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Full Length Research Paper

Microbiological and physico-chemical properties of some commercial Nigerian honey

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A total of 10 honey samples from different geographical locations of Nigeria were evaluated for their physico-chemical properties and microbiological quality. The honey samples were examined for antimicrobial activity against *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Bacillus cereus, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella spp, Shigella spp, Clostridium sporogenes and Candida albicans.* The study revealed that the honeys had an average pH of 3.86, moisture content of 14.09%, ash content of 0.28% and electrical conductivity of 34.09 µS/cm. The free acidity was about 27.00 meq/kg, lactone acidity 10.55 meq/kg and total acidity of about 37.20 meq/kg. Results of the microbiological characteristics showed total coliform counts of 0 - 3.0 × 10 cfu/g and total aerobic mesophilic bacteria (TAMB) between 1.0 × 10^3 and 5.0 × 10^3 cfu/g. Yeasts and moulds were not detected. The *Bacillus* species detected were identified as *B. cereus, B. megaterium, B. polymyxa, B. licheniformis, B. firmus,* and *B. pumilus.* The honey samples showed inhibitory activity at the various concentrations against *Salmonella* spp, *Shigella* spp, *E. coli* and *Proteus vulgaris* with the zones of inhibition increasing with honey concentration. *B. cereus, K. pneumoniae and Clostridium sporogenes* were inhibited at higher concentrations (50 - 100%) of the honey samples. The honey samples showed no inhibitory activity against *S. aureus, P. aeruginosa* and *C. albicans.*

Key words: Honey, physico-chemical properties, antimicrobial activity, *Bacillus* sp.

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 excretions 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 alimentarius, 2001). Although the precise composition of honey varies according to the plant species on which the bee forages, the main constituents are the same in all honeys. On the average, honey is composed of: moisture (17.2%), fructose (38.19%), glucose (31.28%), sucrose (1.31%), disaccharides calculated as maltose (7.31%), higher sugars (1.5%), free acid as gluconic (0.43%), lactone as gluconolactone (0.14%), total acid as gluconic (0.57%), ash (0.16%) and nitrogen (0.041%) (Jeffrey and Echazarreta, 1996). Honey is reported to contain little or no fat, but free fatty acids like palmitic (16:0), oleic (18:1) and linolenic (18:3) have been detected in white clover honey (Tan *et al.,* 1988; Singh and Kuar Bath, 1997). The protein content of honey has been reported to vary between 1.0 to 4.0 g/kg with proline, lysine, phenyl-alanine, aspartic and glutamic acid most readily detected (Bosi and Battalglini, 1978; Mincione and Leuzzi, 1993). A number of B-group vitamins are also reported present in honey but their concentrations are generally low (National Honey Board, 2003).

Honey has been reported to have several important properties. Honey is a solution of high osmolarity which inhibits bacteria (Efem, 1988). The predominant acid found in honey is gluconic acid which originates largely form the activity of glucose oxidase (which the bees add at ripening) and, to a lesser extent from the bacterial action which occurs (Ruiz-Argueso et al., 1973). Glucose oxidase is of considerable interest since it causes the production of hydrogen peroxide which not only stabilizes the ripening of nectar against spoilage but also has micro bactericidal action (Malika et al., 2005). In addition to glucose oxidase, honey contains polyphenols which are

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antibacterial (Bogdanov, 1983). The floral source of honey may also be responsible for some of the anti-bacterial activities of honey (Molan and Russell, 1988). Medicinally, honey is used to enhance wound- healing in humans (Adesunkanmi and Oyelami, 1994; Cooper, 2001; Aysan et al., 2002), treatment of gastric ulcer (Kandil et al., 1987) and shortening of the duration of diarrhea (Salem, 1981; Haffejee and Moosa, 1985).

