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Original Article

Assessment of bacterial quality of honey produced in Tamale metropolis (Ghana)

Parise Adadi [a,*], *Abraham Kusi Obeng* [b]

[a] *Department of Technology for Organic Synthesis, Institute of Chemical Engineering, Ural Federal University, Yekaterinburg, Russia Federation*

[b] *Department of Biotechnology, Faculty of Agriculture, University for Development Studies, Tamale, Ghana*

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ABSTRACT

The bacterial quality of honey from different production sites within Tamale metropolis, Ghana, was estimated using standard microbiological methods. Honey samples were bought from six different production sites within Tamale metropolis and labeled. Samples that were taken from location B recorded the least mean bacterial count of 6.0×10^4 colony forming units/mL with samples taken from location D showing the highest, 1.1×10^5 colony forming units/mL. However, samples from production sites E and F recorded no bacteria growth. Bacteria isolated included *Escherichia coli*, *Staphylococcus* spp., *Shigella* spp., *Streptococcus* spp., and *Bacillus* spp. The pH values of honey samples from the various locations were found to be directly correlated to the average bacteria load. The variation in bacteria load and species at the various production sites and the absence of bacteria growth in two production sites is an indication of the differences in production practices, as well as hygienic conditions at these sites. The presence of these isolates is a cause for concern as pathogenic strains of these bacteria can cause serious health related problems.

1. Introduction

According to Codex Alimentarius commission [1], honey is defined as a natural sweet substance produced by honey bees from the nectar blossoms or the secretion of the living part of plants, which honey bees collect, transform, combine with specific substances of their own, store, and leave in the honeycomb to ripen and mature.

The composition of carbohydrate (82.3%) in honey is more than any other animal product [2]. Honey is composed primarily of the sugars glucose and fructose (monosaccharides). It also contains numerous other types of sugars, disaccharides, like maltose, sucrose, kojibiose, turanose, isomaltose, and maltulose, which make up over 7% of its composition. In addition, honey also contains carbohydrates known as oligosaccharides [3,4]. It also subsumes other ranges of elements such as minerals, proteins, carbohydrates,

* *Corresponding author*. Department of Technology for Organic Synthesis, Institute of Chemical Engineering, Ural Federal University, Mira 19, Yekaterinburg, Russia Federation.
E-mail address: pariseadadi@gmail.com (P. Adadi).

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vitamins, enzymes, free amino acids, and numerous volatile compounds [5,6]. The composition of honey has been shown to depend largely on its floral source, and also varies greatly according to its geographical origin [7,8].

Honey can be used as a natural "sweetening agent" without further processing [9]. Honey is considered as one of the sweetest natural foods in Ghana in terms of its nourishment and therapeutic properties [10]. It can be used as food, for religious ceremonies, and as medicine for both humans and animals [11,12]. It also serves to feed animals and for sweetening drugs for children [10].

The benefits obtained from the consumption of honey can be overshadowed by adulteration. Adulteration of honey occurs by the addition of different materials. Addition of foreign substances such as molasses, starch solution, glucose, sucrose, water, and inverted sugar to honey has been reported [11]. The addition of some of foreign substances can microbiologically contaminate honey [12]. Microorganisms in the honey may arise from the nectar and parts of plant flower, as well as from the processing area.

Good quality honey must lack pathogenic microorganisms that cause enteric illnesses [12]. The present study was therefore carried out to evaluate bacterial quality of honey from production sites, and also to determine the type of bacteria implicated in honey contamination within Tamale metropolis.

2. Methods

2.1. Study location

The study was carried out in the Tamale metropolis. Tamale is the capital town of the Northern region of Ghana.

2.2. Sampling

Honey samples were aseptically collected in sterile bottles from different production sites A, B, C, D, E, and F within Tamale metropolis, Ghana. The samples were then transported in an ice chest containing ice to the Spanish Laboratory of University for Development Studies, Nyanpkala campus for immediate analysis.

2.3. pH analysis of honey samples

The pH of the honey samples was determined using a pH meter (Crison, Barcelona, Spain). Ten mL of each honey sample was measured into a clean beaker. The pH electrodes were first immersed in standard solution to calibrate the pH meter before putting in the honey sample. The pH value was then recorded.

2.4. Microbial analysis

2.4.1. Media preparation

All media were prepared as indicated by the manufacturers. The media used include MacConkey agar (Oxoid Ltd., Basingstoke, Hampshire, England), nutrient agar (Techno Pharmchem, Vardhman, India), and *Salmonella Shigella* agar (Techno Pharmchem, Vardhman, India). All of the media were autoclaved at 121°C for 15 minutes. Then, they were cooled to about 45°C and poured into sterile Petri dishes to solidify.

2.4.2. Preparation of sample

With the aid of the laminar flow hood, serial dilution of the honey samples was carried out with 10 mL of each honey sample in 90 mL of sterile 0.1% peptone water. This was stirred very well using a sterile glass rod.

2.4.3. Inoculation and incubation

One mL each of 10^{-4} and 10^{-5} dilutions was taken aseptically under the lamina flow hood and inoculated on a solidified nutrient agar for total plate count. The inoculated plates were inverted and incubated at 37°C for 24 hours. After 24 hours of incubation, plates with countable colonies [30–300 colony forming units (cfu)] were removed and counted using the colony counter (J.P. Selecta, Barcelona, Spain).

The number of colonies was recorded as cfu/mL. The number of cfu/mL of the sample was calculated as follows:

$$\text{cfu/mL} = \text{cfu} \times \text{dilution factor} \times 1/\text{aliquot}. \quad (1)$$

2.4.4. Bacteria isolation, identification, and confirmation

Sixteen colonies were randomly selected from sampled nutrient agar plates and streaked on fresh nutrient agar plates. These plates were then incubated at 37°C for 24 hours. This was carried out to obtain pure cultures for identification purposes. Morphological characteristics, gram staining, and other biochemical tests were also executed to identify the isolates.

One mL each of 10^{-4} and 10^{-5} dilutions was also inoculated on the solidified McConkey agar and incubated at 37°C for 48 hours. Unique colonies were selected and streaked on fresh McConkey agar plates to obtain pure cultures. Morphological characteristics of the pure cultures as well as other biochemical tests were then used to confirm the species.

One mL each of 10^{-4} and 10^{-5} dilutions was again inoculated on SS agar and incubated at 37°C for 48 hours. Again, distinct colonies were selected and streaked on fresh SS agar plates to obtain pure culture. Pure cultures were again identified and confirmed using morphological features as well as other biochemical tests.

2.4.5. Gram staining and biochemical tests

Biochemical tests carried out included, catalase, modified oxidase, oxidative-fermentative, furazolidone and bacitracin susceptibility, oxidase, sugar fermentation, indole, citrate utilization, urease, and motility tests. Gram staining and all biochemical tests were carried out according to [13].

3. Results

The microorganisms counts ranged from 6.0×10^4 cfu/mL (Location B) to 1.1×10^5 cfu/mL (Location D). Samples taken from locations E and F showed no growth. Different genera of bacteria were isolated from honey samples at different production sites. *E. coli* and *Shigella* spp. were isolated from all the samples except samples from locations E and F.