## AMERICAN UNIVERSITY OF NIGERIA DEPARTMENT OF NATURAL AND ENVIRONMENTAL SCIENCE

Senior Research Project

# EFFICACY OF DISINFECTION TECHNIQUES ON MICROBIAL CONTAMINATION OF FRUITS AND VEGETABLES SOLD IN MARKETS IN YOLA-JIMETA, NORTHEASTERN NIGERIA



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**AMERICAN UNIVERSITY OF NIGERIA DEPARTMENT OF NATURAL AND ENVIRONMENTAL SCIENCES**

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## DEDICATION

I dedicate this project to my dearest sister Ms. Khadija Yusuf (Uwan Yaya) for being not just a sister but a mother to me. I also dedicate this work to Jibril Lawan Kawu (J. Kawu), Shamsuddin Dayyabu (Laba), Yahaya Ya’u Haruna (Yasayyadi), and Tata for helping me and being part of my life when I needed you the most and for shaping me into the person I am today, I would not have made it this far without your support and guidance.

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# EFFICACY OF DISINFECTION TECHNIQUES ON MICROBIAL CONTAMINATION OF FRUITS AND VEGETABLES SOLD IN MARKETS IN YOLA-JIMETA, NORTHEASTERN NIGERIA

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## ABSTRACT

In order to determine the microbial quality of fruits and vegetables sold in Yola- Jimeta markets and the efficacy of vinegar in decontaminant, the microbial contaminations of 16 samples of cabbage, carrot, lettuce, and tomato obtained from Yola-Jimeta market was determined before washing, after washing with water, after washing with vinegar and rinsing with water, and after soaking in vinegar for 5 minutes and rinsing with water. A significant reduction in the microbial loads of the samples was observed after washing with vinegar and rinsing with water, while no microbial growth was observed after soaking in vinegar water for 5 minutes and rinsing with water. Further tests revealed harmful microbes among the microbial growth observed. These results indicated that fruits and vegetables sold in Yola- Jimeta markets are contaminated with harmful microbes and that washing with water does not reduce the microbial load of the samples tested while a decrease in the

microbial loads was observed only after washing with vinegar and rinsing with water. These results suggest that the use of vinegar is an effective decontamination method for fruits and vegetables.

*Keywords*

Bacterial contamination, disinfection, fruits, Jimeta, microbial contamination, vegetables, vinegar, Yola.

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**CHAPTER 1 INTRODUCTION**

The importance of fresh fruits and vegetables as the primary natural source of vitamin and fiber for humans cannot be overemphasized. However, fruits and vegetables are produced, marketed, and consumed with little or no sanitary measures (Fig. 1) in developing nations (Eni, Oluwawemitan, & Solomon, 2010). The use of manure that has not been composted and sewage water that has not been treated as fertilizers further increases the possibility of microbial contamination (Eni et al., 2010) and this practice has led to several outbreaks resulting from the consumption of fresh produce in Europe and the United States (Soon, Manning, Davies, & Baines, 2012). Nonetheless, fresh fruits and vegetables cannot be replaced by any other food source; hence there is a need to make sure that they are safe before consumption. To this end, many decontamination techniques have been devised to counter the effect of harmful microbes. However, the efficacy of many decontamination methods in commercial settings are still doubted (Fonseca & Ravishankar, 2007).



Figure 1 A typical African fruit and vegetable market in Kenya (credit: alamy)

*Fresh fruits and vegetables*

Being recognized as one of the most important source of vitamins, nutrients, and fiber for humans has made fresh produce popular in the world. The world has seen a large increase in the production of fruits and vegetables by 94% between 1980 and 2004 (Fig. 2) (Olaimat & Holley, 2012). The United States’ importation of fresh produce doubled to 12.7 billion dollars from 1994 to 2004 (Aruscavage, Lee, Miller, & LeJeune, 2006), and the daily sales of fresh produce reached 6 million packages in 2005 (Jongen, 2005) as cited in Olaimat & Holley, 2012.

This increase in the level of consumption of fruits and vegetables and the surge of various locally produced and imported fruits and vegetables in all seasons might be attributed to peoples’ growing attention to staying healthy and eating right as well as the convenience provided from prepared products (Warriner, Huber, Namvar, Fan, &

Dunfield, 2009). The world’s fruits and vegetable consumption has increased at an annual average of 4.5% from 1990 to 2004, and in the United States alone, the annual consumption of fruits and vegetables between 1997-1999 increased by 25% relative to the years 1977-1979 (Olaimat & Holley, 2012).

People became more interested in the consumption of fresh fruits and vegetables after the release of information highlighting the health benefits of the consumption of fruits and vegetable (DuPont, 2007). For example, in a report by the World Health



Figure 2 Global production of fruits and vegetables from 1982 to 2004 (sourced from EU, 2007).

Organization (WHO), it’s recommends at least 400 grams of fruits and vegetables are eaten in a day for protection against the risk of non-communicable diseases and improvement of overall health (Soon et al., 2012). Additionally, Healthy People, a

U.S. government program, aims at increasing the intake of fruits and vegetables for people aged 2 years and above to two daily servings of fruits and three daily servings of vegetables to 75% and 50%, respectively (DuPont, 2007).

However, this increase in consumption of fruits and vegetables has been followed by an increase in outbreaks of foodborne illnesses linked to the consumption of fresh fruits and vegetables (Warriner et al., 2009). This increase in the consumption of fruits and vegetables was associated with change of personal dietary habits increased availability of fresh produce with some coming from sources having uncertain sanitary practices (Beuchat, 2002). The use of manure that has not been composted, untreated sewage, irrigation water contaminated by pathogens, increased contact between livestock and fresh produce due to their proximity to areas of high produce production, and also increased number of immunocompromised consumers further worsens the situation (Beuchat, 2002). The most reported pathogens associated with foodborne illnesses related to the consumption of fresh produce are *Salmonella* sp. and *Escherichia coli* O157:H7 (Warriner et al., 2009).

Fresh fruits and vegetables that receive little or no processing and thus do not undergo effective microbial decontamination and elimination steps usually carry microbes, some of which could be harmful to human health (Harris et al., 2003). Contamination can occur at any stage from the farm to the consumer due to environmental, human, or animal contact during production, storage harvesting, and transportation (FDA, 2014).

