KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

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DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

Effectiveness of UV Sanitization of Vegetables using Polymerase Chain Reaction and Microbial Culture Techniques

By

Abena Adutwumwaah

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By

Abena Adutwumwaah (BSc. Molecular Biology and Biotechnology)

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DECLARATION

I declare that I have wholly undertaken the study reported herein under the supervision of Dr. Peter Twumasi and Dr F. C. Mills-Robertson, and that except portions where references have been duly cited, this thesis is the outcome of my research.

Signature: …… Date: ……

Abena Adutwumwaah

(Student)

Signature: …… Date: …….

Dr. Peter Twumasi

(Principal Supervisor)

Signature: …… Date: ……

Dr. F. C. Mills Robertson

(Co-supervisor)

Signature: …… Date: ……

Dr. A. Y. Tetteh

(Head of Department)

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This thesis is dedicated to my father, Mr. Joseph Akomeah Nyamekye for being my inspiration and fortress. Daddy, I pray that God sustains you with a productive and a healthy long life to see me aspire to the greatest level in life which has always been your wish for me.

ABSTRACT

Vegetable related food poisoning is a very serious issue in Ghana and has been linked to a number of diverse health effects such as cholera and deaths. This study focused on assessing the effectiveness of ultraviolet (UV) irradiation to sanitize three vegetables (carrot, cabbage and lettuce) for consumption. Microbial cultures were performed on raw non-sanitized carrots, cabbages, and lettuce to enumerate bacterial colonies, and yeasts and moulds. Biochemical tests were carried out using various media to quantify Gram negative bacteria and enteric pathogens present on raw or untreated vegetables. Also selective sub culturing was carried out to determine the presence of *Salmonella* spp. and *Escherichia coli*. The three vegetables were subjected to UV irradiation at different times of 1 hr, 3 hrs, 6 hrs and 9 hrs. The microbial loads of vegetables exposed to the different times of UV irradiation were assessed using microbial culturing for bacteria, yeasts and moulds. Vinegar as a control was used to sanitize the raw vegetables and microbial analysis carried out. Polymerase Chain Reaction (PCR) and gel electrophoresis were used to confirm the effectiveness of UV irradiation. USP gene of size 615 bp specific for *E. coli O157: H7* was amplified by PCR using primer sequences of *usp-F-5' CGGCTCTTACATCGGTGCGTTG-3', and R-5'-GACATATCCAGCCAGCGAGTTC-3'*. The results obtained revealed that the untreated raw vegetable samples recorded high microbial loads and tested positive for all microbial assays. The vinegar treated vegetables (control) showed significant reduction in microbes as compared to the untreated raw vegetables. UV irradiation was able to eliminate residual microbes on the three vegetables giving a positive sanitizing effect. It reduced a large number of microbes found on these vegetables than the sanitizing effect of vinegar, though statistically, there was no difference between the sanitizing effects both had on the vegetables. Microbial counts reduced with increase in periods of UV exposure, and this was seen in lanes with bands of *E. coli* representing untreated raw vegetables and vegetables that have been exposed to 1 hr, 3 hrs and 6 hrs of UV irradiation while 9 hrs of UV exposure had no counts of bacterial colonies as well as yeasts and moulds. UV is thus an effective and convenient approach to reducing microbes on vegetables in various markets in Ghana. It also had no suspected bands of *E. coli*. Microbial culture techniques and PCR proved to be effective measures for monitoring the efficiency of UV as a vegetable sanitizing agent.

