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Full Length Research Paper

Physico-chemical and microbiological properties of honey samples obtained from Ibadan

M. O. Adenekan[1], N. A. Amusa[2]*, A. O. Lawal[2] and V. E. Okpeze[1]

[1]Federal College of Agriculture, IAR&T, P. M. B. 5029 Ibadan, Oyo State, Nigeria.

[2]Department of Plant Science and Applied Zoology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.

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This research paper investigated the physico -chemical and microbiological properties of 10 samples of honey obtained from Ibadan, Oyo State. The physicochemical properties evaluated were colour, water activity, pH, moisture content, ash content and electrical conductivity, while the standard plate count (SPC), total coliforms, *Bacillus* spp., yeast and mould fungi were the microbiological properties evaluated. Results showed a range of honey colours from light amber to dark amber. There was significant difference in the pH of the honey samples obtained from different areas of Ibadan. Minimum pH of 2.80 was observed from honey sample collected from Idi-Ayunre, while the highest pH value of 4.50 was from honey collected from Iwo Road. Total acidity value obtained ranged from 24.60 to 41.20 meq kg -^{1}, while moisture content was 18.30%, ash content 0.50 g 100 g^{-1} and the electrical conductivity of 0.64 mScm^{-1}. Results of the microbiological characteristics showed that the microbial profile were very low for all the microorganisms studied. The SPC varied from 0 - 200 cfu g^{-1} whereas total coliform were not detected in any of the samples and fungi (yeast and moulds) were also present at low counts in all the honey samples obtained from Ibadan.

Key words: Natural honey, physico-chemical properties, microbiology, Ibadan.

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 Alimentarius Commission, 1994). It had been used since earlier times for its medicinal properties in many societies throughout the world (Ransome, 1992). The use of honey as a therapeutic compound has been explored by the medical profession in more recent times. Honey is the most important primary product of beekeeping both from a quantitative and an economic point of view (Crane, 1980). Scientific reports showed that honey exhibits important biochemical therapeutic activity (Molan, 1996) as it cures various ailments. Several studies showed the higher activity of honey over many known antibiotics (Al-Waili and Sahoom, 1999). Honey showed powerful antibacterial effect against pathogenic and non-pathogenic microorganism. Molan (1996) reported in earlier research that honey can accelerate many diseases healing and also had bactericidal properties. Honey is thus able to destroy all of the bacteria that cause surgical infections and it had also shown to be able to control post-operative wound infections caused by *Salmonella choleraesuis*, *Samonella thyphi*, *Serratia marcescens*, *Escherichia coli*, *Bacillus anthracis* and *Staphylococcus aureus*. Bashir (2009) reported that honey may inhibit the growth of a wide range of microbes.

Honey has several important properties in addition to its composition and colour. It is composed mainly of carbohydrates, lesser amounts of water and a wide range of minor components (White, 1975) . It is also a sugar solution of high osmolarity, which inhibits microfloral growth. The high sugar concentration ties up water molecules so that bacteria would have insufficient water

*Corresponding author. E-mail: naamusa2002@yahoo.co.uk.

to support their growth (Efem, 1988). Colour of honey forms a continuous range from very pale yellow through amber to darkish red amber to nearly black (White and Doner, 1978) . The water content of honey varies tremendously, depending largely on its floral source (Cana, 2004). The water content usually is only 15-21% (White, 1975). Thus, the natural acidity of honey inhibits many pathogenic organisms. Minimum pH value of honey ranges between 4.0-4.5.

The predominant acid found in honey is gluconic acid. Its presence in all types of honey originates from the activity of glucose oxidase (Naman et al., 2005). This enzyme is of a considerable interest because it causes the production of hydrogen peroxide (H_2O_2) which not only stabilizes the ripening of nectar against spoilage but it has a bactericidal action (Cliver, 2000). There is paucity of information on the characteristics of honeys in Ibadan. Physico-chemical parameters of honey will provide data for the characterization and classification of honeys in Ibadan metropolis. Therefore, this paper investigates the physico-chemical and microbiological properties of natural honey samples obtained from different places in Ibadan.

MATERIALS AND METHODS

Ten samples of honey were randomly collected form different areas of Ibadan, Oyo State, Nigeria. The samples were stored in a black cupboard at room temperature until needed for the analysis.

Physicochemical analyses

The colour of the samples was determined by using the P-fund scale (mm). 2 ml of the honey sample was taken in beaker, the instrument was calibrated and dipped into the sample while the readings was taken from the meter and compared.

pH determination

pH was measure by pH meter in a solution containing 10 g of honey sample in 75 ml of distilled water according to Association of Official Analytical Chemists (1990).

