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Full Length Research Paper

Bacteriological assessment of the quality of *Brassica oleracea* var. *capitata* grown in the Accra Metropolis, Ghana

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Bacterial and other microbial contamination of fresh vegetables from the farm or garden to the market and to the final consumer remain a problem worldwide. This study was designed to evaluate the various possible bacterial species responsible for the contamination of *Brassica oleracea* var. *capitata* (cabbage) grown in the Korle-Bu vegetable garden and sold at the Agbogbloshie market in the Accra Metropolis, Ghana. Sixty (60) cabbage samples were collected and investigated bacteriologically using standard Food and Agriculture Organization (FAO) of the United Nations (UN) total aerobic plate count methods.Cabbage samples from the vegetable garden were found to be more contaminated than the market with a total mean colony count of 2.43×10^6 CFU/g and 1.53×10^6 CFU/g respectively. *Staphylococcus aureus* was the most predominant bacteria isolated with a high percentage occurrence of 51% followed by *Escherichia coli* (28%), *Bacillus* sp. (12%), *Streptococcus* sp. (5%), and *Pseudomonas aeruginosa* (4%). From the results of the study, bacterial contamination of cabbage grown at the Korle-Bu vegetable garden and the Agbogbloshie market were all above the recommended standard levels especially *E. coli* which should be less than 10 bacteria per gram. Therefore it is recommended that these vegetables be thoroughly washed with safe water or saline solutions before processing and consumption especially where they are not going to be heated or cooked before consumption.

Key words: Vegetables, Cabbage, *Staphylococcus, Escherichia*.

INTRODUCTION

Bacteria and other microbial contamination of fresh vegetables from the farm or garden where they are grown to the market where they are displayed, sold, and finally to the consumer still remain a major problem throughout the world especially in the developing countries including Ghana. Cabbage known scientifically as *Brassica oleracea* var. *capitata* was first identified by Linn. The plant is one of the major vegetables grown in most parts of Ghana and beyond. There are three main varieties namely, white headed cabbage, red headed cabbage,

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and savoy headed cabbage around the world. Leaves can be eaten fresh in salads, as green cooked vegetables or fermented. In Ghana and other parts of the world, the leaves of the white headed cabbage is one of the components that are used for preparing ready-to-eat (RTE) foods like salad and also for the preparation of vegetable stew in the various houses. The vegetable has a high nutritive value capable of supplying the human body with essential vitamins, proteins, carbohydrates, vitamins, and also can signal genes to increase the production of enzymes involved in detoxification (Leja et al., 2006; Mariga et al., 2012).

The city of Accra is the national capital of the Republic of Ghana and is predominantly an industrial community. Sanitation in Accra is a major problem due to overpopulation and also several industrial waste produced by most of the industries. As a result, most of the river bodies as well as the lagoons have been turned into dumping sites. Therefore it is difficult for the vegetable farmers in the Accra Metropolis to get a suitable water to water their farm produce and end up using the dirty or contaminated water from gutters for watering the vegetables. It is possible that several microorganisms that are harmful to the human health in the dirty water may find their way into the vegetables and infect humans after eating such vegetables. Agbogbloshie market is located at the central part of Accra and is not far from the Korle lagoon which has been turned into a dumping site.

The mode of harvesting, transportation, and storage methods used for the vegetables can also contribute a lot in the introduction of those microorganisms into the vegetables. For instance, there are reports that cross-contamination can occur by the use of dirty harvesting equipments, unhygienic handling during sorting, packaging, transport, improper storage, and display (Beuchat, 2006). The presence of cut surfaces provides an increased surface area for contamination growth and allows microbial infiltration of the tissue. Exposing vegetables to various types of cutting has been shown to result in a six to seven-fold increase in microbial numbers (O'Brien et al., 2001).

The health hazards associated with the eating of fresh vegetables like cabbage is underestimated due to the several nutritional benefits obtained from that vegetable. Gastrointestinal infections, for example, are the most common diseases caused by enteric bacteria. Although a lot of studies have been done on bacteriological analysis of fresh vegetables in several countries, bacterial contamination of food remains a risk factor for gastrointestinal infections in Ghana and with the recent outbreak of cholera in Ghana (Anonymous, 2012) there is the need to conduct this study to find out the level of bacterial contamination of cabbage which is a major component of food in Ghana. Therefore this study was designed to find out the various possible bacterial species responsible for the contamination of cabbage in the Korle-Bu vegetable garden and the Agbogbloshie market of the Accra Metropolis, Ghana.

