



Physicochemical properties of honey samples from Ondo state, Nigeria, and their bioactivity against spoilage and pathogenic organisms

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Abstract

Honey can be defined as the natural sweet substance produced by honeybees (*Apis mellifera*) from the nectar of blossoms or from the secretion of living parts of plant or plant sucking insects living on parts of plants. The medicinal property of honey has been an area of interest to researchers in recent times. This study focused on assessing the physicochemical components of honey samples and their bioactivity on some food spoilage organisms. One hundred samples of honey were collected from different locations in Ondo state, Nigeria. Their physicochemical components which include conductivity, ash content, moisture content, pH, mineral contents and colour were determined. There was variation in the physicochemical components of some of the honey samples with reference to international standards. The assessment of the honey samples as an antibacterial agent revealed its inhibitory potency on both bacteria and fungi isolated from food sample. The inhibitory effect was compared with that of standard antibiotic. The honey samples were seen to display a higher inhibitory effect on the tested organisms than the employed antibiotic.

Key words: Bioactivity, honey, physicochemical, food spoilage organisms, antibiotics.

Introduction

Honey is a sweet food made by bees using nectar from flowers ¹⁸. The variety produced by honey bees (the genus *Apis*) is the most commonly referred to and is the type of honey collected by beekeepers and consumed by humans ¹². Honey produced by other bees and insects has distinctly different properties. Honey bees form nectar into honey by a process of regurgitation, and store it as a primary food source in wax honeycombs inside the beehive. Beekeeping practices encourage overproduction of honey so the excess can be taken from the colony. Honey gets its sweetness from the monosaccharides fructose and glucose, and has approximately the same relative sweetness as that of granulated sugar ¹⁸. It has attractive chemical properties for baking, and a distinctive flavor that makes some people to prefer it over sugar and other sweeteners. Most microorganisms do not grow in honey because of its low water activity of 0.6 ¹³. However, honey sometimes contains dormant endospores of the bacterium *Clostridium botulinum*, which can be dangerous to infants, as the endospores can transform into toxin-producing bacteria in the infant's immature intestinal tract, leading to illness and even death ²².

Honey has a long history of human consumption, and is used in various foods and beverages as a sweetener and flavoring ¹². It also has a role in religion and symbolism. Flavors of honey vary based on the nectar source, and various types and grades of honey are available. It is also used in various medicinal traditions to treat ailments. The study of pollen and spores in raw honey can help to determine floral sources of honey. Furthermore, because bees carry an electrostatic charge, and can attract other particles, the same technique of its pollen and spores study can be used in area environmental studies of radioactive particles, dust or particulate pollution ¹⁶. The term traditional medicine (indigenous

medicine or folk medicine) essentially represents a natural form of health care which has been used through generations ¹.

Traditional medicine practices existed in Africa and other cultures for centuries since man came into being but until recently, has been neglected or even outlawed in some cases due to undue pressure from practitioners of modern medicinal practice and unscientific background of its method of operation ⁸. The 21st century is witnessing serious scientific effort to discover major active ingredients in medicinal plants through research and development. According to Osermene *et al.* ²⁰, this could help orthodox medicine to comprehensively address most disease conditions plaguing mankind or it may be a response to the clarion call by the World Health Organization ¹⁷ that developing countries should endeavour to develop and utilize local medications that are most appropriate to their local circumstances especially for Primary Health Care (PHC) in order to reduce the cost associated with incessant drug importation ¹⁷. Traditional medicine is the sum of all knowledge and practical application, whether explicable or not used in diagnosis, prevention and elimination of physical, mental or social imbalances and relying exclusively on practice and experience and the sociological environment ^{5, 23}. Most of these medicines produced traditionally entail herbs from different plants, honey, *Aloe vera*, and from bitter tasted plants ¹⁵. This present study was aimed at investigating the physico-chemical properties of honey samples in Ondo state, Nigeria and their inhibitory potency on spoilage bacteria isolated from food samples.

Materials and Methods

Study area: The Ondo state of Nigeria is one of the states in south west Nigeria (7°10'0"N and 5°5'0"E). It covers 15,500 km², with an estimated population in the 2006 census of 3,440,000

inhabitants. The climate condition of this state is classified as tropical, with a mean temperature ranging from 25°C to 29°C year round. It has a minimum and maximum temperature between 21.6 and 33.8°C respectively; and a mean relative humidity of 85%.

