.040-0.058	2.295-2.299	3.635-3.745	8.705-9.804	0.640-1.126
(Demaku et al., 2023)						

Table 2: Comparison of heavy metals (mg/kg) obtained in this study with previous studies.

All the mean concentrations of Cu obtained in the samples collected across the states for this study were below the maximum permissible limit of 73 mg/kg. When compared with previous studies, the highest mean concentration of Cu was reported as 32.7 mg/kg in honey samples collected in Ogun State while the lowest was 21.56 mg/kg in Oyo State (Achudume and Nwafor, 2010). Copper is necessary for growth, maintenance of bone, connective tissue, brain and heart, formation of red blood cells, absorption and utilization of iron, stimulation of immune system to fight infections, repair injured tissues

and promote healing. It is an essential micronutrient (Kolbaum *et al.*, 2023) but excess can lead to tissue injury, anaemia, liver and kidney damage, stomach and intestinal irritation, oxidative cell damage and cell death (Bost *et al.*, 2016).

Iron had a mean concentration of 7.91 ± 4.8 mg/kg obtained in samples collected in Oyo State and the highest in this study. This was followed by 6.19 ± 1.0 mg/kg in samples collected in Ekiti State. The lowest mean concentration, 0.75 ± 0.0 mg/kg, was obtained in samples collected in Ogun State. The Recommended Daily Allowance (RDA) of Fe in food is 15 mg/day for male adolescents, 11 mg/day for female adolescents, 15 mg/day for male adults, and 18 mg/day for female adults (Institute of Medicine, 2001; Piskin et al., 2022). The maximum tolerable intake in food set by World Health Organization and Food and Agriculture Organization (WHO/FAO) was 48 mg/kg (Olayiwola et al., 2017). All the mean concentrations of Fe obtained across the states were below the maximum limit of 48 mg/kg. Achudume and Nwafor, 2010 reported the highest mean concentration of Fe as 43.2 mg/kg in Ogun State and the least was 19.4 mg/kg in Ekiti State. The range of iron obtained by Ukom et al was 1.03 to 3.02 mg/kg in honey samples from Southern Nigeria (Ukom et al, 2019). Iron deficiency anaemia occurred when there is lack of iron in diet but excess can be harmful causing nausea, gastrointestinal bleeding, abdominal pain, diarrhoea, vomiting, coagulopathy, hepatotoxicity, shock and metabolic acidosis (Charlebois and Pantopoulos, 2023).

The highest mean concentration of Zn was found in samples collected in Oyo State (2.64±0.5 mg/kg), closely followed by Ekiti State (2.61 ± 0.7) mg/kg). The lowest mean concentration was 1.64±0.1 mg/kg in samples collected in Osun State. The established zinc concentration limit in food is 50 mg/kg by Health Ministry of Brazil, while the maximum tolerable intake in food set by WHO is 40 mg/kg Tolerable Upper Limit (TUL) and 12-15 mg/kg Recommended Daily Allowance (RDA). All the mean concentrations of Zn obtained across the states were below the maximum limit of 40 mg/kg. The highest mean concentration of Zn reported in Achudume and Nwafor was 0.22 mg/kg in Oyo State, closely followed by Ogun State with 0.13 mg/kg while the lowest concentration was 0.06 mg/kg in Ondo and Lagos State (Achudume and Nwafor, 2010). The range of zinc was 0.43 to 1.15 mg/kg in honey samples from Southern Nigeria (Ukom et al., 2019). Zinc is important for the health of humans and all living organisms (Hassan et al., 2022), but high concentrations may interfere with physiological functions and cause diseases in humans (Idoko et al., 2016).

The highest mean concentration of Ni was 0.91 ± 0.5 mg/kg in samples collected in Oyo State.

The lowest detectable mean concentration was 0.36 ± 0.1 mg/kg in samples collected in Ogun State but not detectable in Lagos and Ekiti State. The highest mean concentration of Ni reported was 2.60 mg/kg in honey samples collected in Ogun State while the lowest was 0.85 mg/kg in samples collected in Lagos State (Achudume and Nwafor, 2010). There has not been standard maximum tolerable intake of Ni in food currently available (Silva et al., 2017). Ni is needed by human body in small amounts to produce red blood cells. However, excess exposure can have effects on the respiratory tract and immune system, leading to decreased body weight, heart and liver damage, skin irritation, and potentially lung cancer (Begum et al., 2022).

