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Gamma irradiation effect on the microbial load and physicochemical properties of honey from Ghana

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Abstract. *Larbi DA, Klutse CK, Adotey DK. 2022. Gamma irradiation effect on the microbial load and physicochemical properties of honey from Ghana. Cell Biol Dev 6: 94-107.* The honey's high sugar concentration and low pH give it antimicrobial properties and make it difficult for microorganisms to grow. This study is to ascertain microorganisms' presence in Ghanaian honey, the sources of microbial contamination, the physico-chemical properties of honey, and the effect of gamma radiation on the microbial load. Furthermore, 90 honey samples were collected from Brong Ahafo, Ashanti, and Greater Accra, Ghana, with 30 from each region. Honey was sampled directly from the beehive with the comb before the farmer harvested, and honey was sampled from retailers who buy directly from the farmer. The effect of gamma radiation on the microbial load was studied using a 60Co source gamma irradiation facility at doses of 20 kGy, 30 kGy, and 40 kGy on the presence of microbes and the physicochemical properties (pH, reducing sugar, apparent sucrose, and ash content) of honey. The pH values obtained for the Honey Comb samples were in the range of 3.6-3.9, and the pH for the Retail samples was in the range of 4.9-5.6. Microorganisms were not detected in about 70% of the honey sampled directly from the honeycomb. The mean microbial count in the remaining 30% was within the range of 30-35%, whereas all the honey sampled from the retailers was contaminated with microbes. The mean microbial counts in the retailer samples were 148 CFU/g, 183 CFU/g, and 271 CFU/g for Ashanti, Brong Ahafo, and Greater Accra Regions, respectively. These values were significantly higher than the required maximum relative to the MERCOSUR (Mercado Comun del SUR) standard (≤ 100 CFU/g). The low level of microbial detection in the Honey Comb samples (30-35 CFU/g) could be due to their relatively low pH levels (3.6-3.9) compared to the retailer samples with pH within 4.9-5.6. The ash content of all the honey sampled and analyzed was within the required standard, with an average of 0.16% in the honeycomb samples and an average of 0.62% for the retail samples. The apparent sucrose concentration (in percentage) in honey sampled from the retailers was within the range of 22-33%, which is beyond the required maximum as stipulated by the CODEX Alimentarius Commission (≤ 10%). The study showed that a 20 kGy gamma radiation dose was enough to denature the microbes and preserve the honey's essential qualities. Finally, to ensure good quality honey on the Ghanaian market, it is recommended that honey meant for human consumption should undergo gamma irradiation (cold pasteurization).

Keywords: Antimicrobial, honey, primary and secondary sources, radiation

INTRODUCTION

Honey is a food product consumed by many people throughout life due to its high nutritional value. It is essentially composed of reducing sugars hence a major source of energy. It is used as a substitute for sugar by people and helps digestion and removal of free radicals from the body, among other benefits. Besides sugars, honey also contains proteins, organic acids, amino acids, vitamins, and lipids, making it a rich source of other nutrients (White 1975; Da Silva et al. 2016; Agussalim et al. 2019).

Research has shown that honey physicochemical has the potential to prevent cancer (Beretta et al. 2007), can also be used to cure some eye defects (Kwapong et al. 2013) and other physicochemical (Suntiparapop et al. 2012; Truzzi et al. 2014; Biluca et al. 2016; Chuttong et al. 2016; Nordin et al. 2018; Ranneh et al. 2018; Agus et al. 2019; Villacrés-Granda et al. 2021). Economically, honey is a product of international value serving as a source of foreign exchange for many countries, including Ghana, contributing significantly to the gross domestic product. It is also a major source of livelihood for many people who are into apiculture. But honey is only as good as its quality, and honey quality cannot be judged just by its physical appearance. For these reasons, there is a need to ensure that honey is free from microorganisms and that it is wholesome for human consumption.

Honey is a flavourful product consumed globally as a high-nutritional food. It is composed of a complex mixture of carbohydrates (glucose and fructose account for nearly 85-95%, the rest being sucrose) and other minor substances such as organic acids, amino acids, proteins, minerals, vitamins, and lipids (White 1975). Due to its high sugar concentration, high osmotic pressure, and low pH, it is difficult for microorganisms to grow. However, research has shown that microorganisms have been detected in honey, including pollen, molds, yeasts, and the spores of *Clostridium* sp. and *Bacillus* sp. (Snowdon and Cliver 1996). There are two main sources of microorganism contamination: primary sources include pollen, digestive tracts of honeybees, dust, air, soil, and nectar; The next sources are those arising from animals, such as some rodents, insects, etc., that may visit the beehive while honey is maturing. Secondary sources of contamination are manipulation by people, including food handlers, cross-

contamination, equipment, and materials where harvested honey is stored (Snowdon and Cliver 1996).

The microorganisms are inactive in honey, but they could present different results when transferred into a living host through ingestion. Sulfite-reducing *Clostridium* is an indicator organism whose presence in honey provides evidence of contamination or pollution (Snowdon and Cliver 1996). Clostridium spores are especially dangerous for infants and small children (Centorbi et al. 1999). Botulism is a neuroparalytic disease caused by *Clostridium botulinum* which can survive in honey and be transferred into an infant.

Some North American and European countries have recorded reports of anaphylactic shock in people with allergens (pollen) who eat raw honey. At its worst, anaphylactic shock could cause breathing difficulties, low blood pressure, dizziness, fainting, heart failure, weakness, sweating, nausea, vomiting, and prickling sensations in the brain (Bartkowski 2014). Symptoms of less severe allergic reactions resulting from raw honey include itching, puffy skin, and rash.

Honeybees obtain their nectar from flowers of different plants, including Rhododendrons, with the nectar containing a substance called grayanotoxin, according to the America Food and Chemical Toxicology journal (Koka and Koka 2007). It is explained in this article that grayanotoxins are chemicals that are toxic to the nervous system; they prevent nerve cells from functioning effectively. In addition, pollen grains stick to the bees' bodies during foraging, are transferred into the honeycomb, and mature as part of the honey. This pollen causes allergic reactions in people when exposed to pollen-contaminated honey.

There is a scarcity of published information on the microbiological properties of Ghanaian honey; and very limited information on the physicochemical characteristics of Ghanaian honey. Therefore, the main objective of this research was to investigate the presence of microorganisms in Ghanaian honey and to assess how to improve the quality of Ghanaian honey through cold pasteurization (Gamma irradiation).

MATERIALS AND METHODS

Sampling locations

This research was conducted in Ghana, West Africa, in three (3) regions; Brong Ahafo, Ashanti, and Greater Accra Table 1 and Figure 1 show the regions and sample locations and the map of the locations, respectively. Two (2) of the regions-Brong Ahafo and Ashanti-are believed to be among the largest honey production centers in the country (Alaazi et al. 2010). Brong Ahafo has an estimated 2188 apiaries, the Ashanti has 2243 apiaries, and the Greater Accra region has 1536 apiaries (Alaazi et al. 2010).

The sampling locations were small towns with relatively low vehicular and construction activities compared to the major cities in the regions. The beehives are mostly located away from residences or places where human activities are predominant to protect the residences and ensure minimal human influence.

The sampling started from August through December 2014, samples were taken from the sample points in the Ashanti and Brong Ahafo Regions. From January through March 2015, samples were taken from the Greater Accra Region. Sampling was conducted with the available resources (period within which to complete the project, beekeepers, and finance), and most of the places visited were rural communities. Considering the complex nature of the sampling process and the locations, sampling within the same month proved quite difficult with the available resources. These informed the decision to spread the sampling across this period.

Questionnaire administration

Based on the objectives of this study, a questionnaire was designed to find out the activities and apiculture practices that contribute to honey contamination. In addition, oral interviews were conducted based on the questionnaire with those who could neither read nor write.

