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HOUSEHOLD ANTISEPTICS VS. MICROBIAL QUALITY: INVESTIGATING ELIMINATION EFFICACY ON EDIBLE GREEN VEGETABLES

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Abstract
Green leafy vegetables are essential components of a balanced diet, rich in vital vitamins and minerals. However, concerns arise from the potential contamination of both imported and locally grown green leafy vegetables, making them a primary source of foodborne pathogens. Contamination can result from various factors, including the use of natural organic fertilizers, tainted irrigation water, direct exposure to livestock, wild animals, and birds, as well as inadequate food handler hygiene practices. This contamination poses a significant risk of entero-pathogenic infections, particularly from pathogens such as Escherichia coli and Salmonella.
Escherichia coli encompasses a range of strains, with most being nonpathogenic and naturally occurring in the intestinal tracts of animals, including humans. However, certain strains, such as O157:H7, produce potent toxins that can cause severe intestinal damage, leading to life-threatening conditions. Salmonella, on the other hand, represents a group of bacteria responsible for Salmonella infection, leading to various intestinal illnesses, including typhoid fever, food poisoning, gastroenteritis, and enteric fever.

Keywords: Green leafy vegetables, Foodborne pathogens, Escherichia coli, Salmonella, Contamination sources

1. Background

Green leafy vegetables are an essential part of a balance diet, providing the vitamins and minerals necessary for the body to function properly. Imported and local vegetables have been linked to be the main source of food borne pathogens (Murray, Wu, Shi, Xue, & Warriner, 2017). Contamination of green leafy vegetables including a variety of lettuce, cabbage and cilantro may be the result of application of natural organic fertilizers, contaminated water during the irrigation process, direct contamination by livestock, wild animals and birds and improper hygiene of food handlers(Jones, 2007).The consumption of these vegetables can thus be a potential risk factor for entero-pathogenic infection caused by pathogens such as *Escherichia coli* and *Salmonella*.
The most common enteric pathogens found on fruits and vegetables are *E. coli* and *Salmonella*. Most *E. coli* strains are nonpathogenic and found in the intestines of all animals including humans. There are strains such as O157:H7 which produce large quantities of potent toxins causing severe damage to the lining of the intestines becoming life threatening.
Salmonella, on the other hand, refers to the group of bacteria that can cause *Salmonella* infection or salmonellosis in the intestinal tract. *Salmonella* infection include typhoid fever, food poisoning, gastroenteritis, enteric fever amongst other illnesses (Murrell, 2017).

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In 2018, sixty-two people were infected with the outbreak strain of Shiga toxin-producing *E. coli* O157:H7 from 16 states and the District of Columbia (Centers for Disease Control and Prevention, 2019). Green leafy vegetables which are normally consumed fresh are difficult to decontaminate which are common sources of enteropathogenic infection. A detailed study was conducted to estimate the level of microbial contamination in three vegetables that are commonly consumed as raw vegetables in Belize namelyCilantro (*Coriandrum sativum*), Cabbage (*Brassica oleracea*), local and Mexican lettuce (*Lactuca sativa*). This study also attempted to find the effect of three household chemicals with antimicrobial properties such as soap solutions, vinegar (acetic acid) and Clorox (sodium hypochlorite) in eliminating or reducing the microbial contamination in the three vegetables.

2. Methodology

The concentration of enteropathogenic bacteria on 3 types of green vegetables were determined by culturing the microbes on selective media, namely MacConkey agar and Brilliant Green Agar. The concentration *Escherichia coli* and other lactose fermenting bacteria was enumerated from the cultures grown on MacConkey Agar and the concentration of *Salmonella*and other non-lactose fermenting bacteria were enumerated from the cultures grown on Brilliant Green Agar. The concentration of these enters opathogenic bacteria was determined by dislodging the bacteria from the surface of the vegetables. Triplicate samples of 100g of each of the vegetables were chopped up using sterilized knives under laminar flow chamber. Sample was added to 100ml of distilled sterilized water and placed in a shaker for 5 minutes, after which 100µl(0.1ml)of the water containing the dislodged bacteria were cultured the two types of selective media. To determine the effectiveness of the three household chemicals containing antimicrobial agents triplicate samples of 100g of each of the 3 types of chopped green leafy vegetables were added to 100ml of each of the following solutions:

