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Research Article

MICROBIOLOGICAL ANALYSIS OF HONEY FROM DIFFERENT APIARIES IN THE SOUTHWEST AND NORTH-CENTRAL PARTS OF NIGERIA

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A B S T R A C T

Honey is the most important and primary product of beekeeping from an economic point of view. The global use of honey is on the increase partly because of the recent craving for natural products. Previous research has shown that microbial presence in honey is not unusual. The microbial analysis of honey obtained from 5 apiaries revealed the presence of bacteria and fungi. The bacterial and fungi load was within the range of 15-20 x 10^4 cfu/g and 3-4 x 10^4cfu/g respectively. The bacterial isolates were *Staphylococcus aureus* and *Escherichia coli* while the fungi were determined to be *Rhizopus* spp, *Aspergillus niger* and *Mucor* sp. The values obtained show a bacterial count that is markedly elevated than the acceptable limits. This indicates that certain apiaries lack proper sanitary practices during collection and packaging resulting in honey that is not safe for consumption.

INTRODUCTION

Honey is the natural sweet viscous liquid substance produced by honey bees, *Apismelifera.* It is made from plant nectar by the removal of excess water and the conversion of most of the sucrose in nectar into simple sugars (Hill, 2012). The use of honey pre-dates modern civilization. Thousands of years ago, honey was a precious commodity that was sought after for food, as a sacred material in rituals, and for medicinal purposes (Cooper, 2007). The inscription on a piece of clay tablet carbon dated to around 4500 years ago described a recipe for an ointment requiring honey as an ingredient (Cooper, 2007). Honey is the most important primary product of beekeeping from a qualitative and economic point of view. Its main commercial use is for sweetening a wide variety of food (Hill, 2012).

Honey primarily contains sugar and water which accounts for 95-99% of its contents (Moundoi *et al*., 2001). Majority of the sugars are monosaccharides such as glucose (31.3%) and fructose (38.2%), and also disaccharides such as maltose, sucrose and isomaltose (Moundoi *et al*., 2001; Olaitan *et al*., 2007). A few oligosaccharides are also present in honey. The constituents of honey vary depending on the plant from which the nectar is obtained and the environmental conditions.

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Another constituent of honey, gluconic acid, is used for enzymatic digestion of glucose and it is also responsible for the acidity and characteristic taste of honey.

Other substances found in honey include small amounts of pollen, some minerals, vitamins, amino acids, and plant oils (Hill, 2012). The water content of honey ensures its preservation and storage. Minerals present in honey include potassium (most abundant), calcium, copper, iron and phosphorus (Engel, 1999). Vitamins C, riboflavin, nicotinic acid and pantothenic acid are also found in honey (Frasnelli *et al*., 2014).

Honey has been described as having antimicrobial properties and is often used by medical practitioners in cases where modern therapeutic agents are not effective against a pathogen (Arias *et al*., 2005). Studies have reported that honey can be used against a broad spectrum of bacteria and fungi (Cooper *et al*., 2002; French *et al*., 2005; Lusby *et al*., 2005; and Irish *et al*., 2006). It has also been reported that honey promotes healing of wounds (Lusby *et al*., 2002; Ahmed *et al*., 2003). The antimicrobial activity of most honey is derived from the production of hydrogen peroxide but, manuka honey, which is a non-peroxide honey, exhibits significant antimicrobial activity using its low pH and high osmolality (Mandal and Mandal, 2011). Others however, scorn the use of honey to treat wounds or as an anti-microbial agent (Olaitan *et al*., 2007). They indicate that the presence of microbes in honey is a reason to stop its use. Indeed, honey is a contradiction as it is active against many microbes yet, it has been shown to be a

reservoir for several microbes including *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Olaitan *et al*., 2007; Thompson *et al*., 2014). Any microbe that survives in honey must be able to withstand the concentrated sugar, acidity and the microbicidal activity of honey (Olaitan *et al*., 2007). Even with the reported high amounts of microbes found in some honey samples, there is yet to be any report regarding development of microbial resistance to the effects of honey (Dixon, 2003; Mandal and Mandal, 2011).

Objectives of the study

To determine the presence of microorganisms in honey.
To identify the types of organism present in honey.

MATERIALS AND METHODS

Samples collection: Five 200ml bottles of honey were purchased directly from different apiaries in the southwest and northcentral parts of Nigeria. The samples were temporarily stored in a refrigerator in the laboratory at (4^{o}C).

Microbial analysis: The microbiological analyses were carried out in the laboratory, Achievers University Owo, Ondo state. Serial dilution of the honey sample was done by sterilizing six test tubes which were labeled 10^{-1} to 10^{-6} and kept in a test tube rack; 9ml of distilled water was then measured into the six test tubes respectively. 1ml of honey sample was introduced into the first test tube labelled 10^{-1} and mixed thoroughly, 1ml was taken from the test tube and transferred to the second test tube labelled 10^{-2}. This was continued until the 10-6 dilution was obtained. The 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} honey samples were inoculated on Nutrient agar, Potato dextrose agar using the streak plate method, and incubated aerobically at 37^{o}C for 24 hours. The samples were tested for the presence of coliforms up to 45^{o}C. Other conventional microbiological tests like; Gram staining, citrate, indole, oxidase, Carbohydrate fermentation test (Sugar Fermentation Test), catalase and Methyl Red (MR)-VogesProskaeur (VP) were carried out as well.

