Many anthropological and biological studies detail indigenous people’s use of stingless bee honey to treat various ailments, such as bacterial infections, sore throats, and digestive diseases. The purpose of this study was to examine the optimum storage method of stingless bee honey produced by *Meliponula bocandei* and *Meliponula ferruginea* in Central Ghana. Additionally, another of the study’s goals was to determine whether increased shelf-time would affect the honey’s antimicrobial properties during a series of bioassays against *Pseudomonas aeruginosa*, an infectious bacterial species showing increasing resistance to man-made antibiotics. Over three weeks, no significant difference was observed between two types of storage—covering the bottle in aluminum foil to prevent sunlight from entering or leaving the honey exposed to the light (p>0.05). There was, however, a significant difference between the two species’ honey in terms of pH, moisture content, and antimicrobial efficacy (all p<0.001). Honey produced by *M. ferruginea* had a significantly higher pH and moisture content than *M. bocandei* honey and did not have any antimicrobial activity against *P. aeruginosa*. Additionally, the mean zone of inhibition for the *M. bocandei* honey significantly increased between the initial and final bioassays (p=0.001). These data indicate that *M. bocandei* honey contains antimicrobial compounds that can be used against *P. aeruginosa* to fight bacterial infections as a substitute to man-made antibiotics. Understanding the antimicrobial properties of *M. bocandei* honey may help in the fight against *P. aeruginosa* and other common infectious bacteria that are quickly gaining resistance to antibiotics.

**INTRODUCTION**

Stingless bees (Hymenoptera: Apidae) are found in the tropical regions of Africa, South America, Australia, and Southern Asia, but are believed to have originated in Africa (Daly et al. 1998). Because they lack a vestigial sting, stingless bees are essentially harmless to humans, rarely going out of their way to attack individuals, and only employ fighting swarms when their colony is directly attacked (Gloag et al. 2008). Across the world, various cultures use stingless bees for honey and wax production, as well as for the bees’ outstanding pollination capacity. Numerous studies describe indigenous cultures’ and tribes’ use of stingless bees to increase the yield of crops important for the community’s subsistence (Buchmann et al. 1996; Byarugaba 2004; Karikari and Kwapong 2007; Mendes dos Santos et al. 2008). Furthermore, 177 crops in the world are dependent on stingless bees for pollination (Karikari and Kwapong 2007).

In Ghana, crops such as cocoa have been found to produce a better yield when pollinated by stingless bees. A study by Karikari and Kwapong (2007) found that an overwhelming percentage of farmers noticed an increased crop production when using native stingless bee species to pollinate their farms. Beyond the agricultural significance, propolis production, that is a resinous mixture of sap collected by bees, and honey provide naturist treatments against digestive, respiratory, cardiac, and rheumatic disorders (Pino 2006; Farnesi et al. 2009). Stingless bee honey has been found to contain antimicrobial agents such as flavonoids (DeMera et al. 2004; Guerrini et al. 2008; Oddo et al. 2008). Additionally, studies have shown that stingless bee honey contains polyphenols, making it more resistant to fermentation than the honey produced by the common honeybee, *Apis melifera* (Guerrini et al. 2008). These two components of stingless bee honey make it a useful medicinal aid in the fight against bacteria infections that cause thousands of deaths worldwide each year. One of these infectious bacterial species, *P. aeruginosa* is an aggressive Gram-negative pathogen found in wounds, particularly burns. *P. aeruginosa* is rapidly and continuously gaining resistance to common man-made antibiotics typically used to treat bacterial infections (Estahbanati et al. 2002; Irfan et al. 2008; Kohler et al. 2009, Pappas et al. 2009; Strateva et al. 2009). Although there is a wealth of information available about stingless bee honey from South America, the equivalent knowledge about the African bee is missing (Wagner et al. 2008). The purpose of this study was to ascertain the optimum storage technique for *M. bocandei* and *M. ferruginea* honey, the two common stingless bee species in Ghana, determine its average shelf-life, and investigate the antimicrobial activity of the honey with the course of time. We hypothesized that as stingless bee honey was stored for a longer time period, the antimicrobial efficacy will decrease due to the

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breakdown of flavonoids and other antimicrobial compounds. Additionally, we hypothesized that covering the honey, therefore preventing the contact with light will decelerate the flavonoid breakdown and, therefore, the antimicrobial potency loss.