2ml of dish washing liquid +100ml distilled sterilized water
50% concentration of household vinegar (5% acetic acid)
30% concentration of household vinegar (5% acetic acid)
20% concentration of household vinegar (5% acetic acid)
10% concentration of household vinegar (5% acetic acid)
5% concentration of household vinegar (5% acetic acid)
20% concentration of Chlorine bleach (5.2% sodium hypochlorite)
10% concentration of Chlorine bleach (5.2% sodium hypochlorite)
5% concentration of Chlorine bleach (5.2% sodium hypochlorite)
3% concentration of Chlorine bleach (5.2% sodium hypochlorite)

Samples were left to soak for 3 minutes, then shake on a shaker for 5 minutes after which the vegetable were removed from the solutions using a sterile tweezer. Each of the samples were then added to 100ml of distilled sterilized water, shake for 5 minutes, after which 100µl of the water containing any dislodged bacteria were cultured on the two types of selective media namely MacConkey Agar and Brilliant Green Agar.

3. Results and Discussion

*E. coli*is found naturally in the microflora of intestines in humans and other mammals. Major groups of *E. coli* are designated as enterotoxigenic, enteropathogenic, and so forth and the vegetables can be contaminated with one or more of these groups while in the field or during post-harvest handling (Beuchat, 2018).

Infections with *E. coli* O157:H7 are known to cause several diseases ranging from uncomplicated diarrhea to life-threatening hemolytic uremic syndrome (HUS) being a leading cause of acute renal failure. *E. coli* can be gotten from direct or indirect contact with infected animals/individuals or

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contaminated surfaces. (Franz, Tromp, & Fels-Klerx, 2009). A summary of the initial concentration of *E.coli*and other lactose fermenting bacteriaobtained from the three types of green leafy vegetables grown on MacConkey agar is shown in table 1.

Table 1: Initial Concentration of *E.coli* and other lactose fermenting bacteria collected from Green Leafy Vegetables (GLV) cultured on MacConkey agar.

Type of GLV	Initial concentration of Lactose Fermenting Bacteria
Lettuce A Mexican	2.6×10^4
Lettuce B Local	3.0×10^4
Cilantro	4.5×10^4
Cabbage	1.9×10^4

The results indicated that 100% of the samples were contaminated. Initial concentrations ranged from 1.9×10^4 to 4.5×10^4 cfu/ml. Cilantro leaves showed the highest contamination. These results were even more alarming than the study conducted by Mritunjay and Kumar (2016) which indicated that 85.4% of the samples surpass the limit for aerobic count done by standard protocol.Organic farming practices including the use of animal manures have been linked to increasing the risk of microbiological contamination since manure can act as a vehicle for transmission of foodborne pathogens (Kuan, et al., 2017). Khiyami et al., (2010) investigated the concentration of coliforms found in salads served at restaurants. The colony counts for the total coliforms were in the range of 2.8×10^4 CFU/g.Thus, these vegetables normally eaten raw in salads and certain sea food cocktails such as ceviche can carrylargeconcentrations of enteropathogenic bacteria. Although*E.coli* is part of the body's normal microbiota, high concentrations of *E.coli* and other lactose fermenters indicates the presence of other enteropathogenic bacteria.

The consumption of contaminated food may cause gastrointestinal disorders; for example, enterohemorrhagic *E. coli* (EHEC) may cause hemorrhagic colitis and the hemolytic uremic syndrome because of their production of Shiga toxins, whereas entero-aggregative *E. coli* (EAEC) are associated with persistent diarrhea in children in less-developed countries (Roy M. Robins-Browne et.al., 2004).

