Microbiological Evaluation of Commercial Honey from Edo State, Nigeria.

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Abstract- In this study, fifteen commercial honey samples were collected from different sources within Edo State, Nigeria and evaluated for their microbiological safety. From each sample, total viable counts, molds and yeast were determined. Result of microbiological analysis of commercial honey revealed that honey samples collected directly from bee keepers and the branded samples were completely free of microbial contamination. However, microbial counts were recorded for samples collected from local markets and roadsides. Coliforms were counted in only one of the samples investigated. Bacterial counts ranged from 1.0×104 to 2.0×104 Cfu/ml whereas yeast count ranged from 1.0×104 to 1.2 × 105 Cfu/ml in samples for which they were observed.

Keywords- Microbial contamination, microbial counts, Yeast, mould, fungi, commercial honey,

1 INTRODUCTION

Honey is produced by honeybees from nectars extracted from the nectaries of flowers (Adebiyi *et al*, 2004). Natural honey is one of the most widely sought products due to its unique nutritional and medicinal properties, which are attributed to the influence of the different groups of substances it contains (Buba et al., 2013). It is essentially composed of a complex mixture of carbohydrates (of which fructose and glucose are major ones) and other minor substances, such as organic acids, amino acids, proteins, minerals, vitamins and lipids (Saxena et al., 2010).

Honey has numerous uses and functional applications worldwide such as in food systems, religious and magical ceremonies as well as in human and veterinary medicine (Joseph et al., 2007).

Despite the usefulness of honey, due to its adulteration 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 Cliver 1996).

Due to the natural properties of honey and control measures in the honey industry, honey is a product with minimal types and levels of microbes (Ayansola and Adedoyin, 2012).

Most honeys are supersaturated solutions of fructose and glucose with low pH between 3.2 and 4.5. This relatively acidic pH level prevents the growth of many bacteria. (Kebede et al., 2012).

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. 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. (Agbagwa et al., 2011).

Microbial contamination during and or post processing can also result in spoilage or persistence of some bacteria in honey. (Tchoumboue *et al.*, 2007).

2 MATERIALS AND METHODS

2.1 Collection of Samples

Fifteen (15) natural honey samples were collected in May, 2013. Natural honey (Three) samples were obtained directly from bee keepers labelled H_1, H_2 and H_3, ten samples were obtained from retail outlets across the state (H_4 to H_{13}) and two branded samples (H_{14} and H_{15}) were purchased from a supermarket in the state. All samples were stored at room temperature prior to analysis.

2.2 Microbiological Analysis

This was carried out using the method described by Harrigan and McCance, (1976). The viable cell count of samples was determined by carrying out serial dilutions of the stock solution of each honey sample to obtain dilutions of 10^1- 10^4. That is, 10ml of each honey sample was measured and transferred

aseptically into 90ml of 0.1% sterile peptone water to form the stock solution.

One milliliter of each stock solution was transferred into 9ml of 0.1% sterile peptone water (10^1) and this was serially diluted until 10^4 dilutions were obtained. A solution of 0.1ml of 10^4 dilution was then aseptically transferred into sterile plate count agar (PCA), Saboroud dextrose agar (SDA) and MacCkonkey agar (MAC) for bacterial, fungal and coliform counts respectively.

A sterile bent glass rod was used to spread the inocula (diluted sample) on the surface of the culture media. The inoculated plates were then incubated at 37^0C for 24hrs (Plate count agar and MacConkey agar plates for bacteria and coliform counts) while the plates of Saboroud dextrose agar for fungal count was incubated at room temperature (locker) for 3-5 days.

After the incubation period, the number of the bacteria colonies and fungal growth were then counted using a colony counter and the results were recorded and expressed as colony forming unit per milliliter (Cfu/ml) of the sample the using equation

$$\text{Cfu/ml} = \frac{\text{Total number of colonies counted} \times \text{dilution factor}}{\text{Volume of inocula}}$$

3 RESULTS

Microbial (Bacterial, Fungal and coliform) Counts for Honey Samples from Various Sources in Edo State

Sample Identity	Bacterial count (Cfu/ml)	Coliform count (Cfu/ml)	Fungal count (Cfu/ml)
H_1	—	—	—
H_2	—	—	—
H_3	—	—	—
H_4	—	2.0×10^4	1.2×10^5
H_5	1.0×10^4	—	—
H_6	—	—	—
H_7	—	—	1.0×10^4
H_8	1.0×10^4	—	—
H_9	—	—	—
H_{10}	2.0×10^4	—	—
H_{11}	—	—	1.0×10^4
H_{12}	2.0×10^4	—	—
H_{13}	—	—	1.0×10^4
H_{14}	—	—	—
H_{15}	—	—	—

4 DISCUSSION

Microbiological Assessment of Honey Samples

Bacterial counts were recorded for only four samples, obtained from market and roadsides. The total bacterial count (TBC) showed that honey samples H_5, H_8, H_{10} and H_{12} had a count of 1.0×10^4, 1.0×10^4, 2.0×10^4 and 2.0×10^4 Cfu/ml respectively while all other samples had none.

No bacterial count was recorded for samples collected from primary sources and the branded honey samples.

The total plate count (TPC) or fungal count showed low level for the honey samples for which they were detected; Samples H_4, H_7, H_{11} and H_{13} with values of $1.2 \times 10^{5,}$ 1.0×104, 1.0×10^4 and 1.0×10^4Cfu/ml respectively.

The total coliform count (TCC) were not recorded for honey samples except sample H_4 with a count of 2.0×10^4 Cfu/ml.

The study revealed that the freshly harvested honey samples were completely free of microbiological contamination whereas the samples obtained from the market and other retail outlets recorded some

level of contamination, indicating that the sanitary conditions during extraction and handling in the apiaries were quite efficient and that the microorganisms may have been introduced during handling, manipulation by beekeepers, primary honey treatment and storage.

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).

These results are in agreement with the report of Popa *et al.,* (2010) in Transylvania honeys and the reports of Omafuvbe and Akanbi (2009) in their study of the microbiology and physico- chemical properties of some commercial Nigerian honey.

According to Tchoumboue *et al.*, (2007), the contamination with fungi and bacteria indicate inadequate hygienic conditions during collecting, manipulating, processing and storing. Microbial contamination during and or post processing can also result in spoilage or persistence of some bacteria in honey.

5 CONCLUSION

The presence of mould and bacteria in some of the samples may be attributed mainly to contamination due to poor handling at harvest, packaging or storage. Honey obtained from the local markets were contaminated with fungi and bacterial and coliform organisms indicating inadequate hygiene conditions.

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