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Case Study.

MICROBIOLOGICAL QUALITY OF SELECTED NIGERIAN HONEY

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Fourteen commercial honey samples from different geographical locations in Nigeria were obtained and examined for the Microbiological quality of the samples. From each sample, total viable counts, moulds and yeast were determined. Results obtained showed that total viable counts varied between 2.0 x10^2 and 8.2 x 10^2. Yeast and mould were between 0 and 9.0 x 10^2 respectively, yeast and mould counts were not detected in commercial honey samples of International origin except in one. Microorganisms identified include *Bacillus cereus, Bacillus badius Bacillus. laterosporous,* Aerococcus sp, *Eubacterium* sp, *Micrococcus* sp, *Aspergillus niger*, *Aspergillus. flavus*, *Penicillium* sp. *Mucor* sp, *Saccharomyces rouxii and Saccharomyces mellis*. The most predominate microorganisms were *Bacillus badius* and *Saccharomyces rouxii.*

Key words: Commercial honey, microorganisms, microbiological quality, total viable count.

Introduction

Honey is a viscous liquid, sweet and flavoured foodstuff collected and produced by bees from nectar or sweet juice that can be found in different parts of plants and trees (Marghitas et al. 2008). Honey has an average pH of 3.9, while the water activity varies between 0.5 and 0.6.Iurlina and Fritz 2005 stated that the low pH and high sugar content of undiluted honeys prevent the growth of microorganisms. The intrinsic properties present in honey affect the growth and survival of many species of microorganisms by bacteriostatic or bactericidal action, therefore honey is expected to contain small number and limited variety of microorganisms (Snowdon and Oliver 1996, Olaitan et al.,2007).

The microorganisms found in honey come from the nectar and pollen, from the processing area, machines and containers that are not properly washed (Popa et al. 2010). These microorganisms found are those that can tolerate high sugar content, acidity and antimicrobial properties of honey, they include certain yeast and spore- forming bacteria. During the production of honey, honeybees interact with various environmental factors such as microbes and air suspended particulate matter. This could be retained in the hair on their body surface, or inhaled and attached to their trachea. These factors are primary sources of microbial introduction in honey. Microorganisms could also be introduced into honey during postharvest mainly from bee farm soils, equipments, handling and honey- house air (Snowdon and Oliver 1996). The most encountered sporulated microorganisms are the *bacillus spp* which are the most prevalent in honey followed by Gram – variable plemorphic bacteria (Gilliam,1997).In 2007 Tehoumboue et al., stated that normal honey must lack some level of microorganisms or pathogenic microorganisms that produce enteric illnesses. The presence of microorganisms in honey can affect the stability and the hygienic quality of the product. Al Somai (1994) reported that various bacteria have being inoculated into aseptically collected honey held at 20^0C and the result showed loss of bacterial viability within 8-24 days. Spore forming microorganisms only can survive in honey at low temperature (White, 1996). Yeast present in honey could cause fermentation thereby resulting in formation of alcohol and carbon dioxide, the alcohol further gets oxidized into acetic acid in the presence of oxygen, thus causing sour taste.

Honey has been used right from ancient times as a natural antioxidant in food and an emollient in wound healing (McKibben and Engeseth 2002). Despite the usefulness of honey, due to its adultration and the presence of natural organisms present in honey, it is unsuitable for consumption for infants and children who lack a fully developed immunological system and are prone to alimentary infections (Snowdon and Oliver 1996). It is also unsuitable for therapeutic purpose as bacteria may contaminate wound. Honey is universally available and practitioners may consider using table and commercial honey from supermarkets as available alternative to more expensive, regulated, honey-based wound care products. This work is therefore designed to assess the microbiological quality of selected Nigerian honey samples.

Materials and methods

Source and collection of honey

Fourteen samples of honey produced locally in Nigeria were examined for the presence of microbial flora. Samples were obtained from various geographical locations in Nigeria. Eight commercial honeys were purchased directly from retailers in Imo, Enugu, Bayelsa, Delta, Rivers, Calabar, Oyo and Taraba states. Three samples were obtained from Crop Research Institute Umudike, Abia state, and three honey samples of International origin were purchased from local supermarkets and designated as HSI 1, HSI 2, and HSI 3. Commercial honey samples were analyzed from original containers while the others were collected

aseptically into sterile containers; all samples were stored in the dark.

Microbiological analysis

Microbial counts

This was carried out by the method described by Harrigan and McCance (1976), briefly as follows: 10g of each sample was aseptically introduced into 90 mL of sterile distilled water and was shaken and serially diluted. From appropriate dilution, 0.1 mL was cultured into sterile plates and agar poured on it employing the pour plate method. Plates were incubated appropriately.