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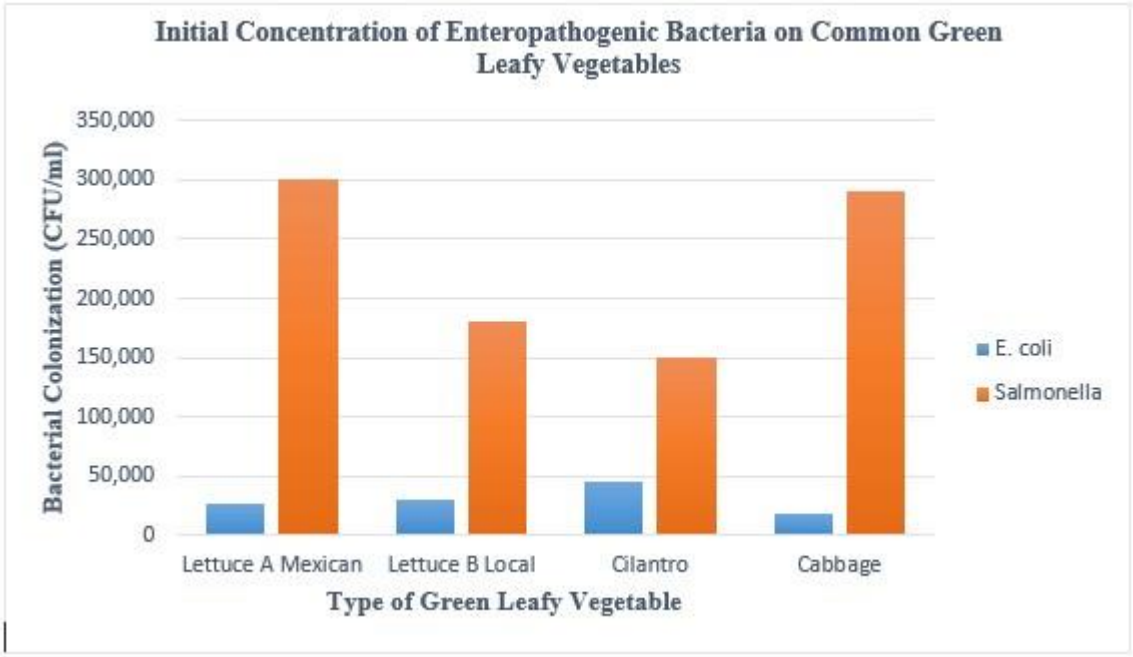

Figure 1: Initial Concentration of Enteropathogenic Bacteria from Green Leafy Vegetables on MacConkey agar and Brilliant Green agar.

The initial concentration of *Salmonella* and other non-lactose fermenting bacteria cultured on Brilliant green agar ranged from 1.8 – 3.0 x10^5 CFU/ml (Figure 1). *Salmonella* is the group of bacteria causing Salmonellosis in the intestinal tract. Salmonelloses include typhoid fever, food poisoning, enteric fever amongst other illnesses (Nordqvist, 2007). Another study by Rivera et al., (2015) reported that contamination of leafy vegetables grown in hydroponic system was attributed to water sources used for irrigation of those vegetables. Although most people assume that rinsing with water will get rid of the bacterial load, several studies have shown that this is not effective. Therefore, an effective and practicable method is required to remove pathogens as well as dirt and debris to prevent further contamination in the home (Temiz, Bagci, & Togay, 2011).

In this study three house hold chemicals with anti-microbial properties were tested to determine their efficiency in the removal of *E. coli*. and other lactose fermenting bacteria as well as other enteric pathogens. Treatment A which consisted of a mild soap solution proved to be ineffective and cultures grown on all plates were too numerous to count. Treatment B (Table 2), consisting of various concentrations of vinegar (5% acetic acid) was ineffective at 5% and 10% concentrations for Mexican Lettuce, Local Lettuce and Cilantro but reduced the initial concentration of *E.coli* and other lactose fermenting bacteria on cabbage by one magnitude. Concentration of 20% vinegar reduced the initial concentration of *E. coli* in all samples tested by one magnitude. At concentration of 30% and 50% no bacterial growth was found on the samples tested.

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Table 2: Concentration of E.coli and other lactose fermenting bacteria from Green Leafy Vegetables (GLV) cultured on MacConkey agar after treatment with varying concentration of household vinegar.

Type of GLV	Treatment B (Vinegar) (CFU/ml)				
	5%	10%	20%	30%	50%
Lettuce A Mexican	TNTC	TNTC	2.8×10^3	No growth	No growth
Lettuce B Local	TNTC	TNTC	2.2×10^3	No growth	No growth
Cilantro	TNTC	TNTC	5.2×10^3	No growth	No growth
Cabbage	3.0×10^3	2.6×10^3	2.5×10^3	No growth	No growth

In a study aimed to compare the levels of effectiveness of various household antiseptics in decreasing levels of *E. coli* on lettuce, household bleach at 4% had the maximal removal of 1.6 $\log_{10}$ CFU/g for *E. coli*. The highest removal of *E. coli* was 35% white vinegar with a maximum reduction of 5.4 $\log_{10}$ (Vijayakumar, 2002). Treatment C, which consisted of various concentrations of 5.2% sodium hypochlorite (table 3) was very effective at all 4 concentrations tested and no growth was observed at a concentration as low as 3%.