In less developed countries such as Nigeria, contamination is mostly due to the use of manure and untreated water as fertilizers in the production of fruits and vegetables (Eni et al., 2010). A high microbial contamination was observed in fruits and vegetables in a study conducted in Sango Ota, Ogun state, Nigeria. The high contamination was suggested to be due to cross-contamination during the storage

time of the fruits and vegetables, during washing in markets where many fruits and vegetables are washed using the same water that was earlier used, and during transportation or handling by vendors (Eni et al., 2010).

In another study in Sokoto State, northwestern Nigeria, eight pathogenic microbes were found in tomatoes sold in markets. The microbes isolated were *Aspergillus niger*, *A. ochraceous*, *A. flavus*, *A. fumigatus*, *Penicillium citrinum*, *Helminthosporim fulvum*, *Curvularia lunata*, and *Sclerotium rolfsii* (Muhammad, Shehu, & Amusa, 2004). The reality that a significant portion of the Nigerian population are low- income earners and frequently consume rotten tomatoes further aggravates the situation (Muhammad et al., 2004).

In Ghana, urban farmers with a limited choice of irrigation water have no choice but to use polluted water for irrigation and thus, increasing the contamination risk even more for fruits and vegetables that are eaten raw (Amoah, Drechsel, Henseler, & Abaidoo, 2007). The detection of a foodborne pathogen in irrigation water is an indicator of possible contamination risk, although the ability of such pathogen to cause risks might depend on its excreted load, duration of latency period, ability to multiply outside mammal hosts, persistence in the environment, persistence on food, infectious dose, and human response (Steele & Odumeru, 2004).

However, fruit and vegetable contamination is not peculiar to the less developed countries; even in developed countries like the United States, this problem is common. In response to this contamination threat, the U.S. Food and Drug Administration (FDA) published a note titled *Guide to Minimize Microbial Food*

*Safety Hazards for Fresh Fruits and Vegetables* that identifies the main sources of pathogen contamination and ways to address these sources (FDA, 2014). Similarly, in 2011, the United States developed the Food Safety Modernization Act (FSMA), which provides both reactive and preventive approaches to food safety in the US (Collart, 2016).

*History of outbreaks in the US*

Outbreaks associated with fresh fruits and vegetables were first reported in the United States in 1982 (Rangel, Sparling, Crowe, Griffin, & Swerdlow, 2005). Outbreaks related to fresh produce has been on the rise since then (Fonseca & Ravishankar, 2007). In the United States, such outbreaks have accounted for 38 (21%) of 183 outbreaks related to foodborne illnesses and 34% of 5,269 cases are food related. These outbreaks usually reach their peak in the summer and fall such that 74% of reported cases occurred between July and October (Rangel et al., 2005). Lettuce was the cause of 13 (34%) of fresh fruits and vegetable associated outbreaks, while apple juice contributed to 7 (18%), salad 6 (16%), coleslaw 4 (11%), melons 4

(11%), sprouts 3 (8%), and grapes 1 (3%) in the United States between 1982 and 2002 (Rangel et al., 2005). The main fruits and vegetables affected in outbreaks between 1990 and 2003 were sprouts, tomatoes, and melons (Fonseca & Ravishankar, 2007). Foodborne illnesses related to the consumption of fresh fruits and vegetables have increased rapidly with the increase in their consumption (Warriner et al., 2009)).

The U.S. Center for Disease Control (CDC) has estimated that contaminated produce has contributed to more than 47.8 million illnesses; 127,839 hospitalizations; and

over 3,000 deaths between 2000 and 2006 (Scallan, Griffin, Angulo, Tauxe, & Hoekstra, 2011). In 1995, 40 confirmed cases of *E. coli* O157:H7 infection associated with lettuce consumption were reported in the US State of Montana (Ackers et al., 1998). This increase in outbreaks has been attributed to an increase in the demand for minimally processed and ready-to-eat fruits and vegetables and to the increased presence of out-of-season fruits and vegetables in the United States (Heaton & Jones, 2008).

*Pathogenic microbes of concern and their pathways*

Organic manure has been identified as a possible route of microbial contamination in fruits and vegetable, with slurries and animal manure as the leading source. Irrigation water that has been contaminated with fecal material and sewage overflow is a direct way of introducing pathogens to farm produce. Soil, which is a natural habitat for most pathogens, can introduce the pathogens directly to the surface of fruits and vegetables during heavy rain or when mixed up in organic manure (Heaton & Jones, 2008). It is common today to find coliform bacteria, which is normally found in human feces, in the fresh waters with little or no human contact (Higgins & Gbakima, 2008).

The bacterium *Salmonella typhi* (Fig. 3) is one of the most prevalent pathogens associated with outbreaks in fresh fruits and vegetables around the world between 2006 and 2008 (Lynch, Tauxe, & Hedberg, 2009). It causes salmonellosis, also called salmonella infection (Fonseca & Ravishankar, 2007), which has symptoms such as vomiting, nausea, fever, and abdominal cramps. *S. Typhi* caused one out of five fresh produce-related outbreaks between 1990 and 2003 in the United States

(Fonseca & Ravishankar, 2007). Some fresh fruits and vegetables, such as melon, tomatoes, sprouted seeds, and lettuce, have been identified as major vehicles for salmonella infections (Heaton & Jones, 2008). For example, uncooked tomatoes caused several outbreaks of salmonellosis in the US States of Illinois, Michigan, Minnesota, and Wisconsin of the United States in 1990 (Hedberg et al., 1999).

Several other outbreaks associated with serotype Thompson of this pathogen were associated with the consumption of fresh cilantro in California in 1999 (Campbell et al., 2001).



Figure 3. A microscopic view of salmonella image

Another pathogen commonly isolated from fresh fruits and vegetables is *E. coli* O157:H7 (Fig. 4)*.* It is categorized into a group of bacteria called coliforms, which are bacteria known for causing gastrointestinal diseases such as diarrhea (Nkere, Ibe, & Iroegbu, 2011) and have an incubation period of 3-5 days (Holton, 2002).