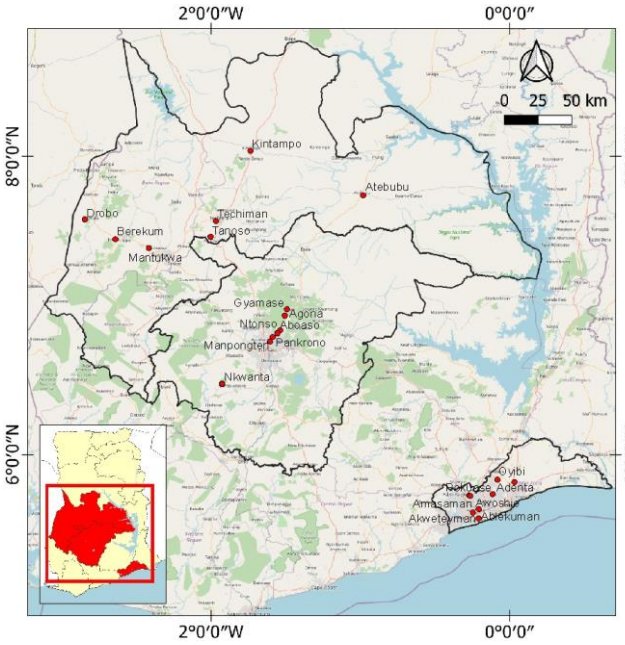

Figure 1. Map of Ghana showing sampling locations

Table 1. Sampling locations in Ghana

Region	Towns
Ashanti	Aboaso, Agona, Ahwiaa, Asenua, Fawode, Jamasi, Mampongten, Nkwanta, Ntonso, Pankrono
Brong Ahafo	Atebubu, Berekum, Drobo, Dumasua, Fiapre, Kintampo, Mantukwa, Nsoatre, Tanoso and Techiman
Greater Accra	Ablekuma, Adenta, Afienya, Akweteyman, Amasaman, Awoshie, Ayi Mensah, Oyibi, Pokuase and Weija

Sampling

The samples were taken along the farmer/hunter-to-trader route. The first set of samples was essentially honey taken directly from the honeycomb before the farmer harvested it for extraction and packaging. The next set of samples was honey harvested, processed, and ready for selling to the retailers.

The last set of samples was taken from the retailers; only the retailers who bought from the sampled farmers were considered; it is efficient because the retailers personally assisted in locating the farmers from whom they obtained their stock. Some of the samples were obtained from honey hunters. At each sampling location, 3 samples were taken; in all, 30 samples were taken from each region. A total of 90 samples were collected. Before the analysis, the honey samples were stored in clean plastic bottles and tightly covered to prevent external contaminants.

The samples were systematically coded based on the regions, sampling site, and collection date. They were then sealed in transparent polyethylene bags (to prevent, as many dust particles as possible, from the bottled samples) and packed into paper boxes for transport to the laboratory. The samples were removed from the bags at the laboratory and kept on clean shelves at room temperature, still in their original packages, before analysis. The temperature conducive for microbial growth varies concerning their type. For example, mesophyllic bacteria grow best within 30^{o}C to 37^{o}C; keeping the honey samples at room temperature and below (when air conditioning is available) could inhibit microbial growth. As much as possible, care was taken to prevent contamination in the storage shelves. For each sampling point, a control was added. The control consisted of an empty bottle treated the same way as the sampling bottles, except that honey was not added. All the plastic containers for the sampling were pre-washed and steam sterilized.

Analysis of honey samples

The flow diagram (Figure 2) illustrates a general overview of the analyses performed on the honey samples;

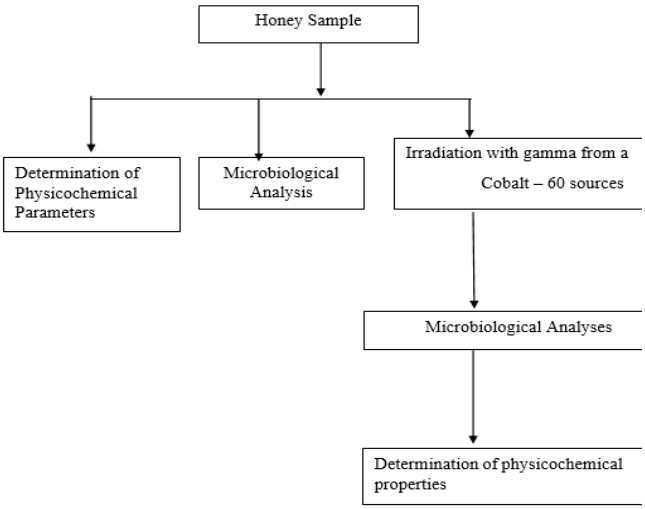

Figure 2. Flow diagram of analyses

Microbial analysis

The microorganisms in the honey samples (farmer-to-trader route) were determined using the Colony Count Technique (ISO 4832: 2006-Microbiology of food and Animal Feeding Stuffs-Horizontal Method for Enumeration of Coliform). That was followed by decontamination of the honey samples by irradiation with gamma rays using a cobalt 60. The samples were analyzed for Total Viable Counts, *Faecal* sp., *Clostridium* sp., *Staphylococcus* sp., and *Salmonella* sp. These are common causative microorganisms of foodborne illness and are also indicator organisms showing the unhygienic processing, packaging, and other treatment of food. The total viable count estimates the samples' possible aerobic and anaerobic microbes.

Media

Media used to culture microbes can be seen in Table 2.

Preparation of agar

The method of preparation was the same for each agar. First, about 22 g of the agar powder was weighed into a 0.5 L Erlenmeyer flask based on the manufacturer"s instruction. Next, about 50 mL aliquot of distilled water was added to the flask's content and then shaken gently to allow for mixing. When it blended, more distilled water was added to the content to reach the 0.5 L mark (Marshall 1993). The prepared media were then sterilized at 100^{o}C for about 30 minutes allowing the mixture to melt further and enhance blending. Next, the media was tempered, which was done whiles ensuring that the water level in the bath was about 1 cm above the level of the medium in the bottle.

Inoculation and incubation

The working surface of the laminar chamber was cleaned with 70% isopropyl alcohol. Duplicate inoculations were done for each sample; hence each duplicate petri dish was marked per sample per microbe analyzed. About 10 g of each honey sample was weighed and homogenized in 90 mL of peptone water solvent by agitating in a dilution bottle for about 10 seconds. About 1ml aliquot of the prepared sample was pipetted into five other 10 mL bottles with peptone water.

A cotton-coated piece of the plastic rod was used to wipe the inner surface of the control plastic bottle and the content dissolved in 5 mL of peptone water which was then transferred into a labeled Petri dish for inoculation with Plate Count Agar and incubation. That was done for each control taken at the sample points visited. The controls were taken mostly at the honey processing areas.

Table 2. Media

Total viable count	**Plate count agar**
Faecal sp.	Eosin Methylene Blue agar
Clostridium sp.	Perfringens agar
Staphylococcus sp.	Baird Parker agar
Salmonella sp.	Xylose Lysine Deoxycholate agar

About 1 mL aliquot of the prepared sample was pipetted into the labeled Petri dishes, and 15 mL of the prepared agar was added. That was repeated for each agar for the various plate counts; Perfringens, Baird-Parker, eosin methylene blue, and Xylose Lysine Deoxycholate. These transfers and sample dilutions were done in a laminar chamber where the temperature was controlled at 45ºC. The agar was transferred 15 minutes after the sample mixtures were pipetted into the petri dishes. In addition, for each sample inoculated for incubation, a blank Petri dish (without sample) was inoculated with plate count agar to verify if the pre-sterilized Petri dishes were free of any possible microorganisms which could influence the results.

The sample-agar mixtures in the Petri dishes were gently swirled to mix thoroughly and left for 15 minutes to settle, after which 4 mL of the respective agar was added to the mixtures as a second layer and left to solidify, which was to ensure that the entire sample was well covered with the agar. The inoculated samples were then incubated at 37ºC for 48 hours, monitoring the changes every 24 hours until the incubation time had elapsed.

Counting of colonies

Colonies were counted immediately after the incubation period. A colony represents cells well separated on the plate and can be distinguished after growth; it could be one cell or several thousand (Sutton 2006). Petri dishes (plates) that have microbial growths up to at least 30 colonies or at most 300 colony units formed per plate are the most favored in colony counting, sometimes the range could be 25 to 250 CFU/plate, and these were selected for counting (Sutton 2006). For plates that contained colonies that spread out, a representative portion of the plate was selected for counting; if a quarter of the plate was counted for colonies, it was estimated that the colonies formed on the plate were four times the count values for the quarter portion.

Counts were calculated using the formula (ISO 4832 formula for the microbial count):

$$N = \frac{(C1 + C2 + C3)}{v} \times d$$

Where C1, C2, C3 = count values for the triplicate plates.