Total viable count was enumerated in plate count agar (PCA, Oxoid) incubated for 24- 48 h. Moulds and yeasts were enumerated on Sabou-aud dextrose agar (SDA, Fluka) supplemented with chloramphenical (100m/l) incubated at 22- 25 ^{0}C for 3- 5 days. Isolates were identified following the methods of Barrow and Feltham(2003), briefly, all colonies that appeared at the end of incubation were counted and results were expressed in colony forming units per gram (cfu/g). Colonies were further observed, isolated, and purified by repeated streaking on nutrient agar. Gram- staining, spore stain and biochemical tests such as; citrate utilization, MR- VP test, indole, starch hydrolysis, motility and growth at 10% Nacl, Utilization of carbohydrates from acid such as: glucose, xylose, galactose, mannitol, mannose, fructose, lactose and sucrose were also determined.

Determination of pH and Moisture content of honey samples

Determination of pH: The pH of honey samples were determined by measuring out 10 mL of each honey sample into a clean beaker and its pH was determined using a pH meter (Equip –Tronics, Digital pH meter model EQ-610)

Determination of moisture content: The moisture content of each honey sample was determined by measuring 5g of the sample and placed into a pre-weighed aluminium drying dish. The sample was dried to constant weight in an oven at 105^{0}C for 4 h under vacuum (AOAC, 1990).

$$\text{Moisture content} = M_1 - M_2 / M_1 - M_0$$

Where:
M_0 = Weight of aluminium dish
M_1 = Weight of the fresh sample + dish
M_2 = Weight of the dried sample + dish

Results

The microbial counts of the various honey samples are reported in Table 1. Total viable bacteria count (TVC) had the highest count. Commercial honey recorded the highest bacteria count with values between 3.1x 10^2 and 8.2 x 10^2 cfu/g respectively. The highest counts were recorded in Bayelsa and Rivers. The lowest mean counts for all the honey samples were recorded in samples from the supermarkets of international origin with values ranging between 3.0 x 10^2 and 6.6cfu/g while yeast count in HSI 3 had counts of 4.3 x 10^3cfu/g. For all the honey samples yeast count had values between 1.8 x 10^2 and 9.2 x 10^4cfu/g. Moulds were not isolated at all from honey samples of international origin and Taraba. Mould counts were low in all the samples examined with values ranging from 0 to 9 x 10^1cfu/g.

The organisms isolated were *Bacillus* spp, *Aerococcus* sp, *Eubacterium* sp, *Aspergillus niger*, *Aspergillus flavus*, *Penicillum* sp, *Candida* spp. *Saccharomyces mellis, Saccharomyces rouxii.* Coliforms were not detected in any of the honey samples. All the honey samples contained *Bacillus* spp, which were identified as *Bacillus cereus*, *Bacillus. firmus*, *Bacillus badius* and *Bacillus. laterosporous* others were not fully identified. The most predominant *Bacillus* spp was *Bacillus badius* which was detected in all the samples. *Micrococcus* sp was detected in honey samples from three locations namely; Imo, Enugu and Umudike. *Eubacterium* sp and *Aerococcus* sp were detected in honey samples of international origin.

The values for pH and the moisture content of the various honey samples are detailed in Table 2. The pH values of the honey samples ranged from 3.10 and 4.25. The pH values correlate with the pH range of 3.2 and 4.5 reported for honey-- (White, 1975).Results of pH obtained in this study was however lower than the range (4.31 – 6.0) reported for Nigeria honey from other locations (Adebiyi et al., 2004).The sample location with the highest pH was Bayelsa with value of 4.25 and the lowest was observed in HSI 2 and Calabar with value of 3.10. Moisture content of honey samples varied between 16.74 and 31.20% as stated in Table 2.

Discussion

The variations observed in microbial count as detailed in Table 1 may be attributed to the type of sample, freshness of the honey, time of harvest and the analytical techniques used (Snowdon And Oliver, 1996). This does not necessarily indicate adulteration or contamination but micro flora associated with honey. When the counts exceed a particular level it can then be suggested that the honey is adultrated, this leads to increase in bacterial counts. Lawal et al. (2009) stated that the adulteration of Nigerian honey may be due to unhygienic handling during processing and storage.

According to previous reports, total aerobic viable count values for honey can range from 0 to thousand - per gram (Iurlina and Fritz 2005). In 1991 Nakano and Sakaguchi worked on 270 honey samples from retail outlets in Japan and their findings for mean aerobic viable count was 83cfu/g and 0 to 72cfu/g of honey of international origin. In 1991 Nakano and Sakaguchi worked on 270 honey samples bought from retail outlets in Japan, and the mean viable count was 83 cfu/g and 35 for honey samples of international origin they reported viable counts from 0 to 72cfu/g with a mean value of 24cfu/g.