Although most strains are not harmful and are found in the digestive tracts of humans and animals where they perform vital functions in our body, such as inhibiting the growth of harmful bacteria and synthesis of vitamins (Holton, 2002), the O157:H7 strain can cause serious health problems such as urinary tract infections, severe anemia, diarrhea and kidney failure and death in some cases (Özpınar et al., 2013)*.*

The harmful strain was confirmed to be the causative organism for enteric diseases by the CDC in 1982 (Holton, 2002). *E. coli* O157:H7 was isolated from both fresh spinach (CDC, 2006) and packaged spinach (Wendel et al., 2009) in Wisconsin and Oregon in 2006. It was also reported to have caused widespread of outbreaks in Atlanta, Georgia, in the United States, due to spinach consumption (Cunningham, 2006).

According to Rangel and colleagues (2005), *E. coli* has accounted for twenty-four multi-state outbreaks of foodborne illnesses in the United States since 1992; all were due to foodborne transmission, and 25% of the total outbreaks were associated with fresh fruits and vegetables (Fig. 5).

Fresh fruits and vegetable associated with outbreaks in the United States mostly originated from restaurants, with 15 (39%) of the reported cases occurring across restaurants, and cross-contamination during food preparation contributed to 7 (47%) of the cases reported in the United States between 1982 and 2002 (Rangel et al., 2005). The average number of cases of outbreaks due to *E. coli* related to fresh fruits and vegetable (20) is much larger than the average number of outbreaks related to ground beef (8). Animal contact has also been reported to have been a source of contamination in the United States (Rangel et al., 2005).



Figure 4 Microscopic view of E. coli (credit: cdc.gov/ecoli).

In less developed countries such as Nigeria, studies have shown that *E. coli, Salmonella* sp.*,* and *Enterobacter* sp. are the most prevalent foodborne microbes in the country (Nkere et al., 2011). *E. coli* is known to be the causative agent of traveler’s diarrhea, an illness experienced by people visiting developing countries; the consumption of contaminated raw vegetables is the main cause of this illness (Harris et al., 2003).

Another pathogen, *Campylobacter jejuni*, which affects mostly raw peas, caused several illnesses in Alaska, United States, in 2005. This pathogen causes Campylobacteriosis, which is associated with most diarrheal illnesses in the United States (Gardner et al., 2011). Campylobacter pathogens are known to be the leading cause of bacterial enteritis in the world. Although they are mainly zoonoses, *C. jejuni* has also been known to contaminate lettuce and salads. While *C. jejuni* contaminates

fruits mainly by cross-contamination, they can survive on fresh-cut melon and papaya for a time long enough to harm consumers (Harris et al., 2003).



Figure 5 . Routes of transmission for *E. coli* O157 by year (Credit: wwwnc.cdc.gov/eid/article/11/4/04-0739-f3).

*Listeria* species is also another group of pathogens associated with fresh fruits and vegetables, notably raw tomatoes and lettuce (Harris et al., 2003). It is known to cause mild gastroenteritis in adults, but their symptoms are more severe in immunocompromised individuals, neonates, and pregnant women (Harris et al., 2003). Because they are very ubiquitous in the environment, *Listeria* spp. can be isolated from vegetables that have been irrigated with contaminated water, feces of livestock, water, and soil samples; therefore, they can contaminate fresh fruits and vegetable (Heaton & Jones, 2008). *Listeria* spp. are also known to cause hemolytic uremic syndrome (HUS), a group of blood-related ailments such as renal injury,

hemolytic anemia, thrombocytopenia, and other related blood diseases (Rangel et al., 2005).

*Shigella* sp. is another pathogen that contaminates fruits and vegetables. There are four species, all of which are pathogenic: *Shigella flexneri, S. bodii, S. sonnei,* and *S. dysenteriae.* They lead to shigellosis which is known to cause severe dysentery and are pathogenic to humans even at low doses. Although transmission is mainly through interpersonal contact, contaminated fruits and vegetables that received little or no heat treatment are known to cause diseases. *Shigella* spp. have been known to cause outbreaks due to consumption of shredded salad and onions (Harris et al., 2003).

*Staphylococcus aureus* is another pathogen detected in fruits and vegetables, it is known to be carried by food handlers, and may grow on peeled oranges (Harris et al., 2003). *Yersinia pseudotuberculosis* O:3 outbreaks were also reportedly associated with the consumption of iceberg lettuce in many countries (Nuorti et al., 2004).

*Survivability of pathogens*

The ability of some pathogens associated with fresh fruits and vegetable to survive in multiple environments is another important factor to be considered when assessing the relationship between microbes and food (table. 1). For example*, L. monocytogene* is known to survive at refrigeration temperatures and can reproduce on stored fruits and vegetables (Heaton & Jones, 2008). *S. aureus* can survive for up to 14 days if stored at 40C to 80C (Harris et al., 2003).

**Table 1.** Survivability, sources, and symptoms of some pathogens (credit: Food Industry Counsel LLC).

|  |  |  |  |
| --- | --- | --- | --- |
| **Common****Pathogens** | **Incubation****Period** | **Common Sources** | **Common Symptoms** |
| ***Bacillus sphaericus*** | 1-6 hrs. (vomiting) 6-24 hrs.(diarrhea) | Soil Organisms typically found in raw dry andprocessed foods | Nausea and diarrhea. Typically resolves within 24- 48 hours |
| ***Botulism******(C. botulinum)*** | 12-72 hrs.(Usually 18-36 hrs.) | Improperly canned home and commercial foods (including cans with dents and punctures), meats, sausage, fish, potatoes, leftoverstews, and water. | Nausea, vomiting, diarrhea, fatigue, headache, dry mouth, double vision, muscle paralysis, respiratory failure. Duration is variable (day to months) |
| ***Campylobacter (c. Jejuni)*** | 2-7 days(usually 3-5 days) | Raw milk and eggs, raw or undercooked beef, poultry andshellfish, and water | Diarrhea (often bloody), abdominal cramps, nausea and headaches, typicallyresolves within 1-10 days |
| ***E. coli O157:H7*** | 24+ hrs. to10 days(usually 3-4 days) | Ground beef, raw milk, and raw produce and vegetables, and person-to-person and person-to-foodtransmission. | Diarrhea (often bloody), abdominal cramps and vomiting; usually no fever. HUS may develop in rare cases. Typically resolves within 1-8 days (in non-complicated cases) |
| ***Salmonella*** | 6-72 hrs.(Usually 12- 36hrs.) | Poultry, eggs, sprouts, person-to- person and person- to-foodtransmission. | Diarrhea, abdominal cramps, nausea vomiting, and fever. Typically resolves within 4 to 7 days |
| ***Listeria*** | 9-48 hrs. (for GIsymptoms) 2-6 weeks (for invasive disease) | Fresh soft cheeses, unpasteurized or inadequately pasteurized milk, ready-to-eat deli meats and hot dogs | Fever, muscle aches, nausea, diarrhea; pregnant women may suffer flu-like symptoms and stillbirth; elderly, immunocompromised and infants can develop sepsis and meningitis. Duration isvariable. |
| ***Shigella*** | 24-73 hrs.(Usually 12-36 hrs.) | person-to-person and person-to-food transmission; contaminated foods, raw vegetables, eggsalads and water/ice | Watery diarrhea, nausea, vomiting, abdominal cramps. Chills and fever, stool may contain blood and mucus.Typically resolves within 4-7 days. |