Sample collection: The honey samples were purchased from different honey sellers from various locations in the Ondo state of Nigeria namely; Akure, Owo, Akungba, Ile-oluji, Ondo town, Ikare-Akoko, Ilara, Igbara Oke, Itaogbolu and Iju. They were taken to the laboratory and screened for their microbiological and physicochemical properties. The local food samples namely rice, beans moin-moin, pounded yam and amala were collected into sterile Petri dishes from selected cafeteria in Akure and were taken to the laboratory for microbiological examination.

Microbiological examination of honey and food samples: The fresh honey samples were conveyed to microbiology research laboratory of Federal University of Technology, Akure. The honey samples were filtered separately with sterile Seitz filter and the obtained filtrates were aseptically streaked on nutrient agar and potato dextrose agar plates and incubated at 37°C for 24 h and 27±2°C for 72 h, respectively, to observe for bacterial and fungal growth. After incubation, the honey plates where no growth were observed were dispensed into sterile Pyrex sample bottles and kept at room temperature prior to further analyses. One g of each of the food samples was weighed and with the use of sterile spatula homogenized inside 9 ml of sterile distilled water. This was serially diluted and 0.1 ml was pour plated on nutrient agar and potato dextrose agar plates and incubated at 37°C for 24 h and 27±2°C for 72 h, respectively, to observe for bacterial and fungal growth.

Physico-chemical analyses of honey samples: The pH of the samples was measured using a pH meter according to the method described by Gonnet ⁹. The moisture content was obtained by drying the granulated pulp in a hot air oven under vacuum at 105°C until a constant weight was attained and the ash content by incinerating dried samples in a muffle furnace at 550°C for 6 h. They were then cooled in a desiccator and weighed immediately ⁴. The mineral contents were analyzed using the atomic absorption and spectrophotometer method as described by Paulwels *et al.* ²¹. The minerals analyzed included calcium, potassium, sodium and phosphorus. Vitamin C content was also analyzed. Electrical conductivity was determined by measuring 20 g dry matter of honey in 100 ml of ultra pure water. This was mixed thoroughly to form a solution. The electrical conductivity cell was immersed at 20°C while reading was expressed in µmhos. The colour of the honey samples was determined by using the P- fund scale (mm). Two ml of the honey samples was poured in a beaker, the instrument was calibrated, dipped into the sample and the reading was taken.

Antibacterial activity of honey samples and antibiotic sensitivity tests: An agar diffusion method was used to access the antibacterial activity of the selected honeys against the 5 isolated bacterial strains. A cell load of 10⁷-10⁸ cfu/ml in their log phase of isolated bacteria were inoculated in Mueller- Hinton broth and incubated for 10 h. Petri dishes containing 1ml of each bacterial isolate in the broth and Mueller-Hinton agar were prepared. Wells were bored with a sterile cork borer on the seeded agar plates. The honey, water and antibiotics were aseptically pipetted into the bored well and the plates were incubated uninverted at 37°C for 24 h. The water and antibiotics served as negative and positive controls, respectively. The sensitivity of the tested organisms was indicated by zones around the wells and diameter was taken as an index of degree of sensitivity.

Results and Discussion

Bacteria and fungi isolated from the tested food samples included *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Aspergillus fumigates*, *Salmonella typhi* and *Varicosporium elodea* (Table 1), most of which are pathogenic microorganisms. However, *Bacillus subtilis* is also a spoilage bacterium. *Varicosporium elodea* is spoilage fungus while *Aspergillus fumigates*, a fungus, has a potential to produce mycotoxins.

Most foods contain viable bacteria and fungi, which could be result of improper handling, exposure to dust, air, flies or result of the food being under heated. These food spoilage organisms can also be pathogenic to consumers. Endospores of *Bacillus* species are more resistant to heat which could be the reason why this species occurred most in the food samples. Physiologically, these organisms could produce chemical changes in foods, such as breaking down of proteins to polypeptides, amino acids, fats to glycerol and fatty acids, and hydrolysis of complex carbohydrates to simple ones. Unpleasant odours resulting from gases formation may occur. Spoilt rice is by nature slimy and rotten. This nature readily provides a veritable and suitable environment for the growth of fungal isolate like *Aspergillus fumigates*. This was in agreement with the work of Leveen *et al.* ¹⁴. Vit *et al.* ²⁶ reported that the pH and temperature of food substrate readily support the growth of mesophilic food spoilage organisms such as fungal isolates.