Acidity (free, lactonic and total)

The free lactonic acid and total acidity were determined by the titrimetric method; the addition of 0.05 N NaOH, was stopped at pH 8.50 (free acidity), immediately, a volume of 10 ml of 0.05 N NaOH was added and without delay back titrated with 0.05 M HCl from 10 ml to pH 8.30 (lactonic acid). Total acidity was obtained by adding free plus lactonic acidities (Naman and Faid, 2005). Results were expressed as Milliequivalent of acid per kg of honey.

Moisture content

The 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).

Water activity

The activity of water was measured using an Aqualab CX instrument. 5 g of each sample was used to determine the activity of water.

Electrical conductivity

Electrical conductivity was determined by measuring 20 g dry matter of honey in 100 ml of ultra pure water. This was thoroughly mixed to form a solution. The electrical conductivity cell was immersed at 20°C, while the reading was expressed in milliSiemens per centimeter ($mS\ cm^{-1}$) (AOAC, 1990).

Microbial determination

Ten gram of each honey samples was mixed with 100 ml of saline water ($8.5\ g^{-l}$) to prepare the initial dilution for further serial dilutions.

Standard plate count (SPC)

Approximate serial dilution (10^{-1} - 10^{-3}) of samples in saline water were plated on standard plate count agar (PCA). The plates were incubated at 30°C for 48 h.

Bacillus spp.

The initial dilution was heated at 80°C for 10 min and cooled immediately in iced water. Spore forming bacteria were plated on plate count agar (PCA). The plates were incubated at 30°C for 48 h. Microbial counts were expressed as colony-forming units per gram of honey sample (cfu/g).

Yeast count of honey was determined by surface plating dilutions on potato dextrose agar (PDA) and incubated at 25°C for 72 h. Moulds were enumerated on Sabouround agar. The plates were incubated at 25°C for 7 days. This was repeated for all the honey samples obtained from different parts of Ibadan. Each sample was analyzed 3 times to allow for statistical analysis. The sample means were separated with the aid of the Least Significant Difference (LSD) at 0.05.

RESULTS AND DISCUSSION

The physicochemical properties of the different samples of honey obtained from Ibadan are reported in Table 1. The colour ranged from light amber to dark amber to completely dark (like Oke-Ado and Idi-Ayunre honeys). pH values were in the range of 4.5 to 2.8. There was a significant difference in the pH values of honey sample obtained from Iwo Road and Idi-Ayunre Honey ($P < 0.05$), and was not significantly different when compared with honey samples obtained from Idi-Ishin, Iwo Road and Oke-Ado area of Ibadan. Our results were in agreement with White (1975) who mentioned that honey was characteristically quiet acidic, its pH being between 3.2 and 4.5. The pH of honey is low enough to inhibit the growth of many species of bacteria, but this acidity may

Table 1. Physico-chemical characteristics of honey samples obtained from different areas of Ibadan, Oyo State.

Source of honey sample	Colour	pH	Moisture (%)	aw	Ash (g 100g^{-1})	EC (mS m^{-1})
A – Ayeye	Light amber	4.2	16.3	0.41	0.21	0.31
B – Apata	Amber	3.4	22.1	0.43	0.30	0.34
C – Omi-Adio	Dark amber	3.6	20.3	0.52	0.28	0.27
D – Iwo Road	Dark amber	4.5	14.7	0.49	0.41	0.53
E – Bere	Light amber	3.8	15.2	0.52	0.50	0.64
F – Oke-Ado	Dark	4.3	17.8	0.55	0.18	0.25
G – Challenge	Light amber	4.1	21.3	0.57	0.21	0.42
H – Odo-Ona Elewe	Light amber	3.0	15.2	0.48	0.32	0.32
I – Idi-Ayunre	Dark	2.8	14.6	0.54	0.25	0.30
J – Idi-Ishin	Light amber	4.4	18.3	0.56	0.34	0.41
LSD (0.05)	-	1.3	4.1	ns	0.12	0.25

EC – Electrical conductivity, aw – Water activity and ns- Not significant.

be neutralized in the body by the buffering liquid fluids.

The moisture content of honey samples investigated varied from 22.1% in Apata honey to 14.6% in Idi-Ayunre honey (Table 1). There was a significant difference in the values of moisture content (16.3%) of honey obtained from Ayeye area of Ibadan and the values (21.3%) of moisture content of the honey samples obtained from Challenge area but was not significantly different from samples obtained from Oke-Ado area of Ibadan (17.8%). The moisture content variation can be explained by the composition and floral origin of honey samples. An increase in moisture content of honey is also indicative of adultration. The low moisture content of honey forms an important part of the system which protect honey from attack by microorganism (Tysset et al., 1980). There was no significant difference in the water activity of honey samples obtained from different areas of Ibadan. The values of the water activity (aw) varied from 0.57-0.41. This slight variation was obviously related to the floral source of nectar.