In general, pathogens associated with fruits and vegetables survive best in the soil, irrigation water, and fertilizer (Alam, Feroz, Rahman, Das, & Noor, 2015). In some microorganisms such as *S. aureus* produce heat resistant toxins, and, therefore, pose a serious threat of infection even at high temperatures (Harris et al., 2003). Cut fruits and sliced vegetables also provide an environment that encourages the survival of pathogens because once cut or sliced, fruits or vegetables provide nutrients for pathogens to multiply (Lynch et al., 2009).

*Shigella sonnei,* another pathogen associated with fruits and vegetable, has been known to survive at 50C on lettuce for as long as three days without any decrease in number and can increase by more than 1,000-fold should the temperature be increased to 220C. This suggests that *S. sonnei* can survive even at refrigerated temperatures. *S. sonnei* can also grow on shredded cabbage and parsley stored at 240C. A combined population of *S. flexneri, S. dysenteriae,* and *S. sonnei* was observed to be able to grow on cut papaya (pH 5.69) and watermelon (pH 6.81) within just 4-6 hours at 22-270C (Harris et al., 2003).

Some laboratory studies have shown that *Salmonell*a sp. can grow on sliced or chopped tomatoes with a pH of 4.5 stored at 200C to 300C (Harris et al., 2003). *E. coli* O157:H7 can grow rapidly on raw fruits and vegetables, especially at 120C. Packaging under pressure does not inhibit the survival and growth of *E. coli.* It is known to have very low infectious dose and can develop resistance to acid (Harris et al., 2003).

Moisture content is also another factor that facilitates the survival and growth of microbes on fresh fruits. For example, fresh fruits and vegetables have an approximate moisture content of 0.97-1.0aw, which favors the growth of microbes (Wadamori, Gooneratne, & Hussain, 2017). Humidity and heat, which are common in tropical regions might also favor microbial growth.

*Commonly used methods of decontamination*

Different physical and chemical methods are used to decontaminate fruits and vegetables. Preventing contamination in the first place is the best way to eliminate pathogens from fruits and vegetables. Nonetheless, this is almost impossible to achieve, also washing and sanitizing fruits and vegetables may even not totally eliminate all pathogens (FDA, 2014).

Washing fresh fruits and vegetable with chlorine after harvest is a reliable way of reducing pathogen contamination (Warriner et al., 2009). However, Fonseca and Ravishankar (2007) have argued that many factors limit the efficacy of chlorine as a decontaminant, including the ability of pathogens to get into plant tissues, the ability of some bacteria to form a biofilm, and the hydrophobic nature of plant surfaces.

Other alternative methods of decontaminating fruits and vegetables from pathogens include the use of ozonated water (Hassenberg, Fröhling, Geyer, Schlüter, & Herppich, 2008), washing under pressure (Segner & Scholthof, 2007), ultraviolet light C (UVC), calcinated calcium, electrolyzed oxidizing water, gamma irradiation, and detergent with water (Fonseca & Ravishankar, 2007). The use of antagonistic bacteria and the use of bacteriophages, or a combination of both, has also been identified as good decontamination alternatives (Olaimat & Holley, 2012). Although

most of these methods may have flaws, studies have indicated that most of them are effective. For example, Segner & Scholthof, found that because apples are washed with clean water under pressure, they had a relatively little amount of microbes (Segner & Scholthof, 2007).

The FDA has further reported that hot water is also used as a decontaminating agent, but pointed out that the method has some adverse effects on color and texture of fruits, and thus decreases the freshness of fruits. The effectiveness of any treatment against microbes depends on the type of the treatment, characteristics of the produces’ surface (hydrophobicity, cracks, and texture), exposure time, temperature, and pH. The ability of some microbes to get to the inside fruit tissues renders many techniques ineffective (FDA, 2014).

In developing nations, inadequacy or nonexistence of sewage treatment facilities, coupled with overpopulated urban areas, make it easy for microbes to get deposited into habitats that support their survival and growth (Higgins & Gbakima, 2008). For example, a study conducted in Ghana showed that all samples of irrigation water contain fecal coliform levels that exceeded the WHO recommended a level of 1 x 103 100ml-1 (Amoah et al., 2007).

However, it is difficult to say with certainty that disease outbreaks in these countries occur due to waterborne or foodborne or fecal-oral contamination. This is because most water-borne diseases can also be spread through fecal, person-to-person, and via contaminated foods (Issa-Zacharia, Kamitani, Muhimbula, & Ndabikunze, 2010). Because some rural areas lack proper sanitation facilities, it is easy for a pathogen,

once introduced into a community, to spread via the fecal-oral route. This makes it likely for developing nations to experience less foodborne contamination and more of fecal-oral contamination (Issa-Zacharia et al., 2010). Some of the ways through which microbes can contaminate fruits and vegetables in developing countries such as Nigeria can be through dust in markets and bacterial soft rot.