V = volume of sample on each plate

d = dilution factor for the plates counted

N = the Colony Forming Unit (CFU) per gram or mL of product. Count results were rounded off to two (2) significant Figures

Determination of physicochemical parameters

The physicochemical parameters (pH, Apparent Reducing sugar, Apparent Sucrose, and Ash content) of 60 honey samples were determined to assess the honey quality. In addition, the results were compared to International Food Standards for honey as stated in the CODEX Alimentarius Standards. The samples selected were the honeycomb samples (S1) and the consumer samples (S3) from all 3 regions. The samples taken after harvesting and treating were not subjected to any physical and chemical test because it was assumed there would be no significant alteration to the physicochemical properties during percolation and sieving. Instead, the physicochemical tests were conducted before and after "decontamination" by gamma radiation to determine how they are affected by the high-energy photons.

The quality of honey depends on several parameters. These parameters include reducing sugar and sucrose concentration, pH, and ash percentage. By CODEX Alimentarius Standards, good quality honey must have less than 60% reducing sugars and not more than 10% sucrose.

Ash content

The Ash dish was heated in an electrical furnace at 600ºC, cooled in a desiccator to room temperature, and weighed to the nearest 0.001 g (M_2). Exactly 5 g of the honey sample was weighed to the nearest 0.001 g into the ash dish that had been prepared (M_0). Two drops of olive oil were added to the sample. Ashing was commenced at a temperature of 350ºC and progressively increased to a maximum of 600ºC. After heating for an hour, the ash dish was cooled in the desiccator and weighed (M_1) (Bogdanov 2009).

The ash proportion (W_A) in $[\frac{g}{100}\ g]$ honey was calculated using the formula below as stipulated by the International Honey Commission;

$$W_A = \frac{M_1 + M_2}{M_0} \times 100$$

M_0 = Weight of sample

M_1 = Weight of ashed sample on the dish

M_2 = Weight of pre-ashed dish without sample

Reducing sugar

"Apparent reducing sugars" are the sugars that reduce Fehling" s solutions from blue to brick red under specified conditions (Bogdanov 2009). Honey primarily comprises reducing sugars (85% to 95%) and apparent sucrose (5% to 10%). The high sugar concentrations give honey its osmotic pressure, which helps inhibit microorganisms' growth.

The Lane and Eynon procedure modified the method employed in this research. This procedure involved reducing Soxhlet"s modification of Fehling"s solution by titration at the boiling point against a solution of reducing sugars in honey. About 50 mL of 1% sample (prepared by diluting 2 g of honey in 200 mL distilled water) were placed into a burette as the titrant (Table 3). In the preparation of the analyte, 10 mL of each Fehling solution A and B with 8 mL of distilled water were transferred into a 250 mL conical flask. That was followed by adding 2 drops of 0.2% Methylene Blue Indicator. The mixture was heated until it started boiling. Titration started at this point until the initial blue color changed to brick red. Titration was stopped, and the volume of titrant was noted and recorded.

The % Total Reducing Sugar was calculated using the equation:

$$\% \text{ total reducing sugar} = \frac{2 \times 10.2}{titre\ value \times mass\ of\ sample} \times 100$$

10.2 = Glucose factor

Apparent sucrose

The apparent sucrose was determined by the concentration of inverted reducing sugar, which was done by measuring about 10 mL of 6.34 M HCL and 50 mL of 1% honey and transferred into a conical flask and kept in a water bath at 60°C for 20-30 minutes. First, the sample was cooled and neutralized with a 5 M NaOH $_{(aq)}$ to a pH of 7 (the pH was monitored with the pH meter). Next, the samples were transferred into a burette. Next, about 10 mL each of Fehling solutions A and B and 8 mL water were transferred into a 250 mL flask and heated till the solution started boiling. Titration started at this stage after adding 2 drops of 0.2% Methylene Blue Indicator and titration was completed while the solution was boiling. At the endpoint, the blue color of the solution changed to brick red.

Calculations

The % Reducing sugar was calculated using the formula

$$= \frac{2 \times 10.2}{titre\ value \times mass\ of\ sample} \times 100$$

From the results obtained, the percentage sucrose was calculated by:

Percent sucrose = % sugar (after inversion) × 0.95

The "Harmonised Methods of the International Honey Commission for honey quality analyses.

pH

Honey is a product of high acidity with pH values ranging from 3.6 to about 6.5 for different types of honey (Bogdanov 2009). The low pH also inhibits the growth of microorganisms in honey, contributing to the honey product's long shelf life. The pH meter was calibrated using buffer solutions of pH 3.7 and 9.0. About 10 g aliquots of the sample were weighed into 75 mL of carbon dioxide-free water in a 250 mL beaker. The solution was stirred to homogenize, the pH electrode was immersed in the solution, and the pH was recorded.

Sterilization of honey by irradiation with gamma rays

Instrumentation. The Gamma Irradiation facility is a ^{60}Co (category IV wet storage gamma radiator) with an initial source strength of 50 kCi. It was manufactured and installed at the Radiation Technology Centre, Gamma Irradiation facility at Ghana Atomic Energy Commission in Accra, by the "Isotope Company Ltd," a company based in Hungary, in 2010 (RTC-GAEC). The cobalt 60 (^{60}Co) source with the current strength of 26 Kci with a dose rate of 1.4 kGy/hr around the shroud.

Packaging of the honey samples for irradiation. About 50 mL of the honey samples were packaged in 100 mL polyethylene bottles for irradiation. Next, 20 bottles, each per dose rate, were secured with masking tape, and the samples were transferred into the gamma chamber by the pneumatic transfer system attached to the facility. The samples were irradiated with gamma radiation from a Co-60 source at varying doses of 20 kGy, 30 kGy, and 40 kGy. The doses were varied in the control room.

The Ethanol Chlorobenzene (ECB) dosimeters were stuck to the samples and subjected to gamma radiation for half the predetermined period for each dose; this is the time taken to irradiate the samples at each dose completely. For example, at a dose rate of 1.4 kGy/hr, irradiating at 20 kGy will take approximately 14 hours to complete. The honey samples were turned through 180 degrees and then subjected to gamma radiation for the other half of the predetermined period to ensure homogenous distribution of the dose delivered under the same conditions.

The ECB dosimeters were removed from the honey samples after the irradiation period. Then the absorbed dose was determined using a calibrated readout instrument (High-Frequency Dosimeter System, Model 2131, version 2.5, produced by SENSOLAB LTD).

The expected and absorbed/delivered doses for the Honey samples are as follows Table 4.

RESULTS AND DISCUSSION

All foods, including honey, should be free of microorganisms. Therefore, the honey samples were analyzed for microorganisms to ascertain whether the treatment given to the product in the percolation, sieving, and packaging processes affected its quality before it finally reached the consumer. The microorganisms considered in this research are very common microbes frequently encountered where food products are treated in conditions suspected to be unhygienic.

Staphylococcus sp. is common in man" s respiratory passages, skin, and superficial wounds. Although the heat of cooking can denature them, the toxins produced by these microorganisms can resist heat and may not be destroyed in the human alimentary, causing food poison (Wagner 2008). In addition, since honey is used raw, it will be dangerous to have these microbes.

Table 3. Apparatus and reagents for reducing sugar and sucrose determination

Apparatus	**A 50 mL burette, Erlenmeyer flask, electric heater**
Chemicals and Reagents:	Fehling solution A (7% CuSO4) and Fehling solution B (25% KOH); 0.2% Methylene Blue Indicator

Table 4. Absorbed and expected doses

Expected dose(kGy)	**Delivered dose (kGy)**
20	20.83
30	33.08
40	41.64

Note: Uniformity ratio: 1.09

Responses from interviews (Through questionnaire administration)

Figures 3 to 6 are graphical representations of some of the responses of the beekeepers regarding honeybee farming and its practices. Most beekeepers (20%) were between the ages of 51 and 60 (Figure 3). For a greater percentage of the age group of 60 years and above, beekeeping was a full-time business, whereas those below 60 years are mostly crop and livestock farmers who keep bees as part of their business.

Most of the apiaries (about 53.3%) were located away from places of residence, as shown in Figure 4. However, some apiaries were not far from fuel stations, and about 20% were located close to quarries, wood processing factories, and places where (human activities) were quite common. In addition, 47% of the beekeepers used metallic and wooden ladles to scoop the honey from their comb (Figure 5). For others, they keep the combs in containers over a period to get the honey out of the combs by gravity.