Risk assessment

Human health risk assessment was estimated for honey consumed in Southwest, Nigeria using hazard indices. Health risk associated with the consumption of honey considered in this study is far from causing harm because the health risk index (HRI) was less than one (<1) as shown in Table 1 except in samples collected in Lagos with HRI (Pb) of 1.03, Ekiti with HRI (Pb) of 1.34 and Ogun State with HRI (Cd) of 1.14. There is no health hazard when health risk index is less than one (<1) but when it exceeds one (1), consumption of such honey may be hazardous causing various ailments. Health risk index (Pb) of 1.03 and 1.34 may have adverse effects on human health causing constipation, weakness, headache and muscles pain, kidney, liver and lung damage. Health risk index (Cd) of 1.14 may cause headache, chest pain, cough and bloody sputum, weakness, kidney and lung disease, bone defect and death. The honey may have been contaminated from polluted environment as a result of anthropogenic activities. However, moderate consumption of honey is imperative considering health risk associated with all the metals studied.

Green analytical method

Analytical eco-scale score of 83 as presented in Table 3 was obtained for heavy metals determination in honey by Atomic Absorption spectroscopy and this indicates "excellent green analysis" which showed reduction in the use and generation of hazardous substances.

Reagents	
-	Penalty points
Nitric acid	4
Hydrogen peroxide	4
, <u> </u>	$\Sigma 8$
Instruments	
	Penalty points
Digestion block	2
AĂS	1
Waste	3
Occupational hazard	3
1	$\Sigma 9$
Total penalty points	17
Analytical eco-scale total score	83

Table	3:	The	penalty	points	for	heavy	metals	determination	in	honey	samples	by	Atomic	Absorption
		spect	roscopy	(AAS).										

General characteristics of honey

The general characteristics (ash content, pH, acidity, electrical conductivity, refractive index, moisture, total solid content) of honey samples collected in the Southwest, Nigeria are presented in Table 4. The highest mean ash content (g/100g)was $0.67\pm0.4\%$ obtained in the samples collected in Lagos State while the lowest was 0.17 ± 0.2 % obtained in samples collected in Ondo State. The standard of ash content given by EU and CODEX draft in honey was 0.6 g/ 100 g (Bogdanov, 2011), which indicates the minerals' content of honey. The mean ash content across the states considered in this study, (except for Lagos with 0.67 g/ 100 g) was below 0.6 g/ 100 g which was stated as maximum limit of ash content. Achudume and Nwafor, 2010 reported the highest mean ash content of 0.39% in samples collected in Ogun State and the lowest value was 0.12% in samples collected in Osun State (Table 5). The range of ash content in honey samples collected in the Southwest, Nigeria was reported as 0.14 to 0.93% (Ayansola and Banjo, 2011). Ash content of honey samples collected in different geographical locations of Nigeria was 0.26 to 0.38% (Ndife and Dandago, 2014). Table 5 shows the comparison of the ash contents obtained in this study with those reported in the literature. The value of ash content present in honey is used to identify the plant source, and it is a direct assessment of the leftover of inorganic residues after honey is carbonized (Ali et al., 2024). Inorganic residues may be the pollutants as a result of heavy metals, and when ash content is above the standard of 0.6 g/ 100 g, health risk may be associated with the consumption of such honey.

The range of the mean pH ranged from 5.27 ± 0.1 in the samples collected in Osun State to 5.45 ± 0.4 in the samples collected in Ekiti State. The standard pH range for honey is 3.4-6.1 by Codex Alimentarius Commission (Abera and Alemu, 2023; Gela et al., 2021); however, Abdi et al. (2024) reported pH of 3.8 in pure honey. The mean pH of honey samples collected across the states and analysed in this study was within the acceptable range of 3.4-6.1. However, Achudume and Nwafor, 2010 reported the pH range of honey collected in the Southwest, Nigeria to be 4.01 -5.10. The pH can inhibit the growth of microorganisms, contributing to the stability of honey (Tischer-Seraglio et al., 2019). The mean acidity (g/100g) ranged from $0.12\pm0.03\%$ in the samples collected in Osun State to $0.56\pm0.4\%$ in the samples collected in Lagos State. The maximum limit of acidity in honey expressed as formic acid percentage (by mass) is 0.20% (Thomson, 2008). Acidity in honey samples from Ondo and Osun State were within the limit while Oyo, Ogun and Ekiti States had values slightly above the maximum limit of 0.20%. Acidity in honey samples collected in Lagos State is above the maximum limit of 0.20%. Honey fermentation causes an increase in acidity (Abera and Alemu, 2023; Bogdanov, 2011) and it occurs as honey gets older. Acidity in honey may also be different due to floras or differences in season of harvesting the honey (Abera and Alemu, 2023). Both pH and free acidity can influence the texture and stability of honey (Tischer-Seraglio et al., 2019).