Many other decontamination methods, such as the use of electrolyzed water (Fonseca & Ravishankar, 2007) (Issa-Zacharia et al., 2010), free chlorine, pasteurization (Cunningham, 2006), hypochlorite, bromine, iodine, quaternary ammonium compounds, acidic compounds with and without fatty acids, alkaline compounds, peracetic acid with and without fatty acids, hydrogen peroxide (Goodburn & Wallace, 2013), ozone (Hassenberg et al., 2008), and irradiation (Cunningham, 2006), are currently in use by various food companies. Biocontrol, such as the use of antagonistic bacteria and bacteriophages, is also an available decontamination method (Wadamori et al., 2017). Other non-thermal technologies, such as the application of pulsed electric light, high pressure, pulsed electric field, oscillating magnetic field, and ultrasound and UV treatments, have also been reported to reduce or, in some cases, eliminate microbes from fruits and vegetables (Goodburn & Wallace, 2013).

However, there are few published studies on the effect of these technologies on fresh fruits and vegetables (FDA, 2014). Furthermore, these methods do have something in common, which is complexity and difficulty to perform. They also require trained and educated personnel and, therefore, may not be used on a wide scale and hence,

the need for a verified low-tech decontamination technique that can be practiced at small scale and household levels.

Addition of detergent to water, which seems relatively easy, has been faulted because it causes infiltration of surface microbes into the inner parts of damaged fruits and vegetables by reducing the surface tension of the water (Beuchat, 2002).

*Food Safety Regulation in Nigeria*

In Nigeria, the National Food and Drug Administration and Control (NAFDAC), established in 1993, is responsible for food safety, and its roles are equivalent to those of the United States’ FDA. Not many studies have been conducted on the effects of harmful microbes on Nigerians. However, some independent research has been done on microbial contamination in Nigeria. For example, microbes have been found on tomatoes sold in markets in Sokoto State (Muhammad et al., 2004), and on fruits and vegetables in Ogun State (Steele & Odumeru, 2004), and in foods across restaurants in Nsukka, Enugu State (Nkere et al., 2011).

No research has been conducted to determine the microbial quality of fruits and vegetables sold in Yola-Jimeta markets. Therefore, I tested fresh fruits and vegetables available in public markets in a small urban center in northeastern Nigeria. My aim was to determine microbial contamination and evaluate the efficacy of two simple and affordable washing techniques that can be used by the general public. I intended to focus on fruits and vegetables eaten raw because they tend to pose more risk of microbe ingestion than the ones that are cooked before eaten. This research is intended to be the foundation upon which subsequent studies will be built.

I will share my finding with the stakeholders so as to give them an insight into the matter

## HYPOTHESIS, AIMS, & OBJECTIVES

**NULL HYPOTHESIS (H0):** Washing fruits and vegetables available in the local markets in Yola-Jimeta has no effect on microbial decontamination.

**RESEARCH HYPOTHESIS (H1):** Washing fruits and vegetables available in the local markets in Yola-Jimeta reduces microbial decontamination.

**NULL HYPOTHESIS 2 (H0):** There will not be harmful microbes on fruits and vegetables available in Yola-Jimeta markets.

**RESEARCH HYPOTHESIS 2 (H2):** There will be harmful microbes on fruits and vegetables available in Yola-Jimeta markets.

## AIMS

* To determine if micro-organisms found on fruits and vegetables sold in Yola- Jimeta markets, northeastern Nigeria, are potentially harmful to people
* To compare the efficacy of simple washing practices in microbial decontamination of fruits and vegetables sold in Yola-Jimeta markets, northeastern Nigeria

## OBJECTIVES

* To isolate microbes found on fresh fruits and vegetables
* To identify these microbes to the species level
* To wash the fruits and vegetables with water to determine the impact on microbial decontamination
* To wash the fruits and vegetables with water and vinegar to determine the impact on microbial decontamination
* To determine how microbial loads found on fruits and vegetables compared to World Health Organization standards

## CHAPTER 2 METHODS

*Study area and sampling*

This study was conducted in Yola and Jimeta metropolitan areas of Adamawa State, northeastern Nigeria.

I collected my samples from both Yola and Jimeta markets which are the two major markets in these areas. I bought my samples in the same way people buy fruits and vegetable for personal usage, in other words, I used targeted sampling. I also ensured that the samples I got were fresh.

Four samples each of: carrots, lettuce, cabbage, and tomatoes were collected from the markets using sterilized leather bags in order to avoid cross-contamination. I randomly bought the samples from different vendors. Half of the samples were bought from Yola market while the other half was bought from Jimeta market. I repeated this sample collection three times on separate days.



**Figure 6**. The map of Nigeria showing Adamawa state, credit: Nigerian Times

*Data collection and analysis*

I divided the samples I collected for each fruit or vegetable into three. I washed the first portion with water and tested the water for microbes; while I washed the second portion with water twice and tested the water obtained from the second wash. For the third portion, I washed with vinegar before rinsing with water and testing for microbes. A Wilcoxon Signed Rank Test, which is a non-parametric test, was used to test for significant differences among the samples before washing, after washing with water, after washing with vinegar and rinsing with water. I used a non-parametric test because the mean does not perfectly describe the center of my distribution due to small sample size and many outliers that cannot be disregarded.

*Materials*

Pipettes, Petri dishes, samples of different fruits and vegetables, autoclave, distilled water, weighing boat, L-shaped spreader, beakers, vials and caps, and loops.

Selective media used; Salmonella-Shigella (SS) Agar, 2.5% basic Fuchsine, Eosin Methylene Blue (EMB) Agar, MacConkey Agar, and Endo Agar

*Lab methods Preparation of Media*

*Salmonella-Shigella Agar for E. coli and S. Typhi identification:*

28.5g of the media was suspended in 0.5 liters (500mL) of distilled water and mixed well. The mixture was then heated with frequent agitation and boiled for 1 minute. No autoclave was conducted. It was then poured into Petri dishes and allowed to solidify. All the samples were then spread across the plates and put in an incubator at 370C for 24 hours.

*Eosin Methylene Blue (EMB) Agar for E. coli and S. Typhi identification:*

18.75g of the medium solute was suspended in 0.5 liters (500mL) of distilled water and boiled until it dissolved completely. The medium was then autoclaved at 1210C for 15 minutes. The medium was then allowed to cool to 600C and then poured onto plates and allowed to solidify. All the samples were then spread across the plates and put in an incubator at 370C for 24 hours.