The intrinsic properties of honey have been reported to affect the growth and survival of microorganisms by bacteriostatic or bactericidal actions (Iurlina and Fritz, 2005). The low pH and high sugar content of undiluted honeys prevent the growth of many species of microorganisms. Honey can therefore be expected to contain a small number and a limited variety of microorganisms. The microorganisms of interest in honey are those that withstand the concentrated sugar, acidity and antimicrobial character of honey. These microorganisms include certain yeasts and spore - forming bacteria; coliforms or yeasts indicative of sanitary or commercial quality (Snowdon and Cliver, 1996). The presence of *Bacillus cereus* has been reported to reflect a generally higher tolerance of the organism among other endospore - forming rods to antimicrobials that are present in honey (Roth et al., 1986).

There are enormous reports on the physico - chemical, antimicrobial, microbiological and medicinal properties of honey from other countries (Singh and Kuar Bath, 1997; Molan and Russell, 1988; Anupama et al., 2003, Iurlina and Fritz, 2005; Iurlina et al., 2006). There is paucity of information on Nigerian honey. There are reports on the healing effect on burns and wounds (Adesunkanmi and Oyelami, 1994) and some chemical and physical properties of Nigerian honey (Adebiyi et al., 2004). The aim of this study was to assess the physico - chemical and microbiological properties, with emphasis on spore-forming bacteria, as well as the antimicrobial activity of some commercial natural honey from different geographical locations of Nigeria.

MATERIALS AND METHODS

Sample collection

Ten samples of commercial honey from five different locations in Nigeria, namely Ewu - Esan (Edo State), Enugu - Ezike (Enugu State), Ile-Ife (Osun State), Osogbo (Osun State) and Saki (Oyo State) were purchased in sterile bottles from retailers and kept in the dark (to protect and conserve any light- sensitive compounds) at ambient temperature (25 - 28°C) until they were analysed.

Physico - chemical analysis

The free, lactonic and total acidity of honey samples were determined by the titrimetric method using 0.05 M NaOH and 0.05 M HCL (AOAC, 1990). Ten grams of honey sample was dissolved in 75 mL CO_2- free distilled water and the pH of the resulting solution was measured using a pH meter (Denver 3015).

Moisture content was determined by drying 2.0 g honey sample at 70°C to constant weight in hot air oven (AOAC, 1990).

Ash content was determined by drying 5.0 g sample in porcelain crucible at 105°C for 3 h in hot air oven to prevent loss by foaming. The dried sample was then ashed in furnace at 600°C to constant weight, cooled and weighed.

Electrical conductivity of samples was measured at 22°C using a conductivity meter (MC 126 Mettler Toledo).

Microbiological analysis

Ten grams of sample was mixed with 90 ml of sterile maximum recovery diluent (MRD, Oxoid CM 733) in a stomacher bag. Subsequent decimal dilutions were prepared in sterile MRD and appropriately diluted suspension of sample (100 ul) was cultured in duplicate by the spread plate method.

Total aerobic mesophilic bacteria (TAMB) were enumerated on plate count agar (PCA, Oxoid CM 325) incubated at 30 ± 2°C for 48 h. Total coliforms were counted on violet red bile glucose agar (VRBG, Fluka 70189) incubated at 35°C for 24 - 48 h. Moulds and yeasts were enumerated on Sabouraud dextrose agar (SDA, Fluka 84088) supplemented with chloramphenicol (100 mg/l) incubated aerobically at 25°C for 3 - 5 days. For aerobic endospore bacteria counts, the diluted suspension of the honey sample was heated for 2 min in continuously boiling water to eliminate any microbial vegetative forms (Thapa et al., 2004). Bacterial endospores were enumerated on nutrient agar (NA, Oxoid CM 003) incubated at 30°C for 48 h. All colonies appearing at the end of incubation were counted and the results expressed as colony forming units per gram (cfu/g). Colonies of endospore - forming bacteria were further observed, isolated and purified by repeated streaking on fresh agar plates of the isolation media. The purified endospore - forming bacteria were identified following the morphological and biochemical standard methods (Harrigan and McCance, 1976; Sneath et al., 1986).

