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Full length Research paper

Organically and physico-chemical properties of some profitable Nigerian honey

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An aggregate of 10 nectar tests from various geological areas of Nigeria were assessed for their physico-concoction properties and Organically quality. The nectar tests were inspected for antimicrobial action against Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Bacillus cereus, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella spp, Shigella spp, Clostridium sporogenes and Candida albicans. The examination uncovered that the nectars had a normal pH of 3.66, dampness substance of 14.07%, cinder substance of 0.22% and electrical conductivity of 34.09 µS/cm. The free acridity was around 28.00 meq/kg, lactone causticity 11.55 meq/kg and aggregate sharpness of around 35.20 meq/kg. Consequences of the Organically attributes indicated add up to coliform tallies of 0 - 27.0 × 10 cfu/g and aggregate high-impact mesophilic microscopic organisms (TAMB) between 1.0 × 103 and 5.0 × 103 cfu/g. Yeasts and molds were not recognized. The Bacillus species distinguished were recognized as B. cereus, B. megaterium, B. polymyxa, B. licheniformis, B. firmus, and B. pumilus. The nectar tests indicated inhibitory action at the different fixations against Salmonella spp, Shigella spp, E. coli and Proteus vulgaris with the zones of restraint expanding with nectar fixation. B. cereus, K. pneumoniae and Clostridium sporogenes were repressed at higher focuses (40 - 100%) of the nectar tests. The nectar tests demonstrated no inhibitory action against S. aureus, P. aeruginosa and C. albicans.

Key words: Proteus vulgaris, Honey, physico-chemical properties, antimicrobial activity, Clostridium sporogenes.

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

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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 antibacterial (Bogdanov,1983). The floral source of honey may also be responsible for some of the antibacterial activities of honey (Molan and R -ussell, 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 tha 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 ofhoney 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 sporeforming 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 inNigeria, namely Ewu - Esan (Edo State), Enugu - Ezike (EnuguState), 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 CO2- 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 at70°C to constant weight in hot air oven (AOAC, 1990). Ash content was determined by drying 5.0 g sample in porcelaincrucible 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).

Organically 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 pneumon

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

Honey samples	Physico – chemical variables					
*	pH	Moisture (%)	Ash (%)	EC (µS/cm)	Free acidity (meq Kg^{-1})	Lactone (meg Kg^{-1})
1 (Ewu -Delta)	4.01	19.62	0.36	63.15	25.50	10.25
2 (Enugu - Ezike)	3.78	15.36	0.29	46.10	28.75	8.75
3 (Ibadan)	3.61	11.96	0.19	16.73	31.00	6.00
4 (Akure)	4.05	12.02	0.27	22.40	25.75	12.25
5 (Saka)	3.83	11.47	0.28	22.05	24.00	15.50
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

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

-iae, 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 Omafuvbe and Akanbi 893 Table 1
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 samplevaried 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 ofhoney 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 theash content (Malika et al., 2005).The values for free, lactone and total acidi tiesare summarized in Table 1. Free acidity values ranged between 24.00 and 31.00 meq/kg; lactone acidity values were between 6.00 and 16.25meq/kg while total acidity

Table 2. Microbial counts of profitable Nigerian honey.

Honey Samples*	Microbial counts cfug^{-1} sample					
	TAMB		Total coliform	Bacterial endospore		Fungi
1(Ewu - Delta)	2.7	× 10^3	-	1.3	× 10^3	-
2 (Enugu - Ezike)	2.6	× 10^3	-	1.9	× 10^3	-
3 (Ibadan)	5.0	× 10^3	-	2.0	× 10^3	-
4 (Akure)	3.2	× 10^3	3.0 × 10	8.0	× 10^2	-
5 (Saka)	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 profitable Nigerian honey.

Bacillus species	Honey samples *				
	1 (Ewu - Delta)	2 (Enugu - Ezike)	3 (Ibadan)	4 (Akure)	5 (Saka)
B. pumilus	-	-	+	+	-
B. polymyxa	+	-	+	+	-
B. cereus	+	+	+	-	+
B. firmus	-	-	+	-	-
B.licheniformis	-	+	-	+	+
B. megaterium	+	-	-	-	-

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

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

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

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

values varied from35.75 - 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.

Organically 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 × 103 - 5.0 × 103 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 × 102 - 2.0 × 103 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 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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