The physicochemical properties of the different samples of honey obtained from different locations in Ondo, Nigeria, are reported in Table 2. The color ranged from light amber to completely dark. The pH values were in the range of 2.90 to 4.40. Although there was significant variation between honey obtained from Itaogbolu and the remaining locations. The values were within the reference value and in agreement with White ¹¹ who reported that honey was characteristically quite acidic. The pH of honey is low enough to inhibit the growth of many species of bacteria but this acidity is neutralized in the body by buffering fluids. The moisture content investigated varied from 13.28-17.98%. The lowest was recorded in Igbara Oke sample while the highest was from Ikare Akoko samples. This could be as a result of the composition and floral origin of the honey samples. The low moisture content property of honey serves as a protection from attack by microorganisms ²⁵. When the moisture content is high, it is an indication of adulteration. According

Table 1. Frequency of microorganisms isolated from selected food samples.

Food samples	<i>Bacillus cereus</i>	<i>Bacillus subtilis</i>	<i>Escherichia Coli</i>	<i>Aspergillus fumigatus</i>	<i>Salmonella typhi</i>	<i>Varicosporium elodea</i>
Beans	+	+	+	-	-	+
Yam	+	+	-	+	+	-
Moin- moin	+	+	+	-	+	+
Amala	+	+	-	+	-	-
Rice	+	-	-	+	-	-

Table 2. Mean values±SE of physicochemical properties of selected honey samples.

Honey samples	pH	Moisture (%)	Ash (%)	Electrical conductivity (µmhos)	Mineral content(µg/g)				Vitamin C (mg/10ml)	Color	Glucose	Fructose	Reference values
					Ca ²⁺	K ⁺	P	Na ⁺					
Akure	4.35±.15	13.40±.02	0.30±.01	23.00±0.2	1.90±.03	0.50±.01	50.00±2	30.31	2.00±.02	Dark Amber	32.42±2	33.12±2	Glucose= 22.0-40.7 Fructose= — pH=3.4-6.1
Owo	3.28±.02	17.00±.01	0.70±.01	51.50±0.2	1.80±.01	1.11±.03	44.90±1	30.91	3.75±.01	Dark Amber	34.23±1	29.42±1	Moisture(%)=13.4-26.6
Akungba	4.40±.02	16.21±.01	0.10±.01	12.50±0.1	1.40±.02	0.65±.01	18.61±1	75.26	1.25±.01	Light Amber	30.23±4	31.22±5	Ash=0.020-1.030
Ile-oluji	4.40±.01	16.75±.01	0.60±.02	45.00±0.2	1.00±.03	1.36±.02	50.43±1	42.515	3.40±.01	Amber	33.45±3	31.00±3	Electrical conductivity= —
Ondo town	3.27±.05	16.00±.02	0.05±.01	19.20±0.1	2.10±.01	0.20±.01	32.42±2	40.22	1.90±.02	Amber	32.42±2	29.12±3	Ca ²⁺ =4.0-30.0
Ikare-Akoko	3.21±.12	17.98±.01	0.13±.02	17.60±0.1	2.60±.01	1.60±.01	22.01±2	17.69	2.35±.03	Amber	32.42±2	27.52±4	K =1.0-47.5
Ilara	4.00±.21	13.61±.01	0.11±.02	4.00±0.1	2.90±.02	0.70±.02	22.98±1	20.28	1.80±.02	Amber	31.33±1	28.42±2	P=2.0-60.0
Igbara Oke	3.89±.11	13.28±.03	0.28±.02	21.00±0.2	1.60±.02	1.32±.02	20.01±2	20.30	3.40±.01	Amber	30.32±3	30.12±1	Na ⁺ = 0.6- 40
Itaogbolu	2.90±.07	14.14±.02	0.18±.01	3.60±0.1	2.00±.01	0.19±.01	21.24±1	9.68	4.30±.01	Light Amber	32.34±1	33.66±1	Vitamin C = —
Iju	3.12±.20	15.21±.03	0.55±.01	38.50±0.2	1.50±.01	0.68±.01	22.45±1	20.28	1.40±.02	Light Amber	35.12±1	32.42±2	Color=light to amber

Reference values from Tchoumboue *et al.*,²⁴ Vinda-Mantos *et al.*,² Anon.³, Crane⁶⁷.