Reports on yeast and mould counts in honey are scanty and its presence in honey is unavoidable because bees collect them with the nectar. Rall et al. (2003) found an incidence of 64% of mould and yeasts in industrial and domestic production of honey with counts ranging from absence to$1.5x10^5$ cfu/g. Our present study showed mould and yeast counts of the honey samples ranging between 9.2 $x10^2$Cfu/g 0 and 9.2. Higher counts of yeast was observed in sample location Imo, Rivers, and Bayelsa, our findings are not in conformity with reports by Popa et al. (2010); Omafuvbe and Akanbi (2009) where no yeast and mould count was observed in all honey samples analysed. Determination of yeasts and moulds count provides information on the shelf life and spoilage potential of the honey, the presence of yeast and mould in honey is unavoidable because the bees collect them with the nectar. Our study revealed that the number of yeast and mould among the commercial were not similar to freshly harvested honey samples indicating that the microorganisms may have introduced during handling, manipulation by beekeepers, primary honey treatment and storage. The most frequently encountered mould was *Aspergillus niger, A. flavus* and *Penicillum* sp.

Microorganisms reported to be associated with bees include *Bacillus, Clostridium, E. coli, Enterobacter, Klebsiella*, and *Proteus*. In resent studies on Nigeria honey *Bacillus* has being detected to have the highest occurrence, the species of *Bacillus* identified include *B. cereus*, *B megaterium, B. polymyxa, B. licheniformis, B. firmus* (Omafuvbe and Akanbi 2009). *Bacillus* sp has also been reported in other studies by various investigators in other countries (Malika et al., 2005; Iurlina and Fritz, 2005). Moulds and yeasts were not detected in these studies; in contrast to our present findings where moulds and yeast were detected in all the honey samples except in HSI-1, HSI-2, HSI-3 and Taraba which might be due to sterilization. The absence of yeasts and moulds and low numbers of bacteria in some of the honey samples suggests that honey has inherent antimicrobial activity that can delay the growth of many microorganisms (Jay, 1992).

In contrast to our present findings *B. pumilis* was not detected in all the honey samples studied but was detected by Omafuvbe and Akanbi, 2009. A study carried out by Piana et al.1991 discovered that 24 out of 50 samples tested positive for *Bacillus cereus,* while Kokubo et al. 1984 found spores in 67 out of 71 honey samples.

If the water content of honey is below 17.1%, the water activity will be too low to support microbial growth and fermentation. Honey samples are stable when the moisture content is between 17% and 20%w/v (Tosi et al., 2004). The highest moisture content was observed in Jos with value of 31.20%. The values obtained in this study for moisture content did not correlate with findings by Omafuvbe and Akanbi 2009, where moisture content values varied between 11.47 and 19.62%. Low moisture content of honey decreases the amount of water made available to microorganisms thereby protecting and increasing the period of storage and processing characteristics. Variations in moisture content of honey is attributed to composition and floral origin (Malika et al., 2005).

Conclusion

The study on the microbiological quality of Nigerian honey from different locations , were determined to provide information on the total viable counts, moulds and yeast present in the samples. Microorganisms recovered were moulds, spore- forming bacteria and yeast. These organisms were adapted to survive in the physico-chemical environment in honey with restricted growth.

Honey in spite of its usefulness has been known to contain microorganisms. It is often described as a reservoir for microbes hence the use of honey for medicinal purposes is viewed carefully. It has now become an accepted principle that honey used for medicinal purposes should be rendered safe by sterilization before use.

Table 1. Microbial counts of selected Nigerian honey

Honey samples	TVC	YEAST	MOULD
Bayelsa	8.2 $x10^2$	9.2 $x10^2$	6 $x10^1$
Enugu	6.0 x 10^3	2.0 $x10^2$	5 $x10^1$
Taraba	7.3 $x10^2$	3.4 $x10^2$	0
Imo	6.0 x 10^3	9.0 $x10^2$	2.4 $x10^1$
Calabar	3.1 x 10^2	4.1 $x10^2$	0
Jos	4.5 x 10^3	3.2 $x10^2$	0
Rivers	8.2 x 10^3	4.7 $x10^2$	9 $x10^1$
Delta	4.2 $x10^2$	6.3 $x10^2$	1.1 $x10^1$
HSI 1	3.0 $x10^2$	0	0
HSI 2	6.6 $x10^3$	0	0
HSI 3	3.0 $x10^2$	4.3 $x10^2$	0
UMU 1	2.0 $x10^2$	5.6 $x10^2$	1.4 $x10^1$
UMU 2	3.2 $x10^2$	2.1 $x10^2$	5 $x10^2$
UMU 3	2.9 $x10^2$	4.9 $x10^2$	0

Key
TVC: total viable count
HSI: honey sample with international origin
UMU: Umudike

Table 2. pH and moisture content of selected Nigerian honey

Honey samples	pH	Moisture Content (%)
Bayelsa	4.25	28.60
Enugu	4.11	19.63
Taraba	3.21	34.65
Imo	3.35	30.85
Calabar	3.10	28.60
Jos	3.60	31.20
Rivers	3.86	27.35
Delta	3.69	27.80
HSI 1	3.45	27.10
HSI 2	3.10	25.80
HSI 3	3.23	26.71
UMU 1	3.21	16.94
UMU 2	4.03	16.75
UMU 3	4.11	19.69

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