MATERIALS AND METHODS

Sample collection

Cabbage samples for the study were collected from the Korle-Bu vegetable garden and the Agbogbloshie market, all in the Accra Metropolis of Ghana. Only cabbage samples grown at the Korle-Bu vegetable garden and cabbage samples from retailers at the Agbogbloshie market who buy their cabbage from the Korle-Bu vegetable garden were analysed. A total of 60 cabbage samples were collected in sterile plastic bags from the Korle-Bu vegetable garden (30 samples) and the Agbogbloshie market (30 samples). In the Agbogbloshie market, the samples were collected from four different retailers in the market who confirmed that they buy their cabbages from the Korle-Bu vegetable garden. The samples were collected in a period of two weeks. Each cabbage sample was given a specific code number which corresponds to number for each media plate used for the analysis. Each day's collected samples were sealed in sterile plastic bags and transported to the laboratory immediately for the bacteriological analysis.

Bacteriological analysis

Total aerobic plate count

The total aerobic plate counts of bacteria from the cabbage samples were evaluated using a modification of the Food and Agriculture Organization (FAO) of the United Nations (UN) standard food and nutrition methods by Andrews (1992). First, the leaves of the cabbage samples were selected with the aid of a sterile forceps and washed with sterile distilled water. Then, 10 g of the leaves of each sample were weighed and rinsed for 8 min in a 250 ml beaker containing 90 ml of sterile distilled water to obtain 10^{-1}. Ten-fold serial doubling dilutions of the samples through to 10^{-5} were made as follows: four additional sterile test tubes were appropriately labelled and serially arranged on the test tube rack for each sample. Sterile distilled water (9 ml) was introduced into each test tube with the aid of micropipette with sterile tips. Using separate sterile pipette tips, 1 ml of the rinsed test sample was introduced into the first test tube (10^{-2}) and mixed thoroughly. After mixing, 1 ml of the contents of 10^{-2} test tube was pipetted and introduced into the second test tube (10^{-3}) and mixed thoroughly. The same procedure was repeated for the rest of the tubes. Then, 0.1 ml of each dilution were pipetted using a micropipette with sterile tips and dropped on the surface of a pre-labelled plate count agar (PCA: Oxoid Limited, Basingstoke, UK) and Difco MacConkey agar (DMA: Becton, Dickinson and Company, Sparks, MD 21152, USA) plates in accordance with the labelling on the cabbage samples. A sterile glass spreader was used to spread the sample dilutions uniformly over the surface of the agar plates. The plates were then incubated at 37°C for 24 - 48 h.

After overnight and subsequent incubations, the plates were examined for evidence of bacterial growth and the number of colonies counted. The isolated bacterial index on each agar plate was expressed as CFU/g by multiplying the number of colonies with the dilution factor. Counting was done with the aid of a hand lens.

Identification of isolates

The identification and characterization of the isolated bacterial species in the present study were done using colonial morphology,

Table 1. Mean bacteria colony count values (×10^6 CFU/g) of isolated bacteria in cabbage from the vegetable garden and the market.

Bacterial species	Garden	Market	Total mean colony count
Staphylococcus aureus	1.19	0.81	2.0
Escherichia coli	0.67	0.42	1.09
Bacillus sp.	0.31	0.17	0.48
Streptococcus sp.	0.13	0.09	0.22
Pseudomonas aeruginosa	0.13	0.04	0.17
Total	2.43	1.53	3.96

Gram staining reactions, catalase, indole, oxidase, motility, citrate utilization, methyl red (MR), VogesProskauer (VP), triple iron sugar (TSI), and coagulase tests according to the FAO of the UN standard food and nutrition methods by Andrews (1992) and cross referenced with Bergey's manual of determinative bacteriology (Holt et al., 1994).

Statistical analysis

Results obtained from the experiments were entered into a database and analysed statistically using Statistical Package for *Social Sciences (SPSS)* version 20 statistical software for windows and a summary was presented using the descriptive statistics such as means and percentages. Factor analysis was performed on samples from the vegetable garden and those from the market to establish their level of correlation or variability in terms of mean colony counts. Also the student's t-test was used to find out significant difference between the parameters studied. P-values >0.05 were taken as statistically insignificant difference.

RESULTS

Mean bacterial counts of cabbage samples from the vegetable garden and the market

Vegetables more especially cabbage are essential part of people's diet all round the world. Sometimes these cabbage are consumed raw and often without heat treatment or thorough washing and as such have been known to serve as vehicle for the transmission of pathogenic microorganisms associated with human diseases. In the present investigations, the highest mean bacterial colony count were observed for the samples from the Korle-Bu vegetable garden (2.43 x 10^6 CFU/g) representing 61.4% of all the isolated bacteria in the study.