RESULTS f production of corn syrup, wine and cider

The entire honey samples were positive for microbial presence. Analysis revealed the presence of bacteria and fungus (molds), however no yeasts were detected. Bacterial testing showed that 60 % of the samples had *Staphylococcus aureus* contamination while the remaining had *E. coli* contamination. According to conducted tests, 40 % of the samples had *Aspergillus. niger*; 20 % showed *Penicillium sp.;* 20 % had *Mucor sp.* and 20 % revealed *Rhizopus sp.* contamination. The values obtained for the fungus ranged from a minimum of 2 x 10^4 CFU/g to a maximum of 4 x 10^4 CFU/g. The bacterial load ranged between 7 x 10^4 CFU/g and 20 x 10^4 CFU/g.

Table 1 Morphology describes the shape edge and color of the organisms

s/n	Number of colonies	Morphology
1	6 x 10^1	Circular, round, yellowish and milky
2	2 x 10^2	Circular, round, serrated, and milky
3	8 x 10^1	Circular, round, serrated, and milky
4	1.7 x 10^2	Circular, round, serrated, and milky
5	2.2 x 10^2	Circular, round, serrated, and milky

Table 2 Total viable count of fungi inoculated on potato dextrose agar

s/n	Number of colonies	Morphology
1	5 x 10^1	Circular, round and milky
2	6.5 x 10^1	Circular, round and milky
3	7 x 10^1	Circular, round, serrated, and milky
4	5.9 x 10^1	Circular, round, serrated, and milky
5	5.3 x 10^2	Circular, round, serrated, and milky

Table 3 Bacteria and Fungi load and identification of microbe

s/n	Bacteria (CFU/g)	Fungi (CFU/g)	Identification of bacterial isolates	Identification of fungi isolates
1	15 x 10^4	2 x 10^4	*Staphylococcus aureus*	*Aspergillus niger*
2	20 x 10^4	3 x 10^4	*Escherichia coli*	*Penicillium spp.*
3	7 x 10^4	3 x 10^4	*Staphylococcus aureus*	*Rhizopus nigricans*
4	12 x 10^4	4 x 10^4	*Staphylococcus aureus*	*Aspergillus niger*
5	10 x 10^4	2 x 10^4	*Escherichia coli*	*Mucor sp.*

DISCUSSION

The most commonly observed microbes in honey are bacteria, fungi and yeast (Sereia *et al*., 2010). This has led to a few concerns being raised over the safety of using microbe-contaminated honey. Except for yeast and molds, the inability of microbes to grow or reproduce in honey prevents the occurrence of many cases of honey-related diseases (Snowdon, 1999). However, by forming spores, pathogenic microbes such as *Clostridiumper fringens*, *Clostridiumbotulinum*, and the commonly observed *Bacillus cereus*, can survive in honey for a long time (Snowdon and Cliver, 1996).

The primary mode of microbial contamination of honey is by air. Transmission of microbes to honey can occur through contaminated pollens, dusts or flowers, which generally occurs during the handling and cultivation of nectar. Humans, digestive tracts of honey bees, equipment and water used in processing have also been reported as sources of microbial contamination (Olaitan *et al*., 2007; Sereia *et al*., 2010). An investigation into the contents of the intestines of some honey bees yielded 1% yeast, 27% Gram-positive bacteria including *Bacillus*, *Streptococcus* and *Clostridium* spp., 70% Gram-negative or Gram variable bacteria including *Enterobacter*, *Escherichia coli*, *Klebsiella*, *Proteus*, and *Pseudomonas* (Olaitan *et al*., 2007). As such, honeybees may play a primary role in the contamination of honey. The bacteria isolated in this report, *E. coli* and *S. aureus*, as well as the isolated fungi, are examples of airborne microbes. *E. coli* are also fecal coliform bacteria (Molina *et al*., 2015). This indicates the low sanitary quality and hazardous levels of pollution in two of the honey samples. Several studies have reported the efficacy of honey against *S. aureus* (Liu *etal*., 2015). However, (Alandejani *et al.* 2009) showed that the bactericidal rate of honey for *S. aureus* strains ranges between 63-82 %. This indicates that certain *S. aureus* strains can possibly survive in honey, which may explain the presence of *S. aureus* in our samples. *Aspergillus* is a fungus that has about 200 identified species. Some of these species are harmful and opportunistic while also beneficial. *Aspergillus niger* is widely used in the fermentation industry for the production of citric acid and gluconic acid, as well as in the production of corn syrup, wine and cider (Bakhiet & Al-Mokhtar, 2015). Pulmonary disease, cutaneous infection and otomycosis have also been associated with *A. niger* (Person *et al*., 2010; Keir *et al*., 2013). *Mucor* spp., *Rhizopus* spp., and *Penicillium* spp. are generally associated with food spoilage (Forsythe, 2010). However, this

does not indicate spoilage of the honey samples because the results of several similar studies also reveal the presence of these fungi except for *Rhizopus nigricans* (Jimenéz *et al*., 1994; Martins *et al*., 2001; Martins *et al*., 2003; Kačániová *et al*., 2012). *Rhizopus nigricans* has a large host range and it is usually found in air and soil samples (Slana *et al*., 2011) thus, it can enter honey through several means.

According to a resolution, honey may contain a maximum microbial content of 100 cfu/g (Sereia *et al*., 2010). The high values obtained imply that our samples are not safe for human consumption. Considering the probability that aflatoxin-producing *Aspergillus* spp may also contaminate honey, more honey producers must strive to show greater care and better hygienic sanitary practices during honey processing.

CONCLUSION

This study revealed the level of hygiene among honey producers or handlers particularly in some locations. The health of the public is at great risk if hygiene rules and practices are not adopted to supervise the activities of handlers in order to ensure that these rules are strictly adhered to.

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