*MacConkey Agar for E. coli and S. Typhi identification*

26g of the solute medium was suspended in 0.5 liters (500mL) of distilled water and boiled with frequent agitation for 1 minute until it completely dissolved. The medium was then autoclaved at 1210C for 15 minutes. The medium was then poured into

sterile Petri dishes and allowed to cool. All the samples were then spread across the plates and put in an incubator at 370C for 24 hours.

*Endo Agar for E.coli identification*

18g of the medium solute was suspended in 0.5 liters (500mL) of distilled water. 8ml of 2.5% Fuchsine solution was then added and boiled until it dissolved completely.

The medium was then autoclaved at 1210C for 15 minutes. The medium was then poured into sterile Petri dishes and allowed to cool. I then spread all the samples across the plates and put in an incubator at 370C for 24 hours.

*Serial dilution and colony counting*

A 5-fold serial dilution was conducted in order to know the amount of colony forming units (CFUs) in the samples. An electric colony counter was used to count the microbial load for each from a selected dilution factor, usually the dilution factor that showed the highest number of growth was selected.

*Bacterial Identification*

Different microbes grow on different selective media and have different colorations (Table 2), these colors were used to identify the microbes on the different plates.

**Table 1.**Different selective and differential media used for the identification of microbes and their respective color indication

|  |  |  |
| --- | --- | --- |
| **Selective media** | **Organisms** | **Color indicating the presence of a microbe** |
| Salmonella-Shigella Agar | *E. coli* and*Salmonella* sp. | Pink color for *E. coli* and pale brown for *S. Typhi* |
| Eosin Methylene Blue Agar | *E. coli* and*Salmonella* sp. | Blue-black or green metallic sheen for *E. coli* and pale purple coloration for *S. Typhi* |
| MacConkey Agar | E. coli and S. Typhi | Pink to red for *E. coli* and light brown for *S. Typhi* |
| Endo agar | E. coli | Red colonies, red medium, and golden sheen |

### Biochemical tests

A standard IMViC tests series was conducted in order to differentiate the different species of Enterobacteriaceae chosen from different plates previously cultured. For this purpose, fresh cultures were subcultured from the selected plates and incubated for 24 hours after which I inoculated each plate culture into the various test tubes containing the indole medium, MR-VP broths, and citrate agar. The test tubes were then incubated at 370C for 24 hours after which the appropriate change in the citrate agar from green to blue was observed and also added Kovac’s reagent for the indole broth. Methyl red was added for the MR test and VP-A and VP-B were for the VP tests and any change in color was observed and recorded

## CHAPTER 3 RESULTS

### Microbial loads in individual samples

In this study, I found microbial growth in all the samples tested before and after treatment with water and with vinegar (Table 3).

**Table 2.**The number of microbial counts found in the samples after each treatment

|  |  |  |  |
| --- | --- | --- | --- |
| Samples | Pre-washing microbial loads (cfu/ml) | Post-washing microbial loads**(water)** (cfu/ml) | Post-washing microbial loads **(vinegar water)** (cfu/ml) |
| Lettuce 1 | 8.60E+06 | 6.06E+05 | 3.66E+02 |
| Lettuce 2 | 6.40E+04 | 8.60E+05 | 1.88E+02 |
| Lettuce 3 | 8.20E+05 | 9.20E+06 | 2.00E+01 |
| Lettuce 4 | 8.20E+05 | 6.70E+06 | 2.60E+01 |
| Carrot 1 | 6.66E+04 | 5.50E+05 | 1.70E+03 |
| Carrot 2 | 8.36E+04 | 8.30E+05 | 9.80E+02 |
| Carrot 3 | 7.40E+04 | 5.00E+04 | 0 |
| Carrot 4 | 9.00E+04 | 6.30E+05 | 2.00E+01 |
| Cabbage 1 | 6.00E+04 | 1.03E+04 | 5.36E+03 |
| Cabbage 2 | 5.74E+04 | 9.10E+03 | 6.84E+03 |
| Cabbage 3 | 8.14E+04 | 2.00E+03 | 0 |
| Cabbage 4 | 1.12E+05 | 6.40E+03 | 0 |
| Tomato 1 | 4.80E+04 | 5.70E+03 | 6.00E+03 |
| Tomato 2 | 4.00E+04 | 8.50E+03 | 5.90E+03 |
| Tomato 3 | 5.20E+03 | 8.50E+03 | 1.12E+03 |
| Tomato 4 | 4.10E+04 | 1.02E+05 | 0 |

However, there was a less microbial load when after washing with vinegar water than before the wash. Furthermore, only one lettuce sample showed microbial

growth after soaking the samples in vinegar water for five minutes but the colonies were not distinct to be counted and thus the colony forming units could not be calculated.

### Bacterial Identification

Different microbes, most which were harmful, have been identified from most of the samples although some samples have higher microbial counts than others (Tables 3, 4 and 5). Some black growth on SS agar suspected to be salmonella was identified. Those black spots were obtained from some carrot samples.

**Table 3**. IMViC test results for some selected samples of carrot

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| sample | Indole test | MR test | VP test | Citrate test | Organism |
| Carrot 1 | + | + | - | + | Salmonella spp. |
| Carrot 2 | - | + | - | + | *Citrobacter Freundii* |
| Carrot 3 | - | - | - | + | *Bacillus sphaericus* |
| Carrot 4 | + | + | - | - | *S. Typhi or S. Paratyphoid* |

**Table 4.** IMViC and phenylalanine deaminase (PDA) tests results

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| sample | Indole test | PDA test | MR test | VP test | Citrate test | Organism |
| Carrot 1 | -(v) | - | + | - | - | *E. coli* |
| Carrot 2 | -(v) | - | + | - | - | *E. coli* |
| Carrot 3 | - | - | + | - | + | *Citrobacter Freundii* |
| Carrot 4 | + | + | + | - | +(v) | *Proteus vulgaris* |
| Carrot 1b | + | - | - | + | + | Cannot be identified |
| Lettuce (vinegar) | - | - | -(v) | + | + | *Serratia marcescens**\** |
| Lettuce | + | + | + | - | +(v) | *Proteus vulgaris\** |
| Tomato 1 | + | + | + | - | +(v) | *Proteus vulgaris\** |
| Tomato 2 | - | - | - | + | - | Cannot be identified |
| Carrot 2b | +(v) | + | + | - | - | *E. coli\** |