After extraction from the combs, the product was sieved and finally stored in containers made of various materials. About 63% of the retailers used plastic containers, of which 43% of these containers were pre-used water bottles. 17% also used glass bottles of different products ranging from beer to soft drinks to store and sell honey (Figure 6).

Retailers carry honey around for sale, from house to house, workplaces, etc. From Figure 7, about 27% of the retailers sell their honey by hawking, and only a few retailers (about 14%) sell theirs in their homes because they have customers who trust their honey and come to the house to purchase. Moreover a combined 46% of the product are sold on markets and roadside, most travelers stop their cars to purchase honey before continuing their journey.

Results of microbiological analyses

Total viable count values for honey ranged from 0 to several thousand per gram. This variation in bacterial counts may be due to the type of sample (Honeycomb samples, finished or retailed), the freshness of the honey, the time of harvest, and the analytical techniques used (Snowdon and Cliver 1996). The microbial quality of honey in this research will be compared with the publication by Sereia et al. (2011). They used the MERCOSUR GMC, number 15/94 technical rules for identity fixation in honey quality, approved by ordinance number 367 on 4 September 1997, which states that honey may contain a maximum of 100 CFU/g.

Figure 8 represents the results obtained for Microbial Counts in honey sampled in the Brong Ahafo Region. The microbial count values in about 80% of the honey samples were below the MERCOSUR stipulated maximum (100CFU/g) for wholesome honey. However, there were samples from a few locations where the microbial counts were significantly above this level. The samples from Fiapre and Berekum were purchased from the market. The honey from these locations was purchased from honey hunters who have specific locations where bees make their honey. On their way from their farms, they visit these locations to extract the product to the market directly for sale.

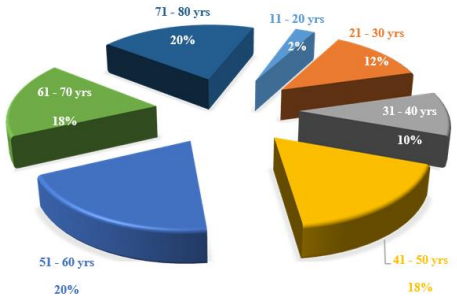

Figure 3. Age distribution of beekeepers for all three (3) Regions

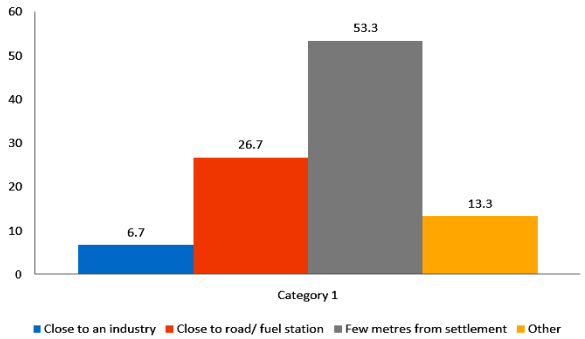

Figure 4. Location of Apiaries

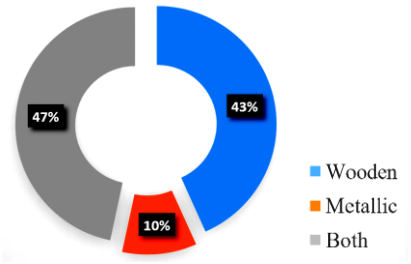

Figure 5. Extraction equipment

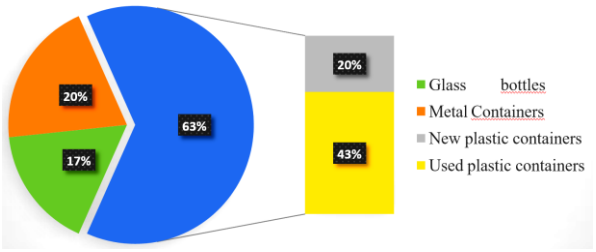

Figure 6. Storage containers for honey

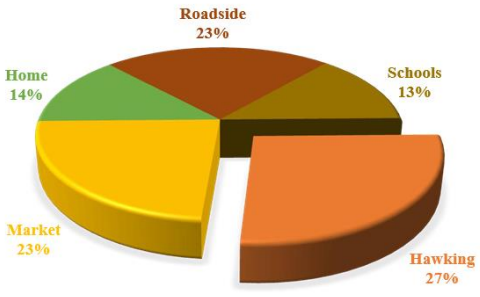

Figure 7. Places where honey is sold

In the Ashanti Region, the apiary in Agona was located just a few meters away from a fuel station, and honey extraction happens on-site when harvested. The beekeepers admitted that it was a good location for marketing since people come around for re-fueling and shopping in the mini-mart. That could have resulted in the contamination of the samples from these locations (Figure 9). The honey sampled from Jamase was purchased from a farmer who had collected some honeycomb into a plastic bucket to be taken home for extraction. Honey was heat-drained from the comb by leaving it in the sun.

In the Greater Accra Region, the retailers from whom honey was sampled were selling in the markets (23%), by the roadside (23%), and hawking (27%) at places where vehicular activities were quite common and where most roads were untarred. In most cases, the retailers transfer the honey into smaller containers after buying it in bulk. These factors could have played a pivotal role in the contamination of the honey hence the relatively higher Microbial Count readings recorded in this region (Figure 10). All the honey sampled from the 3 regions tested negative for *Salmonella* sp.

Relative microbial counts per region

Brong Ahafo Region

The absolute values in Table 5 indicate the total microbial counts in samples from apiaries in this region, whereas the mean values represent the microbial count per sample. After incubation at 37ºC for 48 hours, the Total Viable Count in the honeycomb samples was an average of 3 CFU/g per sample analyzed, the mean values of *Faecal* sp., *Clostridium* sp., and *Staphylococcus* sp. were 1 CFU/g. The mean Total Viable Counts, *Faecal* sp., *Clostridium* sp., and *Staphylococcus* sp., in the "Harvested and Treated samples" (S2), were (5, 2, and 3) CFU/g, respectively. These count values were relatively higher than the microbial counts for the Honeycomb Samples. That indicates that the local treatment methods introduce microbes into the product. Generally, the honey sampled from the honeycombs and that sampled from the treated honey from the beekeeper were low in microbial count and within the MERCOSUR maximum, except for the retail samples, which recorded a mean value of 183±20 CFU/g for Total Viable Count. The most prominent among the microbes detected was *Faecal* sp. with a mean count value of 112±10 CFU/g.

Ashanti Region

Figure 11 represents the mean values of the various microbes analyzed in each sample from the apiaries in the Ashanti Region. It can be observed that the Microbial Counts in the Honey Comb Samples (S1) were relatively very low per sample compared to the Extracted and Sieved Samples (S2), which could be a result of the treatment procedure, the equipment used in collecting honey from the beehives and in sieving, and the method of harvesting from the hive. A higher number of Coliform Units were detected in the Retail Samples (S3). Generally, the microbial count values were within the MERCOSUR maximum level, except for the Retail Samples where mean values of 148 CFU/g and 110 CFU/g were recorded for Total Viable Count and *Faecal* sp. respectively.

Greater Accra Region

The trend in the Greater Accra Region was similar to those observed in the Ashanti and Brong Ahafo regions. *Salmonella* sp. was not detected in any samples, from the apiary to the samples from the retailers. However, *Faecal* sp., spore-forming *Clostridium* sp., and *Staphylococcus* sp. were detected in all the samples (Figure 12). *Faecal* sp. was relatively higher than *Clostridium* sp. and *Staphylococcus* sp. in all the samples analyzed. The significant difference between the Total Microbial Count and the specific microbes analyzed represents the possibility of other microbes, which were not ascertained in this research.

Microbial counts per sample per region

Honeycomb samples (S1)

Table 6 represents the average Microbial Counts in Coliform Units per gram of sample (CFU/g) counted in honeycomb samples after incubation at 37 ºC for 48 hours. The mean value for Total Viable Count was less than 10 CFU/g for all the sample locations, whereas the mean values for *Faecal* sp., *Clostridium* sp., and *Staphylococcus* sp. in these samples were less than 5 CFU/g. Comparing these values with the MERCOSUR standards, they are reasonably below the allowed maximum of 100 CFU/g. The honey sampled directly from the beehive is relatively wholesome for human consumption.