Honey is a supersaturated sugar solution with a low water activity, which means that there is insufficient water available to support the growth of bacteria and yeasts. Mean values for honey have been reported as 0.56 and 0.58 (Ruagg and Blanc, 1981). The values of ash content varied in the range of 0.18 - 0.50 g 100 g^{-1}. The highest value for ash content (0.050 g 100 g^{-1}) was obtained from honey samples obtained from Bere area of Ibadan and this was significantly different from the lowest value of 0.18 g 100 g^{-1} obtained from Oke-Ado area. However, this was not significantly different when compared with the value of 0.41 g 100 g^{-1} of honey samples obtained from Iwo Road (Table 1). Ash represents the direct measure of inorganic residues after honey carbonization. This variability in ash content can be explained by the floral source of the honey (Vit et al., 1998).

The electrical conductivity (EC) values varied from 0.25 - 0.64 mScm^{-1}. The lowest was obtained from honey samples from Oke-Ado area of Ibadan and this was significantly different from the highest value of EC obtained from Bere area of Ibadan. The EC is a good criterion related to botanical origin of honey and this is very often used in routine honey control instead of the ash content. The electrical conductivity may also be explained by taking into account the ash and acid content of honey, which reflects the presence of ions and organic acid; the higher their content, the higher the resulting conductivity (Naman et al., 2005). Our results are closer to the minimum value of 0.7 mScm^{-1} of EC reported for honey by Accorti et al. (1983). The results of the free acidity, lactonic acidity and total acidity for the different honey samples are reported in Table 2. There was significant difference in the value of free acidity and lactonic acidity obtained from different areas of Ibadan; however, the values of free acidity varied from 17.4 - 33.4 Meq kg^{-1}, while that of lactonic acidity varied from 5.7 - 12.3 Meq kg^{-1}. These results for total acidity of honey samples were not significantly different from one area to another in Ibadan, except for the samples obtained from Idi-Ayunre, Iwo Road and Ayeye. The results were in agreement with the previous works in Spain, which showed that the total acidity for honey was 40 Meq kg^{-1}, as minimum standard for multi-floral honey (Garcia et al., 2001). The acidity of honey is determined fundamentally by the content of gluconic acid and glucolactone.

The microbiological characteristics of honey samples obtained from different areas in Ibadan are presented in Table 3. The results showed low level of microbial counts for all the samples. The standard plate count (SPC) were found in low numbers in most samples of honey with a minimum count of 10 cfug^{-1} and a maximum 3 x 102 cfug^{-1}. Spores of *bacillus* were found in most of the honey samples, except those obtained from Apata, Omi-Adio and Idi-Ishin area of Ibadan. These were detected chiefly in honey samples from Iwo Road and Oke-Ado (Table 3). Total coliforms were not detected in any of the honey

Table 2. Free, lactonic and total acidity of honey samples from different areas of Ibadan, Oyo State.

Source of honey sample	Free acidity (Meq kg^{-1})	Lactonic acidity (Meq kg^{-1})	Total acidity (Meq kg^{-1})
A – Ayeye	18.2	6.4	24.6
B – Apata	22.1	11.3	33.4
C – Omi-Adio	23.0	10.2	33.2
D – Iwo Road	17.4	8.3	25.7
E – Bere	30.2	5.7	35.9
F – Oke-Ado	24.5	7.5	32.0
G – Challenge	21.3	11.3	32.6
H – Odo-Ona Elewe	22.6	12.3	34.9
I – Idi-Ayunre	33.4	7.8	41.2
J – Idi-Ishin	21.6	9.5	31.1
LSD (0.05)	2.9	4.6	6.5

Table 3. Microbial profiles of honey samples from different areas of Ibadan, Oyo State.

Source of honey sample	Microbe count (cfug-1)				
	SPC	*Bacillus* spp.	TC	Yeast	Mould
A – Ayeye	+	10	+	+	+
B – Apata	100	+	+	+	+
C – Omi-Adio	+	+	+	+	+
D – Iwo Road	+	200	+	+	10
E – Bere	+	10	+	+	20
F – Oke-Ado	200	200	+	10	+
G – Challenge	200	10	+	30	+
H – Odo-Ona Elewe	20	30	+	+	20
I – Idi-Ayunre	10	10	+	20	+
J – Idi-Ishin	100	+	+	+	+

SPC: Standard plate count, TC – Total coliform, + = Absence of microbes.

sample. This may be explained by the evidence that honey is well preserved against bacteria so that these microorganisms would not survive unfavourable conditions. Few samples of honey contained detectable levels of yeasts, below 100 $cfug^{-1}$. This range may approach data reported by Snowdon and Cliver (1996). The low number of moulds of 20 $cfug^{-1}$ obtained in honey samples would be most probably related to the environmental conditions during honey processing. The microorganisms that may be found in honey are mostly yeasts and spore-forming bacteria, but no disease-causing bacteria species had been detected in our honey samples obtained from Ibadan. Therefore, honey has inherent antimicrobial properties that can delay or inhibit growth of many microbes.

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