The mean colony count values of isolated bacteria ranged between 0.13 to 1.19×10^6 CFU/g for the samples from the vegetable garden and 0.04 to 0.81×10^6 CFU/g for the samples from the market (Table 1). *Staphylococcus aureus* was the predominant bacteria with mean colony count values of 1.19×10^6 and 0.81×10^6 CFU/g from the vegetable garden and the market, res-pectively (Table 1). Other bacteria including *Escherichia coli*, *Bacillus* sp., *Streptococcus* sp., and *Pseudomonas aeruginosa* were also isolated. For example, with *E. coli*, a mean colony count values of 0.67 ×10^6 and 0.42 ×10^6 CFU/g were isolated from the cabbage samples from the vegetable garden and the market whiles for *Bacillus* sp. a mean colony counts values of 0.31 ×10^6 and 0.17 ×10^6 CFU/g were also isolated from the two study sites, respectively. However, for *Streptococcus* sp., a mean colony count values of 0.13×10^6 and 0.09 ×10^6 CFU/g from the vegetable garden and the market, respectively were isolated in the study. A mean colony count values of 0.13 ×10^6 and 0.04 × 10^6 CFU/g were also isolated for *P. aeruginosa* as presented in Table 1.

Bacterial index in cabbage samples

Five groups of bacteria including *S. aureus*, *E. coli*, *Bacillus* sp., *Streptococcus* sp., and *P. aeruginosa* were isolated and identified from the cabbage samples from the Korle-Bu vegetable garden and the Agbogbloshie market of the Accra Metropolis. Factor analysis performed to verify the significance of the differences in counts of bacteria was statistically significant ($p<0.005$). *S. aureus* was the most predominant bacteria isolated with a total mean colony count value of 2.00×10^6 CFU/g of all the bacterial contaminants isolated from all the cabbage samples representing 51% of all the bacteria isolated and identified in this investigation (Table 1 and Figure 1). The second most predominant bacteria isolated in this study was *E. coli* with a total mean colony count value of 1.09×10^6 CFU/g representing 28% of all the bacterial isolates identified. *Bacillus* sp. had a total mean colony count value of 0.48×10^6 CFU/g representing 12% of all the bacterial contaminants isolated and identified. *Streptococcus* sp. and *P. aeruginosa* were the least isolated bacterial contaminants with a total mean colony count values of 0.22×10^6 and 0.17×10^6 CFU/g representing 5 and 4%, respectively of all the bacterial contaminants isolated (Table 1 and Figure 1).

DISCUSSION

Bacterial contamination of cabbage in the Accra Metropolis investigated showed total mean bacterial colony count values of 2.43×10^6 and 1.53×10^6 CFU/g in the vegetable garden and the market, respectively. The results of the present study is similar to a previous research conducted by Frank-Peterside and Waribor (2006) which reported that bacteria load on leafy vegetables increase

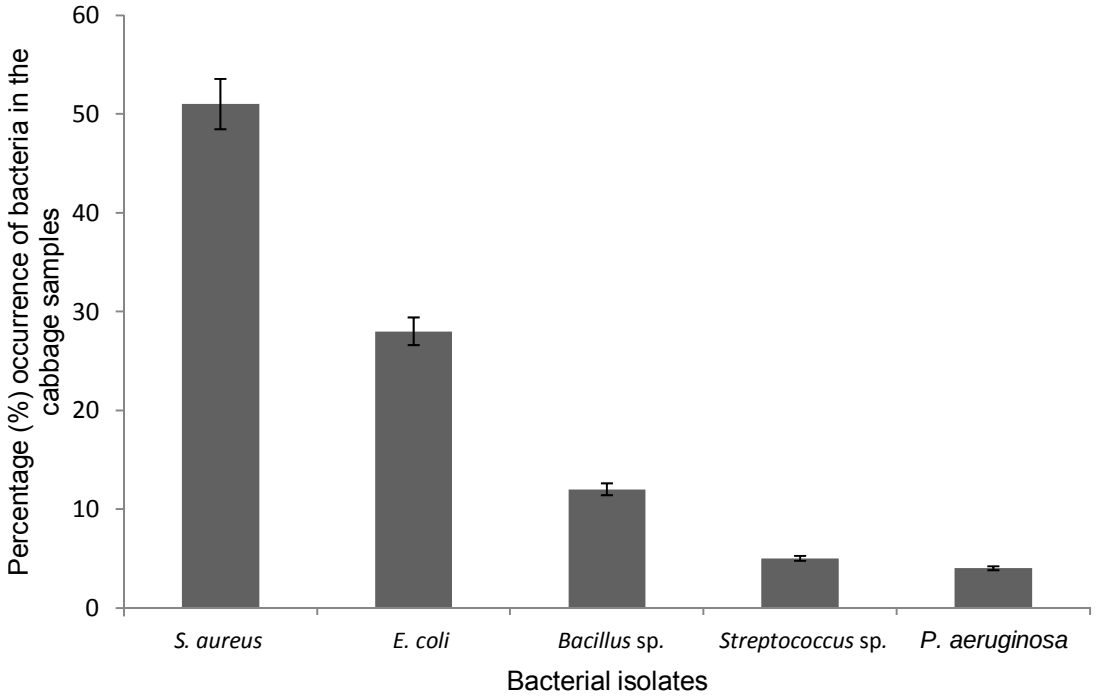

Figure 1. Percentage distribution of bacterial contaminants isolated from the cabbage samples.