MATERIALS AND METHODS

Origins of honey
Honey samples were collected from the International Stingless Bee Centre at the border with Kakum National Park and located approximately 30 km north of University of Cape Coast, Ghana. Three different honey samples were collected. Two were taken from different *M. bocandei* hives—one natural log hive (honey 1) and one man-made hive (honey 3) built from wooden slats. The other honey sample was obtained from a *M. ferruginea* hive found in a natural log (honey 2).

For each sample collection, small incisions were made into the honey pots via a scalpel and the honey was either drained into large containers or extracted with a syringe depending on the shape and size of the honey pot. The honey was then strained through multiple layers of mosquito net to remove any large particles and, immediately afterwards, the initial pH and temperature were measured and recorded with a Hanna HI83141 Portable pH/mV and Temperature Meter. As the honey samples were transported to the laboratory, the containers were covered and kept hidden from sunlight in order to prevent degradation of the honey as suggested by Guerrini et al. (2008).

Bottling and storage
The honey from each hive was separated into transparent 50 mL vials that served as replicates. Half of the vials from each sample were left uncovered to allow sunlight entry, while the remaining half were covered with aluminum foil to prevent the light from entering and possibly degrading some of the honey’s natural compounds. Additionally, six vials—two from each hive with one covered and one uncovered—were set aside for use in the bioassays in order to prevent disturbance of the honey or transfer of foreign molecules from the environment into the vials by the pH meter probe.

Shelf-life quantification
Beginning the day after the honey collection and ending 20 days later, the pH and temperature were measured daily using the pH meter described in section 2.1. All of the measurements were taken at 11:00 GMT each day in order to prevent variations in the pH and temperature due to the time of day and length of time between measurements. Additionally, the moisture content was measured daily using a Stanley-Bellingham refractometer. Due to mechanical problems with the refractometer, the moisture measurements were not started until day three.

The data from the entire 20 days were averaged and multiple one-way analysis of variance (ANOVA) tests were performed, comparing the means for pH vs. uncovered/covered honey, moisture content vs. uncovered/covered honey, pH vs. bee species, moisture content vs. bee species, pH vs. temperature, pH vs. days, and pH vs. moisture content. A confidence interval of *p* < 0.05 was used throughout this study for all statistical analyses.

Assay for inhibition of microbial growth
At the beginning and conclusion of the three-week period, a bioassay was performed with the honey samples to test microbial growth inhibition. Each of the six honey samples was tested against a species of *P. aeruginosa* (G-, ATCC 27858), isolated from a patient with a chronic leg ulcer at Central Regional Hospital in Cape Coast, Ghana. After testing its resistance to a series of common antibiotics, including penicillin and tetracycline, the bacteria was cultured on sensitivity agar (Biotech S.T.A.).

Small 2 cm diameter disks of filter paper were soaked in each nondiluted sample and placed into an oven to dry out, in order to prevent the honey from diffusing outside of the disk and altering the zone of inhibition. After drying overnight, one disk of each honey sample was placed onto the corresponding section of a sensitivity agar plate containing *P. aeruginosa* (Figure 1). Three replicates for each bioassay plate were made, as well as a control plate with *P. aeruginosa* only. The plates were incubated under aerobic conditions at 37°C for 24 hours.

![Figure 1. Diagram of bioassay plates used to test antimicrobial effectiveness of each honey samples. Plates were marked as shown and honey disks were placed in the center of the allotted space.](image)

Quantification of microbial growth inhibition
Quantification of microbial growth inhibition was determined by measuring the diameter of the zone of inhibition from the center of the honey disk. For each bioassay including the replicates, the zones of inhibition for each honey sample were averaged and the means were analyzed by a one-way ANOVA for mean zone of inhibition vs. bee species, mean zone of inhibition vs. bioassay number (initial or final), and mean zone of inhibition vs. uncovered/covered.
RESULTS

Shelf-life properties
Over the course of the three weeks, temperature, pH, and moisture content fluctuated unsignificantly—p>0.05 (Figure 2, 3) and were at levels consistent with the standards set forth by Souza et al. (2006). Temperature of the honey was not graphed because the temperature variation between samples only fluctuated from day to day due to the environmental temperature’s changes. There was, however, a significant difference between the shelf-life measurements of the bee species. For a one-way ANOVA of pH vs. bee species and moisture content vs. bee species, both resulting p-values were significant (p<0.001), *M. ferruginea* honey having the higher pH and moisture content values (Figure 2, 3). Significant p values were also found for one-way ANOVA tests of pH vs. moisture content (p<0.001), pH vs. temperature (p=0.041), and moisture content vs. temperature (p=0.008). All other ANOVA tests returned nonsignificant values (all p>0.05).