State	Sample code	%Ash g/100g	рН	Acidity g/100g	Electrical conductivity mS/cm	Refractive index	Moisture g/100 g	Total solid content g/100 g
Оуо	Oy1 Oy2 Oy3 Oy4 Oy5 Mean±SD	$\begin{array}{c} 0.20 {\pm} 0.01 \\ 0.18 {\pm} 0.0 \\ 0.37 {\pm} 0.04 \\ 0.11 {\pm} 0.1 \\ 0.86 {\pm} 0.0 \\ 0.34 {\pm} 0.3 \end{array}$	$5.19\pm0.015.64\pm0.05.60\pm0.025.20\pm0.015.45\pm0.025.42\pm0.2$	$\begin{array}{c} 0.15 \pm 0.2 \\ 0.18 \pm 0.0 \\ 0.24 \pm 0.0 \\ 0.36 \pm 0.04 \\ 0.27 \pm 0.02 \\ 0.24 \pm 0.1 \end{array}$	$\begin{array}{c} 6.90 {\pm} 0.03 \\ 7.81 {\pm} 0.01 \\ 10.1 {\pm} 0.03 \\ 5.09 {\pm} 0.0 \\ 17.6 {\pm} 0.01 \\ 9.49 {\pm} 4.9 \end{array}$	$\begin{array}{c} 1.47 {\pm} 0.02 \\ 1.48 {\pm} \ 0.02 \\ 1.47 {\pm} 0.01 \\ 1.48 {\pm} 0.01 \\ 1.47 {\pm} 0.02 \\ 1.47 {\pm} 0.02 \\ 1.48 {\pm} 0.001 \end{array}$	$\begin{array}{c} 22.6 \pm 0.04 \\ 22.6 \pm 0.02 \\ 23.8 \pm 0.0 \\ 22.6 \pm 0.03 \\ 23.4 \pm 0.0 \\ 23.0 \pm 0.6 \end{array}$	$\begin{array}{c} 98.2 \pm 0.0 \\ 98.4 \pm 0.03 \\ 98.2 \pm 0.01 \\ 98.4 \pm 0.02 \\ 98.2 \pm 0.0 \\ 98.3 \pm 0.1 \end{array}$
Ondo	On1 On2 On3 Mean±SD	0.05±0.02 0.06±0.03 0.40±0.01 0.17±0.2	5.33±0.0 5.30±0.0 5.70±0.1 5.44±0.2	$\begin{array}{c} 0.15 {\pm} 0.01 \\ 0.19 {\pm} 0.01 \\ 0.25 {\pm} 0.02 \\ 0.20 {\pm} 0.1 \end{array}$	4.49 ± 0.01 4.68 ± 0.0 8.36 ± 0.1 5.84 ± 2.2	1.48±0.0 1.48±0.02 1.48±0.01 1.48±0.001	21.8 ± 0.0 21.8 ± 0.01 22.2 ± 0.02 21.9 ± 0.2	$99.2 \pm 0.01 99.2 \pm 0.04 98.2 \pm 0.01 98.9 \pm 0.6$
Osun	Os1 Os2 Os3 Mean±SD	0.18 ± 0.2 0.16 ± 0.01 0.21 ± 0.03 0.18 ± 0.03	5.32 ± 0.0 5.30 ± 0.0 5.20 ± 0.02 5.27 ± 0.1	0.10±0.1 0.12±0.04 0.15±0.0 0.12±0.03	3.98 ± 0.02 4.72 ± 0.01 6.88 ± 0.03 5.19 ± 1.5	1.48 ± 0.02 1.48 ± 0.01 1.48 ± 0.02 1.48 ± 0.001	22.6±0.0 22.2±0.03 22.6±0.02 22.5±0.2	98.2±0.02 98.4±0.01 98.3±0.01 98.3±0.1
Ogun	Og1 Og2 Og3 Mean±SD	0.47 ± 0.0 0.09 ± 0.01 0.42 ± 0.04 0.31 ± 0.2	5.15 ± 0.0 5.01 ± 0.01 5.90 ± 0.01 5.35 ± 0.5	0.23±0.02 0.17±0.1 0.17±0.1 0.22±0.04	3.66 ± 0.0 2.77 ±0.01 8.39±0.02 4.91±3.0	1.48 ± 0.02 1.48 ± 0.01 1.48 ± 0.0 1.48 ± 0.0	$22.6 \pm 0.0 \\ 22.2 \pm 0.02 \\ 22.6 \pm 0.02 \\ 22.5 \pm 0.2$	98.4± 0.0 98.4±0.01 98.4±0.03 98.4±0.0
Lagos	La1 La2 La3 Mean±SD	$\begin{array}{c} 0.91 {\pm} 0.02 \\ 0.18 {\pm} 0.0 \\ 0.91 {\pm} 0.03 \\ 0.67 {\pm} 0.4 \end{array}$	5.43 ± 0.0 5.32 ± 0.02 5.40 ± 0.0 5.38 ± 0.1	0.78 ± 0.1 0.09 ± 0.1 0.80 ± 0.03 0.56 ± 0.4	$\begin{array}{c} 19.7 \pm \ 0.0 \\ 3.97 \pm \ 0.02 \\ 20.9 \pm \ 0.01 \\ 14.9 \pm 9.4 \end{array}$	1.48 ± 0.0 1.48 ± 0.02 1.48 ± 0.01 1.48 ± 0.001	22.2±0.0 22.6±0.01 20.6±0.01 21.8±1.1	97.4±0.01 98.2±0.02 98.4±0.01 98.0±0.5
Ekiti	Ek1 Ek2 Ek3 Mean±SD	0.32±0.1 0.33±0.03 0.08±0.02 0.24±0.1	5.70±0.2 5.64±0.1 5.01±0.01 5.45±0.4	0.24±0.0 0.27±0.04 0.17±0.1 0.23±0.1	$10.2\pm 0.01 \\ 11.6\pm 0.02 \\ 3.01\pm 0.04 \\ 8.24\pm 4.6 \\ 4.01\pm 14.0 \\ 14.01 \\ 14.0 \\ 14.01 \\ 14.0$	1.47±0.03 1.48±0.02 1.48±0.01 1.48±0.002	23.8 ± 0.02 23.4 ± 0.03 22.2 ± 0.03 23.1 ± 0.8	98.3±0.0 98.2±0.03 98.2±0.01 98.2±0.1
Range a	cross states	0.17- 0.67	5.27- 5.45	0.12-0.56	4.91-14.9	1.48- 1.48	21.8-23.1	98.0-98.9