Figure 13 represents the distribution of microbial contaminants in Honeycomb samples per region. It is observed that the highest mean recorded per honeycomb sample was the samples from the Greater Accra Region. It is worth mentioning that the limit of microbes detected in these samples was significantly below harmful limits for honey relative to the MERCOSUR maximum.

Table 5. Microbes Detected in Samples from the Brong Ahafo Region, Ghana

Microorganisms	Counts (CFU/g)					
	Sample One (S1)		Sample Two (S2)		Sample Three (S3)	
	Absolute	Mean	Absolute	Mean	Absolute	Mean
TVC	30	3.0 ± 1	144	16±4	1462	183±20
Faecal sp.	12	1.0 ± 0.4	37	5±2	895	112±10
Clostridium sp.	7	1.0 ± 0.3	19	2±1	186	24±3
Staphylococcus sp.	7	1.0 ± 0.4	20	3±1	102	13±2
Salmonella sp.	Negative	Negative	Negative	Negative	Negative	Negative

Note: TVC-Total viable count; S1-Honeycomb sample, S2-Treated, S3-Retail sample; Mean values (Average ± SD)

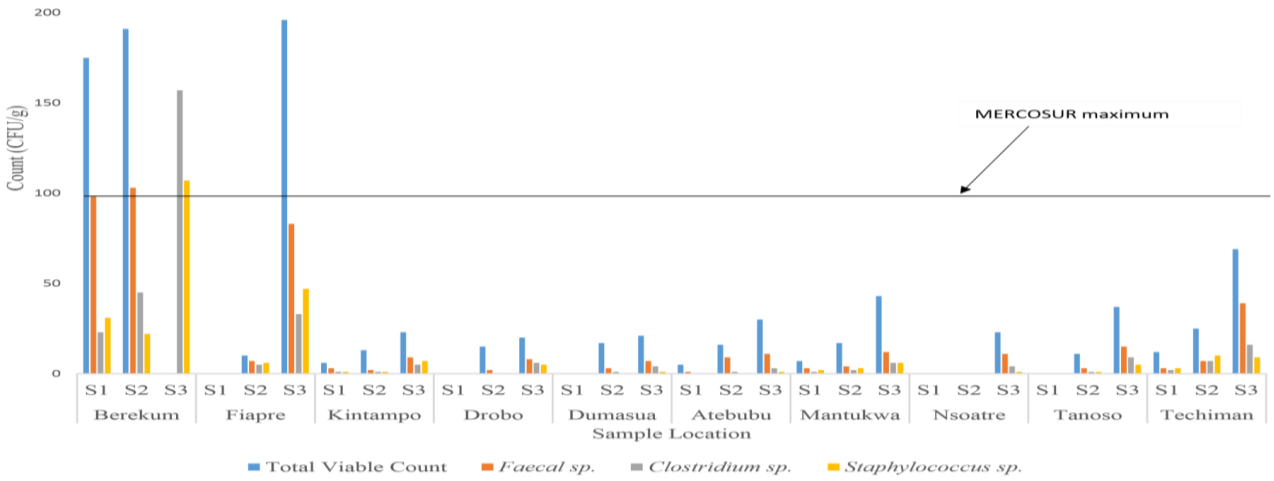

Figure 8. Microbial count in honey sampled from the Brong Ahafo Region, Ghana

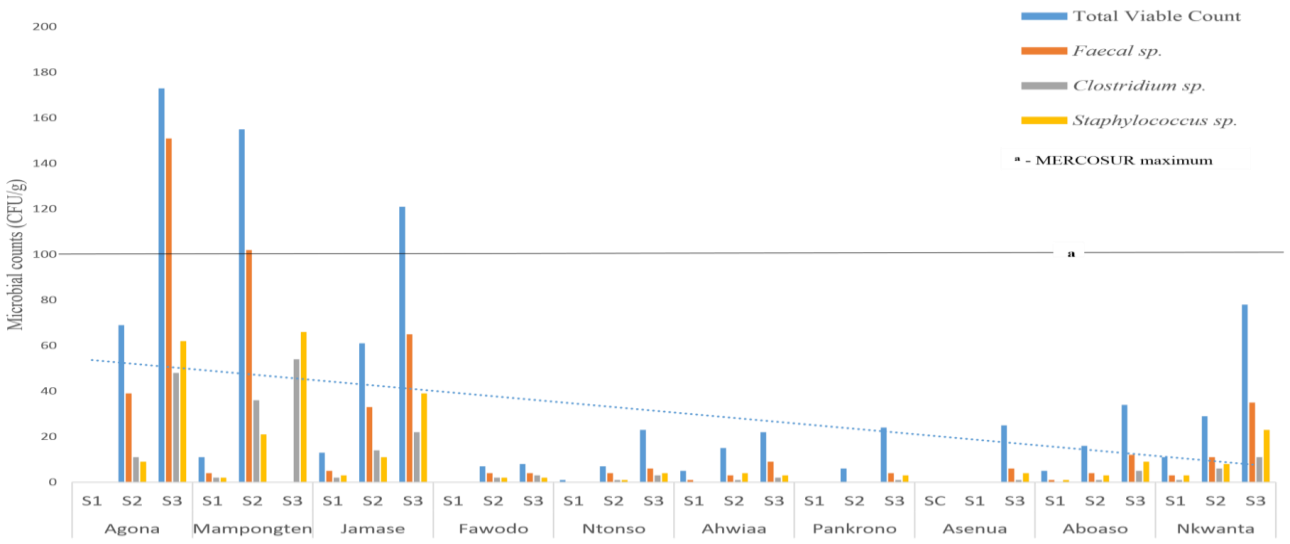

Figure 9. Microbial count per sample in honey sampled from the Ashanti Region, Ghana

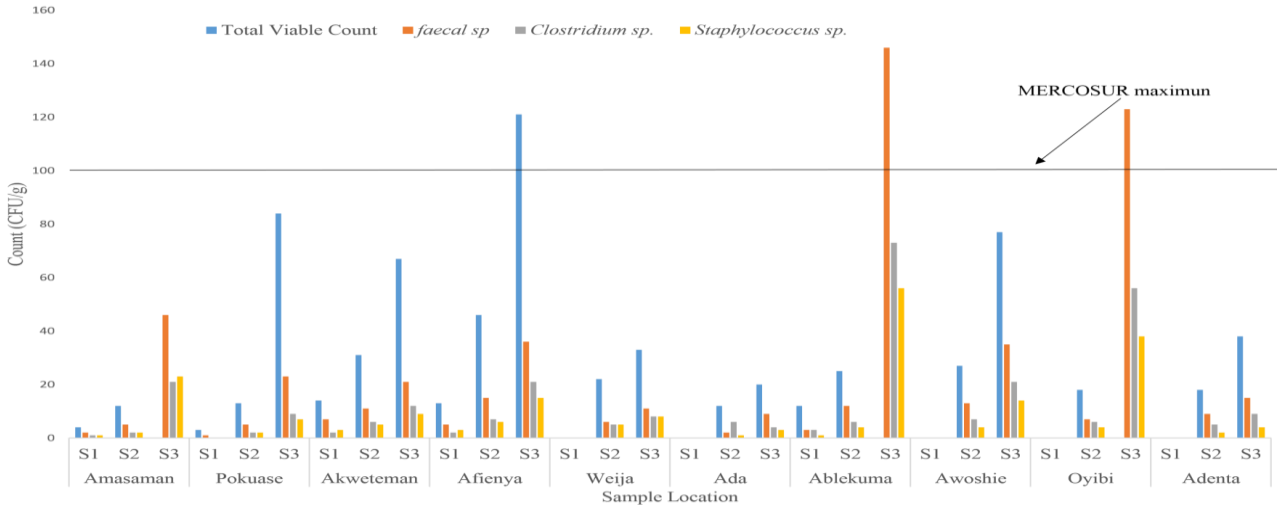

Figure 10. Microbial Count per in Honey sampled in the Greater Accra Region, Ghana

