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Microbial quality of leafy green vegetables grown or sold in Accra metropolis, Ghana

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ABSTRACT

Samples of two exotic (lettuce and cabbage), and four indigenous (African spinach, African eggplant leaves, roselle leaves, and jute leaves) leafy green vegetables were collected from 50 vegetable farms in 12 farming areas (n = 175) and 37 sellers in 4 major market centers (n = 153) in Accra metropolis, Ghana. Microbial quality of collected samples was assessed by isolation of *Salmonella* and enumeration of aerobic bacteria, yeasts and molds, fecal coliforms, and enterococci. Mean aerobic bacteria, yeast and mold, fecal coliform, and enterococcus counts on collected vegetables were 8.80, 4.95, 4.90, and 3.67 log CFU/g, respectively. Approximately 75.4% of the vegetables from 96.0% of the vegetable farms and 84.3% of the vegetables from 97.3% of the vegetable sellers tested positive for enterococci, and 81.1% of the vegetables from 96.0% of the farms and 83.7% of the vegetables from 94.6% of the vegetable sellers tested positive for fecal coliforms. *Salmonella* were isolated from 5.1% of the vegetables from 16.0% of the vegetable farms and 15.7% of the vegetables from 24.3% of the vegetable sellers. Vegetable source and type had significant influence on the microbial counts. Results revealed that the sampled leafy green vegetables had poor microbial quality. Consumption of fresh leafy green vegetables without sanitizing or heat treatment should be discouraged.

1. Introduction

Leafy green vegetables are important components of Ghanaian diets serving as sources of vitamins, minerals, and other nutrients. The vegetables are used as part of a main course or side dish. Both indigenous (cocoyam leaves, African spinach, African eggplant leaves, roselle leaves, and jute leaves, cowpea leaves), and exotic (lettuce, cabbage, spinach, broccoli, Chinese cabbage) leafy green vegetables are currently cultivated and consumed in Ghana (Drechsel, Adam-Bradford, & Raschid-Sally, 2014). Although indigenous leafy vegetables are often cheaper and more nutritious, exotic leafy vegetables are patronized more, especially by urban dwellers due to changes in lifestyle and diets and lack of public knowledge about nutritional benefits of indigenous vegetables (Darkwa & Darkwa, 2013).

Vegetable farming usually takes place in the rural areas of Ghana, and harvested vegetables are transported to markets in urban areas. Over the past decades, vegetable farming activities have increased in the urban and peri-urban areas mainly due to increasing market demands, lack of jobs, and changes in lifestyle and diets. Urbanization and increasing population size has, however, led to scarcity of land and water with most famers having access to smaller land size for farming as observed in other West African countries (Drechsel et al., 2014). The farmers situate their farms close to various water sources such as pipe, wells, streams, and drains for irrigation (Drechsel et al., 2014). Both inorganic and organic fertilizers (poultry and cow manure) are used for vegetable cultivation, with poultry manure being commonly used because it is relatively cheap and easily available (Amoah, Drechsel, Abaidoo, & Henseler, 2007). Leafy green vegetables are harvested by hand with or without knives into buckets, baskets, or sacks, and then transported to market centers and other retail points under non-refrigeration conditions by market women or middle men. Vegetables are sometimes washed with water to remove dirt before

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display for sale.

Increased consumption of fresh produce has been associated with an increasing number of foodborne outbreaks in the U.S., Canada, and European countries (Callejón et al., 2015; Kozak, MacDonald, Landry, & Farber, 2013; Lynch, Tauxe, & Hedberg, 2009). Majority of such outbreaks are caused by bacteria (*S. enterica* and *E. coli*) or viruses (Hepatitis A and Norwalk virus) which can be transmitted through the fecal-oral route (Callejón et al., 2015; De Roever, 1999; Kozak et al., 2013). About 420,000 cases of foodborne illness are reported in Ghana annually with 65,000 deaths (Ababio & Lovatt, 2015). These incidences are believed to be underestimates because most cases of illnesses are not reported to health facilities in Ghana (Ababio & Lovatt, 2015). The number of foodborne illness is likely to increase in Ghana if the consumption of fresh produce increases, a trend that have been observed in other countries (Callejón et al., 2015; Kozak et al., 2013; Lynch et al., 2009).

Indigenous Ghanaian leafy vegetables are mostly used for making stews or soups, which requires minimum to high heat treatments before consumption. Most exotic vegetables are, however, used as side dishes requiring no or minimal heat treatments such as in salads or coleslaws. These leafy vegetables may receive some degree of washing before use. However, research has shown that washing alone is insufficient to reduce microbiological counts on leafy green vegetables to acceptable levels (Almeida De Oliveira, Ritter, Tondo, & Cardoso, 2012; Fishburn, Tang, & Frank, 2012). Consumption of leafy vegetables with no or minimal heat treatments makes them probable vehicles for foodborne infections. The objective of this study was to determine the microbial quality of selected leafy green vegetables that are grown and sold in urban areas in Accra Metropolis, Ghana by isolation of *Salmonella* and enumeration of aerobic bacteria, yeasts and molds, fecal coliforms, and enterococci.