Table 4 : Mean values of general characteristics of hor	nev.
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Table 5:
 Comparison of general characteristics of honey obtained in this study with previous studies.

S/N	%Ash g/100g	рН	Acidity g/100g	Electrical conductivity mS/cm	Refractive index	Moisture g/100 g	Total solid content g/100
Southwest Nigeria	0.17-0.67	5.27-5.45	0.12-0.56	4.91-14.90	1.48-1.48	21.8-23.1	98.0-98.9
(This present study)							
Southwest Nigeria	0.121-0.389	4.017-5.05	-	136-180.71	1.449-1.467	26.39-31.54	-
(Achudume and							
Nwafor, 2010)							
Southwest Nigeria	0.14-0.93	-	0.05-0.12	-	-	11.48-15.83	84.17-84.17
(Ayansola and							
Banjo, 2011)		4 27 4 0 4		0 (1 1 0 2			02 02 104 02
(Ultram at al. 2010)	-	4.3/-4.94	-	0.64-1.05	-	-	93.23-124.23
(UKOM <i>et al.</i> , 2019)	0.26.0.38	4 10 4 58	1 30 1 55			15 60 18 41	82 10 84 31
geographical	0.20-0.30	4.10-4.30	1.50-1.55	-	-	15.07-10.41	02.10-04.01
regions. Nigeria							
(Ndife and							
Dandago, 2014)							
Southwest Ethiopia	0.09- 0.23	3.41-4.0	-	0.25- 0.39	-	18.35-	75.9- 77.5
(Abdi et al., 2024)						21.87	
Northeastern	0.12-0.49	2.93-4.53	-	-	-	11.87-	-
Ethiopia						16.70	
(Abera and Alemu,							
2023)							
Pakistan	0.12-0.34	3.32-5.12	-	0.23-0.68	-	15.66-20.14	-
(Ali et al., 2024)							