with time during storage. However, the decrease in the bacterial contamination in the samples from the market may be attributed to the storage conditions of the vegetable. Some of the market women when orally interviewed confirmed that, they occasionally sprinkle salt water on the cabbage to prevent them from quick spoilage and also to help kill microorganisms that may be present in the cabbage during harvesting as previously proposed by Abdullahi and Abdulkareem (2010). This act by the market women may have resulted in the low bacterial colony count values in the cabbage samples from the market.

Among the isolated bacterial pathogens, *S. aureus, E. coli*, *Bacillus* sp*., Streptococcus* sp*.* and *P. aeruginosa* were the predominant bacterial species found to be associated with the cabbage in the vegetable garden and also during the storage and selling processes in the Agbogbloshie market in the Accra Metropolis*.* This finding indicates gross contamination from the vegetable garden and the market until it finally reaches the consumer. The high prevalence of *S. aureus* on the cabbage samples may be due to pre and post-harvest handling; for it is known that, *S. aureus* is an opportunistic pathogen found living in the nasopharynx and skin of up to 50% of normal people (Enright, 2003; Guignard et al., 2005). Therefore the high frequency of the bacteria found in this study may be attributed to the bacteria being present as a normal flora of humans and can contaminate the vegetables as a result of poor hygiene of farmers and sellers.

E. coli was found in 28% of all the samples from the vegetable garden and the market analysed (Figure 1). The presence of *E. coli* on the cabbage samples from all the sampling sites with a high amounts on those from the vegetable garden may be as a result of faecal contamination because the bacteria is present in sewage, faeces, soil, water, and commonly come in contact with vegetables as result of the water used during the growing processes of the vegetables. During the investigation, the water used by the farmers for watering the cabbage was examined macroscopically on each sampling day and they were found out to be just wastewater from drainages around the vegetable gardens as previously reported by other research workers (Drechsel et al., 2006; Ackerson and Awuah, 2010). Also work by Solomon et al. (2003) reported that repeated spraying of crops with contaminated irrigation water increases the chances of crop contamination and this may also account for the high bacterial contamination of the cabbage samples investigated.

Although *Bacillus* sp. was isolated from the cabbage samples from the vegetable garden and the market, respectively in this study (13 and 11%, p-value > 0.05) other research workers did not isolate the bacteria in their investigations (Ibrahim and Jude-Ojei, 2009; Taura and Habibu, 2009). However, Abdullahi and Abdulkareem (2010) working on RTE vegetables in Sabon-Gari, Zaria, Nigeria also observed the presence of *Bacillus* sp. The isolation of *Bacillus* sp. may be due to environmental factors and the ability of the bacteria to form spores (Gupta et al., 2013; Merghni et al., 2014).

Percentage occurrence of *Streptococcus* sp. from the cabbage samples from the vegetable garden and the market in this study was recorded (5 and 6%, p-value > 0.05) as presented in Figure 1 and Table 2. However, the

Table 2. Percentage occurrence (%) of isolated bacteria in cabbage from each site sampled.

Bacterial isolates	Garden	Market	P-value
Staphylococcus aureus	49	53	0.756*
Escherichia coli	28	27	0.930*
Bacillus sp.	13	11	0.811*
Streptococcus sp.	5	6	0.861*
Pseudomonas aeruginosa	5	3	0.692*
Total (%)	100	100	

*p-values were considered insignificant (> 0.05)

isolation of *P. aeruginosa* (5 and 3%) from the cabbage samples from the vegetable garden and the market, respectively (p-value > 0.05) may come from the environment. In a similar related work done by Itohan et al. (2011) who also isolated the bacteria in cabbage and the vegetables they analysed. *P. aeruginosa* is widely distributed in nature and is commonly present in moist environments. It can also colonize normal humans, in whom it is a saprophyte. It only causes disease in humans with low immune defences system (Stover et al., 2000). Therefore cross-contamination of the cabbage samples by *P. aeruginosa* can occur during storage, preparation, dirty harvesting equipment, unhygienic handling, and improper storage (Codex Alimentarius Commission, 2007).

Conclusion

This study have shown that all the cabbage samples investigated have high bacterial contamination and their persistence and proliferation is a reflection of the use of unsafe or contaminated water in watering these vegetables. It is therefore recommended that these vegetables be thoroughly washed with safe water or saline solutions before processing and consumption especially where they are not going to be heated or cooked before consumption.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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