(V)= variable, \* Further test needed

### Statistical analysis

A Wilcoxon Signed Rank Test showed that washing with water did not elicit a statistically significant change in lowering microbial counts in the fruits and vegetables tasted (Z = -0.827, P = 0.408) and therefore, contrary to what I expected. Another unexpected observation was that the mean microbial count for pre-washing is higher than that of post-washing treatment. However, the Wilcoxon Signed Rank Test showed that treatment with Vinegar water did elicit a statistically significant change in lowering the microbial counts of the fruits and vegetables tasted (Z = - 3.516, P = 0.00). The mean, range, and the standard deviations of microbial loads for

the three treatment showed more variation in the Vinegar treatment than in the water treatment (Table 6).

**Table 5.** Means, Standard deviations, and Ranges of microbial loads for all treatments

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatments | N | Mean | Std. Deviation | Minimum | Maximum |
| Microbial pre-washing | 16 | 6.9 x 105 | 2.1 x 106 | 5.2 x 103 | 8.6 x 106 |
| Microbial loads post-washing with water | 16 | 1.2 x 106 | 2.6 x 106 | 2.0 103 | 9.2 x 106 |
| Microbial loads post-washing with vinegar water | 16 | 1.7 x 103 | 2.5 x 103 | 0 | 6.8 x 103 |

## CHAPTER 4 DISCUSSION

*Microbial load concentration observed compared with WHO standard for irrigation water*

The guidelines for the microbial quality of irrigation water differ from one country to another and between surface waters, treated wastewater, and groundwater. Fruits and vegetables that are most likely to be eaten uncooked, like the ones used in this study, have a higher microbial quality standard than those eaten cooked. Of all the types of irrigation waters, wastewater irrigation quality is usually the strictest due to the likely high number of human pathogens it might contain (Steele & Odumeru, 2004).

The WHO standard for coliforms in drinking water, with which we are making comparison in this study, is zero, in other words, there is zero tolerance for coliforms in drinking water. Nonetheless, some public health experts argue that the mere presence of total coliforms in drinking water is not an indicator of health threat (Edberg et al., 2000). Although the non-pathogenic *E.coli* does not pose a threat to humans and animals, some strains with virulence factors pose serious health threats to humans and animals (Warriner et al., 2009).

The United States has zero tolerance for fecal coliforms in wastewater used for irrigating crops eaten uncooked and 200/ml fecal coliforms for crops eaten processed (Blumenthal, Peasey, Ruiz-Palacios, & Mara, 2000). California however, has a tolerance of 2.2 total coliforms and zero fecal coliforms for wastewater used for irrigation. These standard are similar to the standards for drinking water as set by the US EPA (Anonymous, 2002). However, the *Revised Guidelines for the Safe Use of*

*Wastewater and Excreta in Agriculture and Aquaculture*, as outlined by the WHO, stipulated up to 1000 fecal coliforms per 100ml of water for fruits and vegetables eaten uncooked while it allows up to 100,000 fecal coliforms for fruits and vegetables eaten after processing (Blumenthal, Mara, Peasey, Ruiz-Palacios, & Stott, 2000). This guideline exceeds by far, the microbial load obtained in this study before washing the samples (6.9 x 105Cfu/ml). Nonetheless, this should be a major concern if we compare what we got and the guidelines of the US state of California mentioned earlier.

The guideline for the microbial quality of surface water is, however, lenient compared to those of wastewater because it is assumed that it contains less human pathogens, although it is hard to protect it from being contaminated by non-point sources such as birds and animals (Steele & Odumeru, 2004).

*Potential source of contamination*

Fruits and vegetables can get contaminated with microbes at several points from farm to the table, however, contamination can be greatly reduced if arrested at certain points along this path. Contamination is most likely to occur in the farm, during initial processing, and in the kitchen. Contamination in the farm mostly come from irrigation wile contamination during initial processing and final processing in the kitchen mostly occur due to cross-contamination from various sources (Lynch)

*Salmonella typhi, E. coli* and other potentially harmful microbes discovered in this study also indicate the need for extra care on the fruits and vegetables we consume. Possible sources of such organisms include untreated human waste water which can

contain a significant amount of pathogenic microbes. According to an estimate, a liter of untreated sewage water from developing countries contains 7,000 *Salmonella spp.,* 7,000 *Shigella spp.,* and a lot of other pathogenic microbes (Steele & Odumeru, 2004). However, untreated water is not the only culprit here, surveys conducted on groundwater in Ontario Canada indicated that 31% of the water samples contain pathogenic microbes with a record well above the recommended 10 total coliforms per 100ml for drinking water, while 20% contain fecal coliforms, 17.6% contains *E. coli*, and the remaining 14.8% contains fecal streptococci (Goss, Barry, & Rudolph, 1993). Similar studies in Nebraska, United States (Exner & Spalding, 1985) and Saudi Arabia (Al-Sulaiman, 2016) showed almost the same results.

The fact that farmers in this region use all kinds of water for irrigation explains why

*E.coli* was detected on some of the samples analyzed in this study. The presence of

*E. coli* always connotes a public health concern (Edberg et al., 2000). To this end, the detection of *E. coli* in some of the samples tested should be a source of public health concern.

If the above result is obtained from groundwater, then surface waters such as lakes, rivers, and streams would have a higher tendency of being polluted with pathogenic microorganisms (Steele & Odumeru, 2004). In the US state of Virginia, the presence of cattle was found to be the main source of fecal contamination of stream water and 94% reduction in fecal coliform contamination was observed after installing fences that restrict the cows from accessing surface waters (Hagedorn et al., 1999).

There are many pieces of evidence suggesting that pathogenic microbes present in irrigation water can directly contaminate fruits and vegetables and even cause diseases in humans. Studies found that there were a lot of disease outbreaks in societies that used irrigation water which receives little or no treatment (Steele & Odumeru, 2004). However, treating irrigation water might be difficult in Nigeria because the production of fruits such as tomatoes is mainly practiced near or along rivers where the conditions favor the growth of different types of microbes (Muhammad et al., 2004).