Antimicrobial properties
Overall, there were significant changes in the mean zone of inhibition over the course of three weeks. For both the initial and final bioassay, the *M. ferruginea* honey did not have a zone of inhibition for any of the three replicates, whereas both *M. bocandei* honey samples produced zones of inhibition for all bioassays (Figure 4). All of the one-way ANOVA tests were run once with each sample of honey included. Following those tests, the *M. ferruginea* honey samples were removed from the data to determine if there were any significant differences between initial and final bioassays for the samples of *M. bocandei* honey. One-way ANOVA tests of mean zone of inhibition vs. bee species (p<0.001) and mean zone of inhibition vs. bioassay number of *M. bocandei* samples (p<0.001) were found to be significant. All other ANOVA tests of mean zone of inhibition vs. *M. bocandei* samples only mean zone of inhibition vs. uncovered/covered, and mean zone of inhibition vs. bioassay number for all samples returned nonsignificant values.

DISCUSSION

As demonstrated in section 3.1., there was no significant difference between the storage methods of honey, indicating that sunlight does not affect the honey’s pH, moisture content, or temperature. However, the significant difference of the pH and moisture content of *M. bocandei* and *M. ferruginea* honey points out to a species difference. Kajobe (2006) suggests that different species of stingless bee will forage on different flower species depending on their preferences. Therefore, the differences observed might be due to various chemical compositions in the respective plants. Another possibility is that the rainy and dry seasons of Ghana affect how far the bees will look to forage and what plant species they will pollinate (Kajobe et al. 2005). Additionally, the type of plants and flowers each species of bee forages on can affect the antimicrobial properties of the particular honey. This could explain why honey produced by *M. ferruginea* did not have any antimicrobial effectiveness against *P. aeruginosa*, while honey produced by *M. bocandei* was very effective against this particular bacterial species.

The fluctuations in temperature, pH, and

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**Figure 2.** Graph of the change in the mean pH (units) over time (days) for each of the six honey samples.

**Figure 3.** Graph of the change in the mean moisture content (%) over time (days) for each of the six honey samples.
moisture content over the three-week period are most likely due to the fluctuations in temperature and environmental conditions. There are a few sources of error that could have resulted from these fluctuations and possibly impacted the day-to-day results, but not the comparison between the two species. Because the study was performed during the rainy season, there were extreme variations in humidity and temperature, that could have impacted the moisture content of the honey. However, there was little effect of these fluctuations on the comparison of the honey produced by different species.

As to what caused such a significant difference between mean zones of inhibition between the initial and final bioassay of *M. bocandei* honey, we propose that there is possibly another shelf-life variable significantly changing and causing a change in the antimicrobial properties of the honey. Although sunlight does not affect pH, moisture content, and temperature, there may be a compound that is being affected. Studies have shown that sunlight does degrade honey compounds, which could explain why the positive changes in antimicrobial efficacy (Guerrini et al. 2008).

**Figure 4.** Plot of the mean zone of inhibition (mm) for both the initial and final bioassay of each of the six honey samples. Sample names were abbreviated to uc1 (uncovered honey 1), c1 (covered honey 1), uc2 (uncovered honey 2), c2 (covered honey 2), uc3 (uncovered honey 3), and c3 (covered honey 3).

Efforts need to be focused on conserving stingless bee species worldwide. These insects hold a wealth of knowledge that is virtually untapped in terms of chemical and medicinal properties and are being threatened by deforestation of tropical rainforests. Often, stingless bee hives are destroyed by farmers who believe they pose a threat to their fields and will negatively impact their crop yield (Brown et al. 2001). In order to continue research on stingless bees, their natural habitat needs to be preserved through beekeepers and farmers education around the world and the prevention of deforestation.

Further research needs to be performed on the shelf-life of stingless bee honey over a longer period, taking into account additional variables, such as sucrose levels. More research should also be conducted on the foraging activity of *M. bocandei* and *M. ferruginea*, in order to determine whether *M. bocandei* collects nectar and pollen that contains particular flavonoids, while *M. ferruginea* does not. Another interesting direction would be to investigate whether the antimicrobial effectiveness of honey corresponds to the antimicrobial effectiveness of propolis.

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