Table 3: Concentration of E.coli and other lactose fermenting bacteria from Green Leafy Vegetables (GLV) cultured on MacConkey agar after treatment with varying concentration of Clorox bleach.

Type of GLV	Treatment C –Clorox Bacteria (CFU/ml)			
	3%	5%	10%	20%
Lettuce A Mexican	No growth	No growth	No growth	No growth
Lettuce B Local	No growth	No growth	No growth	No growth
Cilantro	No growth	No growth	No growth	No growth
Cabbage	No growth	No growth	No growth	No growth

A large number of foodborne pathogenic bacteria that is a part of the normal microflora in the gastrointestinal tracts of animals is shed into the environment. spp. can occur in various environments even in soil and thus when organic farmers use manure or contaminated water, the soil and/or the water can act as vehicles to transmit *Salmonella* to the vegetables. Common leafy green vegetables that have been involved in outbreaks from *Salmonella* include cilantro, lettuce, and parsley (Jacobson, Gill, Irvin, Wang, & Hammack, 2012).

The results shown in Table 4, indicated that vinegar (5% acetic acid) was effective in the removal of the non-lactose fermenting bacteria at a concentration as low as 30% for lettuce A, Cilantro and Cabbage. Nonlactose fermenting bacteria was still found on the sample of lettuce B. A concentration of 50% house hold vinegar was effective in the removal of 100% of the pathogens. Treatment C, which consisted of various concentrations of 5.2% sodium hypochlorite was also applied to the sample to determine its effectiveness in the removal of the non-lactose fermenting bacteria. The results in table 5 indicated that a concentration as low as 3% was effective in the removal of 100% contamination.

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Table 4: Concentration of Salmonella and other non-lactose fermenting bacteria from Green Leafy Vegetables (GLV) cultured on Brilliant green agar after treatment with varying concentration of vinegar.

Type of GLV	Treatment B (Vinegar) (CFU/ml)				
	5%	10%	20%	30%	50%
Lettuce A Mexican	TNTC	TNTC	TNTC	No growth	No growth
Lettuce B Local	TNTC	TNTC	TNTC	4×10^1	No growth
Cilantro	TNTC	TNTC	TNTC	No growth	No growth
Cabbage	TNTC	TNTC	TNTC	No growth	No growth

Table 5: Concentration of Salmonella and other non-lactose fermenting bacteria from Green Leafy Vegetables (GLV) cultured on Brilliant green agar after treatment with varying concentration of Clorox bleach.

Type of GLV	Treatment C (Clorox)			
	3%	5%	10%	20%
Lettuce A Mexican	No growth	No growth	No growth	No growth
Lettuce B Local	No growth	No growth	No growth	No growth
Cilantro	No growth	No growth	No growth	No growth
Cabbage	No growth	No growth	No growth	No growth

The increase in outbreaks can be attributed to several issues from the sanitary quality of cultivation water, harvesting, transportation, storage, processing and handling of the produce. Improper sanitary methods can lead to bacteria on the produce to multiply depending on how suitable the conditions are. The agriculture facilities in Belize are lacking in money and facilities for proper sanitization, store and distribution leading to contamination and foodborne illnesses.

4. Conclusion

Consumption of raw green leafy vegetables or as part of a dish can lead to cross contamination to other vegetables or utensils, thus can pose a severe health issue if proper steps are not taken. Prevention of microbial contamination to ensure food safety and prevent foodborne illnesses is crucial and necessary step that should be practiced at all homes. Microbial contamination was observed in all the vegetables sampled at an initial 10^4 to 10^5 concentration of 10 cfu/ml on MacConkey agar and of 10 cfu/ml on brilliant green agar, signifying there is more non lactose fermenting bacteria than lactose fermenting bacteria on the vegetables. Various disinfecting methods were performed to evaluate the effectiveness of the removal of entero-pathogenic bacteria from green leafy vegetables. The results obtained in the study demonstrated that soaking in Cloroxat a 3% concentration and vinegar at a 30% concentration followed by stirring for three minutes were the most effective methods for decontamination.

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