2. Material and methods

2.1. Sample collection

Samples of two exotic, green leaf lettuce (*Lactuca sativa*) and cannonball cabbage (*Brassica oleracea var capitata*), and four indigenous, African spinach (*Amaranthus* sp.), African eggplant leaves (*Solanum macrocarpon),* roselle leaves (*Hibiscus sabdariffa*), and jute leaves (*Corchorus olitorius*) leafy green vegetables (Fig. 1; n = 175) were collected in duplicate from 50 farms in 12 different farming areas in Accra Metropolis district of Ghana from March 2016 to March 2017 (Fig. 2; Table 1). Leafy green vegetable samples (n = 153) were also collected from 37 sellers in 4 major market centers in the same region. The vegetable samples were collected from farmers who cultivate them or market venders who sell them and were willing to participate in the study. Collected leafy green vegetables were placed into sterile, plastic Ziploc bags (Nasco, Fort Atkinson, WI), kept in a car cooler (Rubbermaid; Newell Brands Inc, Atlanta, GA USA) with ice packs (VWR, Lutterworth, UK), and transported to a microbiological laboratory in the Department of Nutrition and Food Science at University of Ghana. The samples were analyzed immediately upon arrival at the laboratory.

2.2. Microbial enumeration

Each leafy vegetable sample (25 g) was placed in a sterile whirl-pak bag, and 225 ml of 0.1 M phosphate buffered saline (pH 7.4) was added to the bag. The vegetable samples were rinsed by shaking on a platform shaker (Lab-Line Instrumental Co., Melrose Park, IL, USA) at 100 rpm for 30 min at room temperature. A 0.1 ml of appropriate dilutions of vegetable-rinsing buffer was inoculated on four different microbiological media including tryptic soy agar (TSA), Enterococcus agar (EA), MacConkey agar (MAC), and potato dextrose agar (PDA) acidified with 10% tartaric acid to pH 3.5 (Becton, Dickinson and Company, Sparks, MD, USA). Inoculated plates of TSA were incubated at 37 °C for 24 h and those of EA were incubated at 37 °C for 24–48 h. The MAC plates were incubated at 44.5 °C for 24 h and plates of PDA were incubated at 25 °C for 48–72 h. Colonies of aerobic bacteria on TSA, yeasts and molds on acidified PDA, presumptive enterococci on EA, and presumptive fecal coliforms on MAC were enumerated after the incubation. Enterococcus colonies were confirmed by culturing selected colonies in tryptic soy broth (Becton, Dickinson and Company) with 6.7% NaCl (Fisher Scientific, Pittsburgh, PA, USA) and fecal colonies were confirmed by growth in EC broth (Oxoid Ltd, Basingstoke, Hampshire, England) with inverted fermentation tubes and incubated at 44.5 °C (Becton, Dickinson and Company).

2.3. Salmonella isolation

For *Salmonella* isolation, a leafy vegetable sample (25 g) was rinsed in 225 ml 0.1% peptone water by shaking on a platform shaker at 100 rpm for 30 min at room temperature. The rinsing buffer was incubated at 37 °C for 24 h and followed by selective enrichment in Rappaport-Vassiliadis (RV) broth with incubation at 42 °C for 24 h. Subsequently, 0.1 ml of RV broth was inoculated on XLT4 agar with supplement (Becton, Dickinson and Company) for isolation of presumptive *Salmonella* colonies. The colonies were confirmed by growth, on triple sugar iron agar (Becton, Dickinson and Company) and lysine iron agar slants (Becton, Dickinson and Company), and slide agglutination test using *Salmonella* O antiserum poly A- I and VI (Becton, Dickinson and Company).

2.4. Statistical analysis

One-way analysis of variance test was performed, and Fisher's Least Significant Difference test was used to compare the means ($p \leq 0.05$) using the Statistical Analysis Software (Version 9.4). The effect of sample source (farm or market) and vegetable type on vegetable-borne microbial counts were determined.

3. Results

The mean aerobic bacteria counts on all sampled leafy green vegetables ranged from 8.30 to 9.20 log CFU/g. The mean yeast and mold counts were from 4.25 to 5.73 log CFU/g (Table 2). Mean fecal coliform counts ranged from 4.28 to 5.81 log CFU/g and enterococcus counts from 2.93 to 4.53 log CFU/g. Cabbage samples had the highest mean aerobic bacteria count. Lettuce samples, nevertheless, had the lowest mean aerobic bacteria count which was significantly ($p \leq 0.05$) different from the mean aerobic bacteria counts from the other five types of leafy green vegetables sampled in the study (Table 2). Lettuce samples also had the lowest mean yeast and mold count while *C. olitorius, H. sabdariffa*, and *S. macrocarpon* samples had higher yeast and mold counts. *C. olitorius* and *S. macrocarpon* samples also had higher fecal coliform counts and enterococcus counts compared to other types of vegetables sampled in the study. Cabbage and lettuce samples had the lowest mean fecal coliform and enterococcus counts, respectively. In general, the indigenous leafy vegetables sampled in the study had higher yeast and mold, fecal coliform, and enterococcus counts than lettuce and cabbage, except the counts on *Amaranthus* sp. which were comparable to those from the two exotic vegetables.

There were significant differences in the microbial counts on vegetables collected from various farming areas or market centers (Table 2). Vegetables from farming areas 1, 3, and 11 had lower