Assay of antimicrobial activity

Honey samples were screened for antimicrobial activity against *Salmonella spp, Shigella spp, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Bacillus cereus, Proteus vulgaris, Pseudomonas aeruginosa, Clostridium sporogenes and Candida albicans* according to the agar well diffusion method (Allen et al., 1991). *Salmonella*, *Shigella* and *Klebsiella* species are clinical isolates. *Candida albicans* was grown in yeast extract broth for 18 h at 25°C while the other test organisms were grown in nutrient broth (NB, Oxoid CM 001) for 18 h at 35°C. The yeast and bacteria cultures (100 ul) were added to 20 ml molten SDA and Mueller Hinton agar (MHA, Fluka 70191) respectively and immediately poured onto sterile plates. The inoculated plates were stored at 4°C for 30 min to set and wells (8 mm diameter) were cut in the agar with the aid of sterile cork borer. Solutions containing 25, 50, 75 and 100% (w/v) of honey were made in sterile distilled water. A 100 ul aliquot of each honey sample was added to each well. Cultures were incubated at 35°C for 24 h except for *Candida albicans* which was incubated at 25°C for 24 h. Antimicrobial activity was assessed by measuring the size of the zones of inhibition surrounding wells.

RESULTS AND DISCUSSION

Physico-chemical characteristics

The physico-chemical properties of the different samples

Table 1. Physico - chemical properties of commercial Nigerian honey.

Honey samples *	Physico – chemical variables						
	pH	Moisture (%)	Ash (%)	EC (µS/cm)	Free acidity (meq Kg^{-1})	Lactone (meg Kg^{-1})	Total acidity (meq Kg^{-1})
1 (Ewu - Esan)	4.01	19.62	0.36	63.15	25.50	10.25	35.75
2 (Enugu - Ezike)	3.78	15.36	0.29	46.10	28.75	8.75	35.75
3 (Ile-Ife)	3.61	11.96	0.19	16.73	31.00	6.00	37.00
4 (Osogbo)	4.05	12.02	0.27	22.40	25.75	12.25	38.25
5 (Saki)	3.83	11.47	0.28	22.05	24.00	15.50	39.50
Mean ± SE	3.86 ± 0.08	14.09 ± 1.55	0.28 ± 0.03	34.09 ± 8.86	27.00 ± 1.26	10.55±1.60	37.20 ± 0.76

Values are means of duplicate samples; SE, Standard error. * Source of honey in parentheses.

samples of honey are given in Table 1. The pH values of the honey samples ranged from 3.61 and 4.05. The pH values correlate with the pH range of 3.2 and 4.5 reported for honey (White, 1975). The pH range obtained in this study was however lower than the range (4.31 - 6.0) reported for Nigerian honey from other locations (Adebiyi et al., 2004) and Argentinean honeys (Iurlina and Fritz, 2005). The acidic pH of honey is desirable since acidification has been shown to promote healing by causing oxygen release from hemoglobin (Leveen et al., 1973). The pH of honey is low enough to prevent the growth of many species of bacteria.

The moisture content of the honey sample varied between 11.47 and 19.62% (Table 1). The moisture content of the samples falls within the range reported for floral honeys (Mincione and leuzzi, 1993; Anupama et al., 2003; Malika et al., 2005). The variations in the moisture content of honey have been attributed to the composition and floral origins of honey (Malika et al., 2005). Moisture content is practically the most important quality parameter, since it affects storage life and processing characteristics. The strong interaction of sugar in honey with water molecules may decrease the water available for microorganisms. The low moisture content of honey also forms an important part of the system which protects honey from attack by microorganisms.

The ash content of the honey samples varied between 0.19 and 0.36% (Table 1) and it falls within the range reported for Nigerian honey samples from other locations (Adebiyi et al., 2004) and other countries (Jeffery and Echazarreta, 1996; Malika et al., 2005). The floral origin of honey has been reported responsible for the variability in ash content (Vit et al., 1998).