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The highest mean value of electrical conductivity (mS/cm) was 14.9 \pm 9.4 mS/cm in honey samples collected in Lagos State while the lowest value 4.91 ± 3.0 mS/cm, was obtained in samples collected in Ogun State. The standard limit of electrical conductivity in honey is 0.8mS/cm (Bogdanov, 2011). The mean value of electrical conductivity obtained in honey samples collected across the states was higher than 0.8mS/cm. The value of electrical conductivity reflects the acidity and mineral content of honey. Honey, containing electrolytes in the form of acids and minerals, exhibits varying degrees of electrical conductivity. The higher the acid and ash contents of honey, the higher the electrical conductivity, therefore, the lower the electrical conductivity the better the quality of honey (Ukom et al., 2019). The mean value of refractive index in honey samples collected across the states in the Southwest, Nigeria was 1.48. The CODEX standard of refractive index is 1.40 - 1.90 (Adams *et al.*, 2010). The higher the refractive index, the lower the moisture content, the lower the fermentation, and the better the shelf life (Albu et al., 2021). The highest moisture content was $23.1\pm0.8\%$ in honey samples collected in Ekiti State while the lowest value was 21.8±1.1% in samples collected in Lagos State. The standard value of moisture content by EU and CODEX draft is 21 g/ 100g (Bogdanov, 2011). In this study, the mean moisture content in the honey samples collected exceeded the limit of 21 g/ 100g. However, moisture in honey determines the stability of honey and spoilage resistance against yeast fermentation (Ukom et al., 2019). The highest mean total solid content (g/100g) of honey samples collected was $98.9\pm0.6\%$ in the samples collected in Ondo State and the lowest value, $98.0\pm0.5\%$ in the samples collected in Lagos State. Total solid content is inversely proportional to the moisture and if the value is more than 80%, the honey can be considered to be high grade and would be highly stable during storage (Laaroussi et al., 2020; Ukom et al., 2019). The honey samples investigated in this study would be highly stable under storage due to total solid content that was more than 80%.

Green analytical method on general characteristics of honey

Analytical eco-scale scores of 98, 97, 91, 96, 98, 100 and 100 (Table 6) were obtained for % Ash, pH,acidity, electrical conductivity, refractive index, moisture content and total solid content, respectively. For each parameters analysed, the method used was categorised as "excellent green analysis" due to high analytical eco-scale total score as a result of the relative low amounts of hazardous substances used and generated.

S/N	Characteristics	Reagents	Penalty points	Instruments	Penalty points	Total penalty points	Analytical eco - scale total score
1	% Ash	None	0	Muffle furnace	2	2	98
				Desiccator	0		
				Weighing scale	0		
				Waste	0		
				Occupational hazard	0		
2	pН	Buffer solution	0	pH meter	0	3	97
	*	Distilled water	0	Waste	3		
				Occupational hazard	0		
3	Acidity	Distilled water	0	Titration	0	9	91
		NaOH solution	4	Waste	5		
		(10–100 mL category)		Occupational hazard	0		
		phenolphthalein	0				
4	Electrical	Potassium chloride	2	Conductivity meter	0	4	96
	conductivity	Distilled water		Water bath	2		
			0				
5	Refractive index	None	0	Flask	0	2	98
				Water bath	2		
				Abbé refractometer	0		
6	Moisture content	None	0	None	0	0	100
7	Total solid content	None	0	Abbé refractometer	0	0	100

Table 6: The penalty points for general characteristics of honey.

CONCLUSION

Results of heavy metals and general characteristics of honey samples collected in Southwest, Nigeria indicated that the honey was rich in essential and beneficial minerals such as Cu, Fe and Zn required for healthy growth and their concentrations were below the maximum limits of 73 mg/kg, 48 mg/kg and 40 mg/kg, respectively. Lead concentration was above the maximum limit of 0.5 mg/kg. In all the honey samples, Cd was below the maximum limit of 0.2 mg/kg. Human health risk assessment confirmed that health hazard is not associated with the honey consumed in the Southwest, Nigeria. The health risk index (HI) was less than 1, except in the honey samples collected in Lagos, Ekiti and Ogun State with (HI) of 1.02 (Pb), 1.34 (Pb) and 1.10 (Cd), respectively. Greenness of methods evaluation confirmed that the methods used were excellent green analysis. The general characteristics of honey studied were also within the permissible limit of 0.6 g / 100 g(ash content), 3.4-6.1 (pH), 0.20% (acidity), 1.40 -1.90 (refractive index) and 80% (total solid content) except the ash content and acidity in honey samples collected in Lagos; electrical conductivity and moisture content in all the honey samples collected across all the States. There should be periodic evaluation of the quality of honey and other agricultural products consumed in the Southwest, Nigeria to monitor, sensitize and implement measures to reduce the challenges pose on human health from ingestion of contaminated food products.

CONFLICT OF INTEREST

The authors declare that they have no competing interest.

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