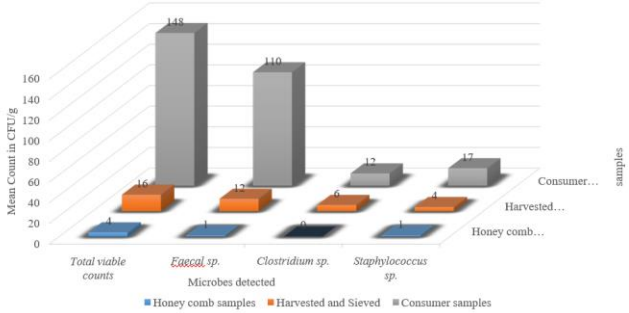

Figure 11. Microbial count per honey sample in Ashanti Region, Ghana

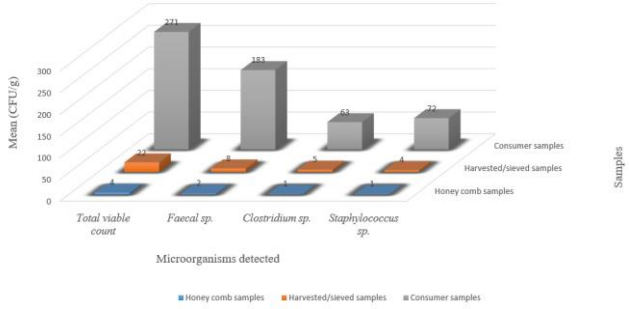

Figure 12. Microbial count per sample in Greater Accra Region, Ghana

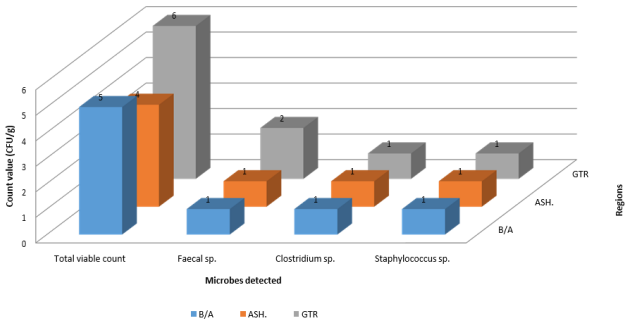

Figure 13. Mean microbial count in honeycomb samples per region

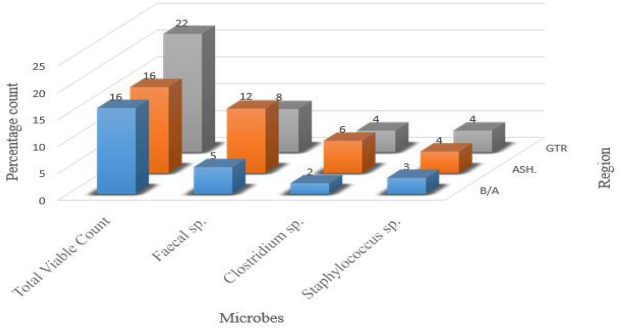

Figure 14. The average count of microbes detected in the treated samples per region

Sieved samples (S2)

Figure 14 represents data for the mean microbial counts in the Extracted and Sieved (S2) samples. The Mean Value (i.e., the Microbial Count per sample) for the Total Viable Count for the Brong Ahafo, Ashanti, and Greater Accra Regions were 16, 16, and 22 CFU/g, respectively. These values fall within 10 to 10^2 CFU/g, as reported by Snowdon and Cliver (1996), hence honey treated by the beekeeper can be deemed wholesome in terms of microbial quality. The Mean Values for *Faecal* sp., *Clostridium* sp., and *Staphylococcus* sp. for each sample in all the sample areas were below 10 CFU/g.

TVC-Total Viable Count

Consumer (Retail) Samples (S3)

In Table 7, it is observed that the mean microbial count for the Retailer Samples (183, 148, and 271) CFU/g for B/A, ASH, and GAR, respectively, were relatively higher than the mean microbial counts of the honey sampled directly from the honeycomb (S1) as well as honey sampled from the farmer after harvesting, extracting and sieving (S2). In addition, the count values were significantly higher than the MERCOSUR maximum standard of 100 CFU/g for all the sample locations, which indicates that the honey sampled from the retailers was contaminated. The honey sampled from the Greater Accra Region had the highest mean microbial count for Total Viable Count.

Table 6. Microbial counts in honeycomb samples per sample region

Microorganisms	Counts (CFU/g)					
	B/A		ASH		GAR	
	Abs.	Mean	Abs.	Mean	Abs.	Mean
Total aerobic count	30	5.0±2	35	4.0±1	35	6.0±2
Faecal sp.	12	1.0±0.3	10	1.0±0.3	14	2.0±0.4
Clostridium sp.	7	<1	3	<1	9	1.0±0.02
Staphylococcus sp.	7	<1	7	<1	9	1.0±0.03

Table 7. Microbial count in retailer samples per sample location

Microorganisms	Counts (CFU/g)					
	B/A		ASH		GAR	
	Absolute	Mean	Absolute	Mean	Absolute	Mean
TVC	1462	183±16	1332	148±12	2440	271±18
Faecal sp.	895	112±10	992	110±9	1465	183±15
Clostridium sp.	186	24±4	196	25±4	425	53±5
Staphylococcus sp.	102	13±3	153	17±5	577	72±6
Salmonella sp.	Negative	Negative	Negative	Negative	Negative	Negative

Results of physicochemical analyses of the honey samples

Figures 15, 16, and 17 represent results from the physical and chemical parameters analyzed for 60 samples comprising the Honey Comb Samples (S1) and the Retail Samples (S3) from all 90 sample locations. It is part of the hypothesis of this research that the harvested and processed samples (S2) would have no significant difference in reducing sugar and apparent sucrose concentrations, ash content, and pH. However, should there be any significant change to honey in terms of these qualities, it would happen during the products" transition to the consumer.

Pre-irradiation

The physicochemical analyses were conducted to identify any significant changes resulting from the dose of gamma radiation used in decontamination. It is the high concentration of reducing sugars and the low pH (acidic nature) of honey that makes it difficult to support microbial growth; in case these parameters alter, the shelf life of honey could be affected because it may improve the conditions of microbial survival and perhaps their multiplication and other food spoilage factors.

As much as decontamination is necessary, it is equally significant to maintain the physicochemical parameters to maintain or improve the shelf life and natural quality of honey. Therefore, the parameters analyzed were total reducing sugars, apparent sucrose, pH, and ash content.

The graphs (Figures 15, 16, and 17) show the concentration (in percentage) of reducing sugar and apparent sucrose in the honey sampled from the 30 sample points. The sucrose concentrations of the Honey Comb samples (S1) from all the sample points in the Brong Ahafo region were found to be within the Codex standards for honey (≥ 60% for Reducing Sugar concentration and ≤ 10% for Apparent Sucrose concentration), except for the Berekum sample (12.24% for Apparent Sucrose and 54.16% for Reducing sugar).

Apart from the sample from Aboaso in the Ashanti Region, all the Honey Comb samples (S1) from this region had apparent sucrose concentrations within the acceptable range. About 20% of the Honey Comb samples from the Greater Accra Region had sucrose concentrations slightly over the required maximum by the Codex standards (≤10%). The Codex standard gives precedence to a type of honey (honeydew) concerning the reducing sugar and apparent sucrose concentrations with reducing sugar concentration (≥53%) and apparent sucrose (≤15%); hence these samples could still be deemed wholesome.

All the Retail Samples (S3) from the thirty (30) sample locations had sucrose concentrations significantly above the average maximum of 10%. The reducing sugar concentrations in the Retail Samples compared to the Honey Comb samples were reduced for all the samples analyzed. The samples' pH was within the Codex standards of 3.8±1 to 6.0±1.

The Reducing Sugar (fructose and glucose) for honey, by Codex standards and the directive of the European Commission (EC Directive 2001/110), should be ≥60 g/100g of sample (i.e., 60%). In contrast, the Apparent Sucrose concentration should be ≤10 g/100g (10%) of the sample. Furthermore, the pH of honey should fall within 3.6 ±0.1 and 6.1±0.1, and the values for ash content by international standards (EC directive 2001; CODEX STAN 12-1981) should not exceed the range of (0.6-1.2)%.

Table 8 shows the physicochemical parameters for honey sampled from the Brong Ahafo Region. The Reducing Sugar values recorded for the Honey Comb Samples were mostly within the required standard, with a mean value of 70%. In comparison, the mean value recorded for the Apparent Sucrose for these samples was 8 %, falling within the required values for wholesome honey. For the Retail Samples (S3), the mean value for "Total Reducing Sugar" content of 54% falls below the expected standard according to Codex (≥60%). Concerning the Apparent Sucrose, the mean value of 23 % is significantly higher than the expected range of ≤10%. The ash content for the consumer samples was all within the required standard values.