Table 3. Antibacterial activity of honey samples and antibiotic sensitivity test on food spoilage organisms.

Tested organisms	Selected honey samples													Antibiotics				
	A	B	C	D	E	F	G	H	I	J	AM	CPX	S	PEF	GRI			
<i>Bacillus</i>	14±2	13±2	11±2	12±2	13±2	12±2	11±2	13±2	15±2	9±2	10±2	13±2	10±2	12±2	10±2			
<i>Cereus</i>	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm			
<i>Bacillus subtilis</i>	13±2	10±2	12±2	13±2	12±2	14±2	10±2	12±2	16±2	11±2	10±2	10±2	12±2	12±2	12±2			
<i>Escherichia coli</i>	18±2	16±2	15±2	15±2	20±2	18±2	15±2	15±2	15±2	21±2	18±2	17±2	13±2	20±2	13±2			
<i>Aspergillus fumigatus</i>	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm			
<i>Salmonella typhi</i>	2±1	NR	NR	NR	2±1	NR	NR	NR	10±2	NR	NR	NR	NR	NR	7±1			
<i>Varicosporium elodea</i>	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm			
	16±2	10±2	10±2	10±2	15±2	10±2	10±2	10±2	18±2	10±2	10±2	18±2	11±2	20±2	NR			
	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm			
	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	5±1			
	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm			

Key: NR= No zone of inhibition A=Akure, B= Owo, C= Akungba, D= Ile-oluji, E= Ondo town, F=Ikare- Akoko, G=Ilara, H=Igbara Oke, J=Itaogbolu, AM=Ampiclox, CPX=Ciprofloxacin, S=Streptomycin, PEF=Penoxacin, GRI= Griseofulvin.

to the international regulatory standard for honey¹⁰, honey with high water content aids fermentation or deterioration. Values for the ash content ranged from 0.10% to 0.70%. This variability in ash content could be explained by the floral source of the honey²⁶. Obtained values are within the reference value. The mineral content from Akungba Akoko and Ondo town turned out to be in decreasing order of Na, P, Ca and K while in samples from the remaining locations, the phosphorus content is highest followed by sodium and the least mineral content is potassium. The presence of these minerals makes it nutritionally suitable for both children and elderly people. Variation in mineral content was recorded from the reference value in some honey samples for instance the sodium ion content samples from Akungba Akoko and Ile Oluji did not agree with reference value. The electrical conductivity values varied from 3.60 to 51.50 μ mhos. According to International Standard of honey (2002), the electrical conductivity of honey is near zero hence higher conductivity indicates adulteration of the honey sample.

The bioactivity of honey samples compared favourably with the tested conventional antibiotics. The honey sample showed more inhibitory potency against the tested bacteria than the fungal isolates (Table 3). Generally honey sample from Itaogbolu displayed high inhibitory potency against all the tested organisms with the highest zone of inhibition recorded against *Escherichia coli* (21 \pm 2 mm), this in no doubt could be because of the physicochemical quality this sample possessed, although its bioactivity against fungal isolates was low compared to the conventional antibiotics. In all the honey samples tested none was able to inhibit *Varicosporium elodea*. The antibiotic griseofulvin, was able to inhibit *Varicosporium elodea* with 5 \pm 2 mm zone of inhibition while (7 \pm 1 mm) was recorded for *Aspergillus fumigatus*, however, honey samples from Itaogbolu showed greater zone of inhibition than this antibiotic (10 \pm 2 mm). This study revealed that the honey samples possess different antimicrobial activities, which agreed with the study conducted by Omoya and Akharaiyi¹⁹ that honey in its saturated solution of sugar will cause osmotic effect on bacteria.

Conclusions

The results confirm that the physicochemical parameters, such as moisture content, pH level and sugar content of different honey samples, influence their bioactivity against microorganisms. The honey samples were able to establish variable inhibitory zones *in vitro*. Favourably comparison was established between the honey samples and the conventional antibiotics employed on the tested spoilage organisms. Honey may therefore be a potential eco-friendly intervention to control food spoilage and pathogenic microorganisms.

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