The electrical conductivity values of the honey sample were between 16.73 µS/cm and 63.15 µS/cm. These values are similar to those reported by Adebiyi et al. (2004) on Nigerian honey from other locations. Electrical conductivity measures all ionisable organic and inorganic substances present in honey. It has been reported to be related to the botanical origin of honey and very often used in routine honey control instead of the ash content (Malika et al., 2005).

The values for free, lactone and total acidities are summarized in Table 1. Free acidity values ranged between 24.00 and 31.00 meq/kg; lactone acidity values were between 6.00 and 16.25 meq/kg while total acidity values varied from 35.75 - 39.50 meq/kg. The total acidity values were below the maximum limits of 40 meq/kg set internationally for honey. The values obtained for total acidity falls within the range reported for Moroccan honey (Malika et al., 2005). The acidity of honey contributes to its stability against microorganisms and to flavour.

Microbiological characteristics

The microbial counts in the different samples of honey are reported in Table 2. The total aerobic mesophilic bacteria (TAMB) counts in the samples ranged from 1.0×10^3 - 5.0×10^3 $cfug^{-1}$. Our result on mesophilic bacteria count is higher than that reported by Iurlina and Fritz (2005) and Malika et al. (2005) but falls within the range reported by Tysset and Roussean (1981). This variation in bacterial counts may be due to the type of sample, freshness of the honey, the time of harvest and the analytical techniques used (Snowdon and Cliver, 1996). The bacterial endospore counts ranged from 8.0×10^2 - 2.0×10^3 $cfug^{-1}$. The result obtained for bacteria endospore indicates that the total aerobic mesophilic bacterial count comprised mainly of spore formers than vegetative cells. This agrees with the reports of Malika et al. (2005). Total coliform count was detected in only one of the honey samples (Osogbo sample). This is an indication of the

Table 2. Microbial counts of commercial Nigerian honey.

Honey Samples*	Microbial counts cfug^{-1} sample			
	TAMB	Total coliform	Bacterial endospore	Fungi
1(Ewu - Esan)	2.7 × 10^3	-	1.3 × 10^3	-
2 (Enugu - Ezike)	2.6 × 10^3	-	1.9 × 10^3	-
3 (Ile-Ife)	5.0 × 10^3	-	2.0 × 10^3	-
4 (Osogbo)	3.2 × 10^3	3.0 × 10	8.0 × 10^2	-
5 (Saki)	1.0 × 10^3	-	1.0 × 10^3	-

Values are means of duplicate samples. *Source of honey in parentheses; TAMB, total aerobic mesophilic bacteria; -not detected.

Table 3. Occurrence of endospore - forming bacteria in commercial Nigerian honey.

Bacillus species	Honey samples *				
	1 (Ewu - Esan)	2 (Enugu - Ezike)	3 (Ile-Ife)	4 (Osogbo)	5 (Saki)
B. pumilus	-	-	+	+	-
B. polymyxa	+	-	+	+	-
B. cereus	+	+	+	-	+
B. firmus	-	-	+	-	-
B.licheniformis	-	+	-	+	+
B. megaterium	+	-	-	-	-

*Source of honey in parentheses; +, detected; -, not detected.

the sanitary quality of the honey. In a similar study, total coliform were not detected in honey (Rall et al., 2003; Iurlina and Fritz, 2005; Malika et al., 2005). There were no moulds and yeasts in all the honey samples studied. This was however not surprising since Malika *et al*. (2005) reported counts of less than 10 cfug^{-1} in Moroccan honey while some French honeys had zero counts of moulds and yeasts (Tysset et al., 1970). The absence of yeasts and moulds and the low numbers of bacteria in the honey samples confirms that honey has inherent antimicrobial properties that can delay the growth of many microorganisms. Generally, honey may contain organisms from bees, soil, air and dust that are introduced during post- harvest handling (Jay, 1992).