Tables 9 and 10 are representations of the physicochemical qualities of the honey sampled from the Ashanti and Greater Accra Regions, respectively. The results trend was similar to those obtained for honey sampled in the Brong Ahafo Region. The mean values of the Total Reducing sugars for the Honey Comb samples were 68% and 66% for Ashanti and Greater Accra samples, respectively. The Apparent sucrose concentrations were 9.0% and 9%, respectively. These results satisfy the Codex standards for honey quality. For the Retail Samples, there was a reduction in the Reducing sugar concentration and a significant increase in the Apparent Sucrose concentrations for both regions, as seen in Tables 9 and 10. The ash content for the honeycomb samples was within the range of 0.1-0.2 (%), which falls below the codex range of 2-4%.

Results from microbiological analysis after irradiation

The effect of gamma-irradiation on the microbiological decontamination of the honey sampled from the sample points in the Brong Ahafo, Ashanti, and Greater Accra regions are shown in Tables 11, 12, and 13.

All the honey samples irradiated tested negative for microorganisms. The gamma radiation at 20 kGy, 30 kGy, and 40 kGy was enough to decontaminate the honey samples.

Physicochemical results after gamma-irradiation

The Table 14 represents the physicochemical results of the irradiated samples. There were no significant changes to the Reducing Sugar, Apparent Sucrose, Ash, and pH of the honey samples after irradiation with gamma energy from a Cobalt-60 source.

Results compared to standards

The microbiological analyses' results were compared with those of similar research undertaken by Finola et al. (2007) in Argentina. These researchers reported that microbial contaminants in honey were within "MERCOSUR stipulated values" of 10^1-10^2 CFU/g of honey.

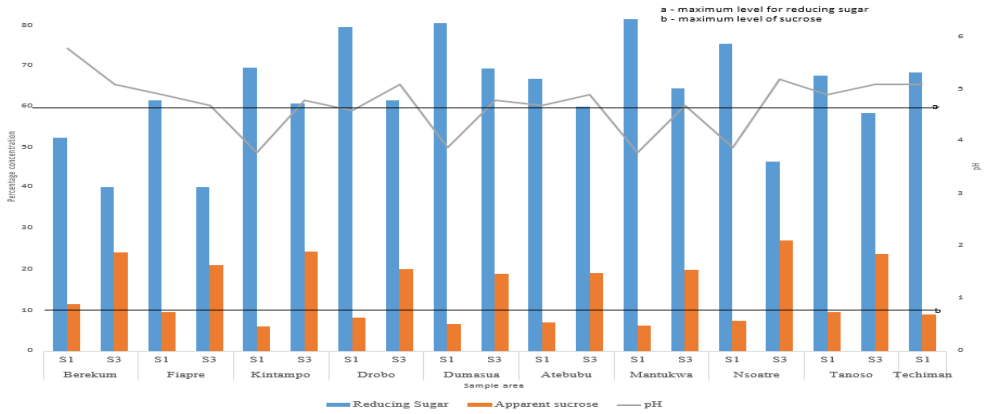

Figure 15. Pre-irradiation physicochemical parameters of samples from the Brong Ahafo Region, Ghana

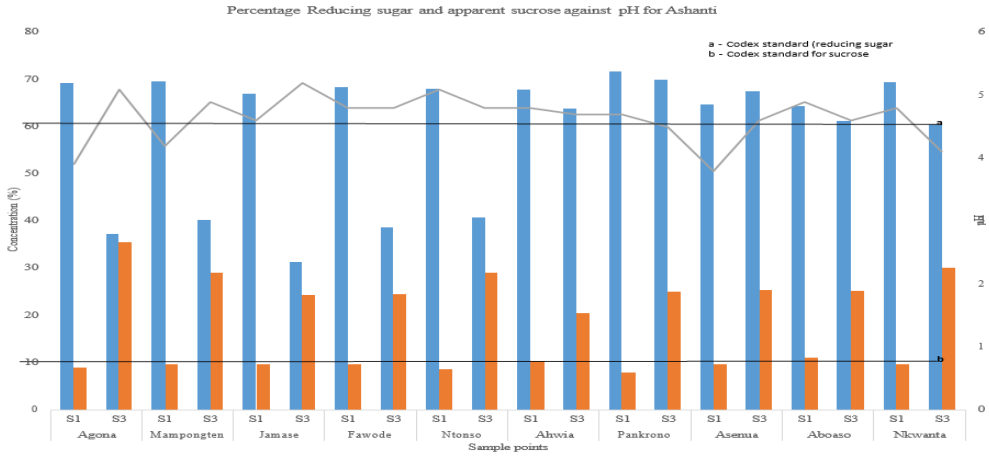

Figure 16. Pre-irradiation physicochemical parameters of samples from the Ashanti Region, Ghana

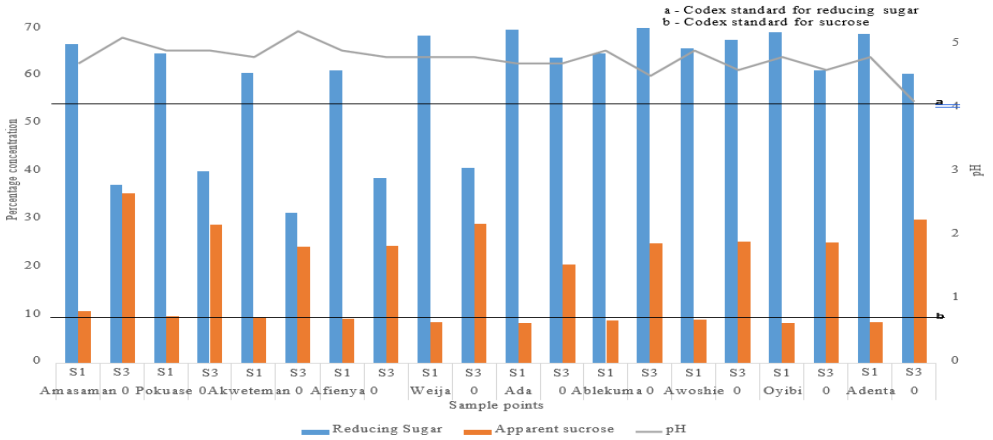

Figure 17. Pre-irradiation physicochemical parameters of samples from the Greater Accra Region, Ghana

Following this standard, the mean viable counts in microbes detected in the honeycomb samples (S1) from all the sample points were within 30-35 CFU/g, which falls within the MERCOSUR stipulated value. The mean count for *Faecal* sp. in all the honeycomb samples was below 10 CFU/g. The count for sulfite-reducing *Clostridium* sp. and *Staphylococcus* sp. were below 10 CFU/g. About 40% (12 out of 30) of the honeycomb samples from all 3 regions sampled were contaminated with sulfite-reducing *Clostridia* sp. In contrast, approximately 43% (13 out of 30) of these honeycomb samples were contaminated with *Staphylococcus* sp. Most of these contaminations were due to the locations of the beehives and a lack of proper maintenance of the hives. However, from the microbiological point of view, the relatively low microbial count in the honeycomb samples indicates the proper management of the beehive by most honeybee keepers.