Another way through which the samples tested in this study may have gotten microbes is bacterial soft rots (Wells & Butterfield, 1997).

*Before and after washing with water*

Although a high microbial load was observed in the samples tested in this study, it is worth mentioning that all the samples were fresh and had no sign of spoilage before and after the test. This suggests that mere physical appearance of fruits and vegetables is not enough as a measure of the microbial quality of fruits and vegetables and therefore, the need for disinfection techniques such as the use of vinegar at all times is recommended (Oie et al., 2008).

An unexpected observation in the result indicates that the mean microbial load of the samples before washing with water (6.9 x 105) was slightly higher than the mean microbial load that I obtained after washing the samples with water (2.6 x 106). That observation could have been due to either cross-contamination by mistakenly using

same water twice during washing the samples or using the same beaker for I used to collect the water from the unwashed samples to wash the samples

*Vinegar wash*

The presence of microbes in fruits and vegetables is an indication of the quality of the sanitary measures they have undergone from farm to consumer and in some cases, it might be just a reflection of the microbes living in the farms from which the samples were harvested (Beuchat, 2002). The results obtained in this research indicated a heavy reduction in the microbial loads of the samples after the use of vinegar. The mean microbial load was found to have been greatly reduced from 6.9 x 105 cfu/ml before washing to 1.7 x 103 cfu/ml after washing with vinegar and rinsing with water.

The results indicated that vinegar water is effective as in reducing the microbial loads of fruits and vegetables. Only one sample showed microbial growth after soaking in vinegar for five minutes hence, there were no numerical values to report. This indicates that increase in exposure time was significant in reducing microbial loads from the samples tested in this study. The efficacy of vinegar is further supported by the fact that most bacteria survive in alkaline solutions while vinegar (a solution of 5% acetic acid) reduces the pH of a solution (Oie et al., 2008).

*Public health implications of the microbes identified*

Some potentially harmful microbes such as *S. typhi, E. coli,* and *Bacillus sphaericus* have been identified in this study. Such observation on fruits and vegetables is not unusual in Nigeria. A study conducted in Sango Ota, Ogun State, southwestern

Nigeria, indicated a high level of microbial presence in fruits and vegetables (Eni et al., 2010).

Salmonella is known as the main cause of salmonellosis, a type of illness that mainly occur after the ingestion of contaminated water, fruits or vegetables. It can also contracted through person-person or person-food contacts. The main symptoms include diarrhea, vomiting, fever, and abdominal cramps. If not treated salmonellosis could be fatal.

*E. coli* is also an indicator organism and has many strains. The identity of the strains identified in this research cannot be ascertained. However, *E. coli* has generally been used as an indicator organism since the late 1970s due to its specific nature and abundance in human and animal feces in which it is estimated to measure approximately 109g-1. It can also be found in sewage, natural waters, and treated effluents (Edberg, Rice, Karlin, & Allen, 2000). Symptoms of E. coli ingestion includes diarrhea which is often bloody, vomiting, abdominal cramps, and no fever follows usually.

*Bacillus sphaericus* is usually found in the soil and this explains why it can easily contaminate fruits and vegetables. It symptoms typically starts between 1 and 24 hours and include nausea, vomiting, and diarrhea.

The identification of these harmful microorganisms will, therefore, make us reject our second null hypothesis and believe with a degree of certainty that fruits and vegetables available in Yola-Jimeta markets can contain harmful microbes.

*Significance of the findings of this study*

The result indicated that vinegar water, which is very cheap and sells at around N300

per bottle, is effective in reducing the microbial load in the fruits and vegetable samples tested. This finding is important due to the fact that the Yola/Jimeta community inhabitants comprises of mostly low-income earners, will go a long way to reduce microbial contamination and fruits and vegetables associated outbreaks.

The findings of this study will also help in reducing the microbial contamination of many fruits and vegetables exported from Africa to other continents. 125 (41.3%) cases of microbial contamination from food items exported from African countries to European countries between 1996 and 1997 have been reported (Unnevehr, 2000).

Generally, fruits and vegetables tested in this study contained microbes and this calls for a redress from the relevant authorities, in this case, NAFDAC. Yola and Jimeta are perfect models of many cities in northern Nigeria. Fruits and vegetable consumers, vendors, and farmers from these parts of the country need to stand up to their responsibilities of providing the populace with healthy produce.

Consumers should also not rely on the safety assurance given to them by vendors and should go the extra mile in making sure that the fruits and vegetables they consume are of the safest microbial quality. Every household should have vinegar water for their routine in order to ensure the safety of fruits and vegetables that are eaten raw

Vendors can also play role in ensuring the safety of fruits and vegetables by adhering to maximum handling standards and by disinfecting their produce with any

of the effective disinfection techniques available. Covering the produce from dust is also another way of reducing the microbial contamination of fruits and vegetables.

The farmers can play their role by making sure they use irrigation water of a high microbial quality that reflects the world standard. Such efforts, if applied, would increase the microbial quality of fruits and vegetables and decrease health issues related to microbial contamination from fruits and vegetables.

## CHAPTER 5 CONCLUSION

This study finds that most of the fruits and vegetables sold in Yola-Jimeta markets are contaminated with microbes, some of which are harmful to humans. The study has also established the efficacy of the use of vinegar water as an effective disinfection technique for microbes on fruits and vegetables. This study highlights the dangers associated with the consumption of fruits and vegetables obtained from markets without subjecting them to further disinfection methods such as the use of vinegar water. This study will serve as a pioneer study in this field in Yola-Jimeta metropolitan areas and by extension Adamawa state. This should serve as an eye- opener to the relevant authorities tasked with the job of ensuring and safeguarding the safety of fruits and vegetables in the state in particular and the nation in general. The aims of the study were to find out whether or not the fruits and vegetables sold in Yola-Jimeta markets are contaminated by microbes and whether or not those microbes are harmful to humans. The results obtained indicated that the fruits and vegetables sold in those markets are contaminated by microbes and that some of the microbes are potentially pathogenic to humans.

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