The *Bacillus* species found in the honey samples were identified as *B. cereus*, *B. megaterium*, *B. polymyxa*, *B. licheniformis*, *B. firmus* and *B. pumilus* and their occurrence pattern is shown in Table 3. *B. firmus* and *B. megaterium* were found in only one honey each. These Bacillus species have been reported in honeys from other countries (Tysset et al., 1970; Iurlina and Fritz, 2005; Malika et al., 2005). *Bacillus* species are among the main spoilage organisms in food due to their versatile metabolism and heat - resistance spores. *B. cereus*, *B. subtilis* and *B. licheniformis* have been associated with food poisoning (Iurlina et al., 2006). Honey has not been involved in food borne outbreaks caused by *B. cereus*, although no firm evidence exists that would exclude honey as a potential vehicle of infection.

Antimicrobial activity

The results of antimicrobial activity of the honeys are shown in Table 4. All the honey samples showed inhibitory activity against *Salmonella* sp. *Shigella* sp. and *E. coli* with the diameter of zones of inhibition ranging from 12 - 27 mm; 14 - 25 mm and 19 - 38 mm respectively. The honey samples except Ewu- Esan sample also showed significant inhibitory activity against *Proteus vulgaris* (diameter of zone of inhibition ranged from 12 mm to 20 mm). Similarly, honey samples from Ewu - Esan, Enugu - Ezike and Ile-Ife showed inhibitory activity against *B. cereus*, *K. pneumoniae* and *C. sporogenes* at higher concentrations (75 - 100% w/v) while Osogbo and Saki honeys showed inhibition at 50 - 100% concentrations. All the honeys were not active against *S. aureus*, *C. albicans* and *P. aeruginosa*. Our result is in agreement with other reports (Iurlina and Fritz, 2005; DeMera and Angert, 2004; Agbaje et al., 2006). The non susceptibility of some of the test organisms to the honey samples could be due to the emergence of resistant strain. In addition, several factors may influence the antimicrobial activity of honey. These factors include its physico - chemical properties, botanical origin, entomological origin and symbioses with beneficial bacteria. For example, DeMera and Angert (2004) reported that honey from different phytogeographic regions varied in their ability to inhibit the growth of bacteria and yeasts suggesting that botanical origin plays

Table 4. Inhibitory activity of commercial Nigerian honey against some microorganisms.

Test Organisms	Honey samples** (% concentration w/v) / diameter of zone of inhibition (mm)																			
	1 (Ewu – Esan)				2 (Enugu - Ezike)				3 (Ile –Ife)				4 (Osogbo)				5(Saki)			
	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
**Shigella sp.*	14	16	19	22	17	18	21	22	17	20	21	22	21	22	23	24	17	20	23	25
**Salmonella sp.*	12	16	21	27	17	18	22	24	14	18	22	25	14	17	20	22	15	20	21	22
S. aureus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. albicans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. aeruginosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. vulgaris	0	0	0	0	14	18	20	20	14	17	20	21	14	17	18	18	12	14	16	18
Bacillus cereus	0	0	12	15	0	0	13	21	0	0	14	17	0	20	23	26	0	22	26	29
**K. pneumoniae*	0	0	0	8	0	0	9	11	0	0	0	14	0	8	12	13	0	8	12	15
C. sporogenes	0	0	0	11	0	0	12	16	0	0	15	17	0	8	11	13	0	10	13	19
E. coli	19	21	23	25	24	29	30	36	26	27	30	33	30	31	32	35	26	30	34	38

*Clinical isolates; **Source of honey in parentheses.

an important role in influencing the antimicrobial activity.

The results obtained in this study showed that the physico - chemical properties of Nigerian honey compare favorably with honeys from other countries. The honeys contained low numbers of aerobic mesophilic bacteria while mould and yeast were not detected. The prevalent spore forming bacilli recovered from the honey samples were *B. megaterium*, *B. polymyxa*, *B. licheniformis*, *B. firmus*, *B. pumilus* and *B. cereus*. The honey samples showed varied antimicrobial activities on the test organisms.

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