Table 8. Physicochemical composition of samples from the B/A before gamma irradiation

Parameter	Type of Honey Sample			
	Honey Comb Sample		Retail Sample	
	Range	Mean ±SD	Range	Mean ±SD
TRS (%)	52.6-81.6	70±9	40.34-69.45	54±10
AS (%)	6.2-11.6	8±2	19.09-27.46	23±4
AC (%)	0.04-0.27	0.1±0.05	0.06-0.27	0.16±0.06
pH	3.8-5.6	5.0±0.6	3.8-5.3	5.0±0.2

Note: TRS-Total Reducing Sugar; AS-Apparent Sucrose; AC-Ash Content

Table 9. Pre-irradiation physicochemical parameters of the honey sampled from Ashanti Region, Ghana

Parameter	Type of honey sample			
	Honey Comb Sample (S1)		Retail Sample (S3)	
	Range	Mean ±SD	Range	Mean ±SD
TRS (%)	61.28-71.85	68±3	30.87-70.50	51.0±6
AS (%)	7.45-10.23	9.0±0.8	21.66-35.46	26.0±4
AC (%)	0.04-0.15	0.1±0.07	0.11-0.75	0.2±0.04
pH	3.9-5.1	5.0±0.3	4.4-5.6	4.9±0.3

Note: TRS-Total Reducing Sugar; AP-Apparent Sucrose; AC-Ash Content

Table 10. Pre-irradiation physicochemical parameters of the honey sampled from Greater Accra Region, Ghana

Parameter	Type of honey sample			
	Honey Comb Sample (S1)		Consumer Samples (S3)	
	Range	Mean ±SD	Range	Mean ±SD
TRS (%)	60.71-69.68	66±3	31.57-66.96	43±12
AC (%)	8.42-10.88	9.0±0.7	21.02-41.49	32±6
AC (%)	0.12-0.24	0.2±0.09	0.45-0.78	0.7±0.02
Ph	4.7-4.9	5.0±0.07	4.9-5.6	5.0±0.2

Note: TRS-Total Reducing Sugar; AP-Apparent Sucrose; AC-Ash Content

Table 11. Post-irradiation microbial analyses of honey from the Brong Ahafo Region, Ghana

Irradiation dose (kGy)	Microbes (CFU/g)							
	Total viable count		Coliform		*Clostridium* sp.		*Staphylococcus* sp.	
	S2	S3	S2	S3	S2	S3	S2	S3
0	16	183	5	112	2	23	3	13
20	ND	ND	ND	ND	ND	ND	ND	ND
30	ND	ND	ND	ND	ND	ND	ND	ND
40	ND	ND	ND	ND	ND	ND	ND	ND

Note: ND-No detection (negative); S2-Extracted/Sieved Sample; S3-Retail Samples

Table 12. Post-irradiation microbial analyses of native honey from the Ashanti Region, Ghana

Irradiation dose (kGy)	Microbes (CFU/g)							
	Total viable count		Coliform		*Clostridium* sp.		*Staphylococcus* sp.	
	S1	S3	S1	S3	S1	S3	S1	S3
0	16	148	12	110	6	12	4	17
20	ND	ND	ND	ND	ND	ND	ND	ND
30	ND	ND	ND	ND	ND	ND	ND	ND
40	ND	ND	ND	ND	ND	ND	ND	ND

Note: ND-No detection (negative)

Table 13. Post-irradiation microbial analyses of native honey from the Greater Accra Region, Ghana

Irradiation dose (kGy)	Microbes (CFU/g)							
	Total viable count		Coliform		*Clostridium* sp.		*Staphylococcus* sp.	
	S1	S3	S1	S3	S1	S3	S1	S3
0	22	271	8	183	5	63	4	72
20	ND	ND	ND	ND	ND	ND	ND	ND
30	ND	ND	ND	ND	ND	ND	ND	ND
40	ND	ND	ND	ND	ND	ND	ND	ND

Note: ND-No detection (negative)

Table 14. Post-irradiation physicochemical analyses

Dose (kGy)	Reducing sugar			Apparent sucrose			Ash content			pH		
	BA	ASH	GAR	BA	ASH	GAR	BA	ASH	BAR	BA	ASH	GAR
Pre-irradiation	54.3	51.13	42.70	22.16	26.23	32.25	0.16	0.22	0.67	4.8	4.9	4.8
20	55.2	51.46	43.10	22.62	26.25	32.31	0.15	0.23	0.67	4.7	4.8	4.8
30	54.7	51.25	42.74	22.40	27.10	32.11	0.14	0.25	0.66	4.8	4.9	4.9
40	52.1	50.3	40.41	20.20	27.9	34.65	0.16	0.23	0.66	4.4	4.3	4.4

The microbial count values in samples harvested and treated by the farmers (S2) were relatively higher for all the 30 samples analyzed than the honeycomb samples. About 90% (27 out of 30) of these samples were contaminated with *Faecal* sp. Approximately 18% of the samples were from the Ashanti Region (the highest among the three regions) for *Faecal* sp. Sulfite-reducing clostridium was detected in all the contaminated samples, and *Staphylococcus* sp. The soil is the main source of *Clostridium* sp., although dust, equipment, buildings, and the environment could also contain this genus of microbe. The presence of these microorganisms indicates contamination or pollution. The chain of manufacturing and maturity at harvest should be monitored to decrease the chances of making honey impure.

Most of the beekeepers used their bare hands to remove the comb from the beehive, and very little attention was given to the possibility of contamination during harvesting. The combs are kept in plastic containers, sometimes metallic containers, and left overnight to drain. During the day, the containers with the honey are brought out into the

sun to heat-drain, exposing the honey to dust and other particles.

Microorganisms were detected in all 30 honey samples from the retailers. The mean count of *Faecal* sp. in the samples was 111 CFU/g, 124 CFU/g, and 183 CFU/g for Brong Ahafo, Ashanti, and Greater Accra Regions, respectively. These count values were higher than the maximum values stipulated by the MERCOSUR regulations, 10^2 CFU/g. Samples from the Greater Accra region had the highest recorded values for microbial counts. The mean values for *Clostridium* sp. and *Staphylococcus* sp. were 53 CFU/g and 72 CFU/g. Comparing the areas sampled in the Greater Accra Region to those sampled in the other two regions, the Greater Accra Region has the highest population density; hence, human activities are higher in this region than in other regions. That is a major factor in the contribution of contaminants to any food sample; honey is not an exception. All the samples were negative for *Salmonella* sp.

Mean values for Clostridium sp. and Staphylococcus sp. for the Ashanti Region were 25 and 19 CFU/g, respectively. In contrast, the mean values of *Clostridium* sp. and *Staphylococcus* sp. detected in samples from the Brong Ahafo Region were 24 and 13 CFU/g, respectively. Therefore, honey sampled from the Brong Ahafo Region can be said to be the most wholesome for human consumption.

Honey from the honeycombs sampled from all the regions had reducing sugar values within the required concentrations as in the standards of the International Honey Commission (≥60%) and the CODEX Alimentarius Commission. About 70% of the sugars in the honeycombs samples from the Brong Ahafo Region were reducing sugars and contained the required sucrose concentrations (average; 8%) according to the Codex standards.

The results showed relatively high concentrations of sucrose in the honey sampled from the retailers. The mean reducing sugar concentration for the honeycomb samples from the Ashanti Region was 68%, within the accepted concentration range, and an average of 9% for sucrose. Honey sampled from the retailers in the Greater Accra Region had the highest mean concentration of sucrose (34%) compared to 26 % and 22% in the samples from the Ashanti and Brong Ahafo regions, respectively. Relative to the Codex, EC directive, and the standards from the International Honey Commission, these results were above the required concentration of sucrose in natural honey. The great disparity in apparent sucrose concentration in the honeycomb samples (S1) relative to the retail samples (S3) indicates adulteration of honey with white sugar. This inference concerns the percentage increase for the samples from the Brong Ahafo Region,

The ash content helps to interpret the honey's origin and indicates the foraging area of the honeybees. Ash content standards are set at a minimum of 0.6 g/100 g of sample and a maximum of 1.2 g/100 g (i.e., 0.6%-1.2%) of honey by the Codex Alimentarius Commission, the IHC, and the EC directive among other standards all of which follow a similar quality assurance criteria. The ash content of all the honey sampled and analyzed was within the required standard, with an average of 0.16% in the honeycomb samples and an average of 0.62% for the retail samples.

This research showed that the honey sampled from all the sample areas was found to have an acidic character. The pH values ranged from 3.8 to 5.6 in the Brong Ahafo Region (Table 11), 3.9 to 5.6 in samples from the Ashanti Region (Table 12), and 4.7 to 5.6 in the samples from the Greater Accra Region. These results conform to the standard values. The results also showed that the pH in the retail samples was higher than in the honeycomb samples, which could have contributed to the fewer detection values of microorganisms in the honeycomb samples. The pH meets the values reported by Bera et al. (2009), with pH values in the 3.8 to 4.2 and the Codex standard range of 3.8±1 to 6.0±1.

Gamma irradiation at a temperature of 25°C at doses ranging from 20 kGy to 30 kGy had no significant effect on the physical and chemical qualities of the honey. The mean reducing sugar concentration reduced insignificantly after irradiating at 40 kGy relatives to the average concentration before irradiation. On average, the change in apparent sucrose concentration was insignificant, whereas there was a slight reduction in pH after irradiation at 40 kGy.

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