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CHAPTER ONE

1.0. INTRODUCTION

Today vegetables are important and vital part of the Ghanaian diet. Fruits and vegetables play an important role in human nutrition and health, particularly as sources of vitamin C, thiamine, niacin, pyridoxine, folic acid, minerals and dietary fibre (Wargovich, 2000). It is also known that fruits and vegetables in the diet supply 16% of magnesium, 19% of iron and 9% of the calories (Liu, 2003). Other vital nutrients supplied by fruits and vegetables include riboflavin, zinc, calcium, potassium and phosphorus. Experts have recommended a daily intake of at least 400g of fruits and vegetables (World Health Report, 2002). Several reports have also shown that adequate intake of fruits and vegetables form an important part of a healthy diet and low fruit and vegetable intake constitute a risk factor for chronic diseases such as cancer, coronary heart disease (CHD), stroke and cataract formation (Van Duyn *et al.*, 2000). Scientific evidence indicates that frequent consumption of fruits and vegetables can prevent oesophageal, stomach, pancreatic, bladder and cervical cancers and that a diet high in fruits and vegetables could prevent 20% of most types of cancer (Crawford *et al*., 1994). A study showed significant reduction in the risk of stroke development among those with the highest intake of fruits and vegetables (He et al., 2006). Also, increasing an individual's fruit and vegetable consumption by 600 grams per day was shown to reduce the global burden of stroke by 19% and decrease the risk of CHD by 31% (Lock *et al*., 2005).

Phytochemical antioxidants function to modify the metabolic activation and detoxification of carcinogens, or even influence processes that alter the course of the tumor cells (Wargovich, 2000). Although antioxidant capacity varies greatly among fruits and vegetables, it is necessary

to consume variety of commodities rather than limiting consumption to a few with the highest antioxidant capacity (Wargovich, 2000). In some countries, consumers are encouraged to eat up to 10 servings of fruits and vegetables per day (USDA, 2000).

Tomato and its products have been linked to reduced carcinogenesis, particularly prostate cancer due to the presence of lycopene, which gives red tomatoes their color (Wertz *et al.*, 2004). Tomatoes also contain a wide array of other beneficial nutrients and antioxidants, including alpha-lipoid acid which aid in blood glucose control (Alpha-Lipoic acid Nutrition 411) and choline, an important nutrient found in tomatoes that helps with sleep, muscle movement and learning, maintaining the structure of cellular membranes, aids in the transmission of nerve impulses, assists in the absorption of fat and reduces chronic inflammation (Donaldson, 2010). Green, leafy vegetables provide healthy doses of vitamin K essential for proper blood clotting, bone mineralization and cell growth (Joshipura *et al.*, 1999).

In Ghana, vegetables are usually bought in local markets in their fresh state or in the form of salads which are purchased from fast-food vendors and restaurants. Most of these vegetables are highly contaminated with pathogenic microbes in quantities that exceed tolerable levels and pose great health hazards to consumers (Amoah *et al.*, 2007). The poor quality of vegetables consumed in Ghana has resulted in serious health implications. Microbial contamination of vegetables is associated with many stages on the supply chain especially post-harvest handling, storage and transport from the farm to the consumer (Awuah *et al.*, 2009). The prevalence of food poisoning caused by the consumption of contaminated raw vegetables even after they have been washed or sanitized has been thoroughly documented (Sapers, 2001). A study carried out on

salad vegetables purchased from food vendors on the street confirmed the presence of pathogenic microbes such as *Escherichia coli O157:H7, Salmonella typhi, Shigella* spp. and *Stapylococcus aureus* in fresh produce (Seow *et al*., 2012). Another survey on even thoroughly washed samples of fresh leafy vegetables, tomatoes, cabbage, and cucumber obtained from local markets in Mumbai, India, revealed a total bacterial count of 10^6-10^7 cfu/g and 10^2-10^5 cfu/g range (Gomez *et al*., 2003). . In the same study, Green leafy vegetables were observed as the samples with the greatest number of contaminants (Gomez *et al*., 2003). The ill effects of these contaminants on consumers range from common symptoms of nausea, vomiting and diarrhoea to lifelong consequences of kidney defects, hypertension and even death (Blackburn and McClure, 2002).

Lately, concerns have been raised concerning the quality of vegetables that are sold on the Ghanaian market (www.ghanaweb.com). Newman (2005) reported high levels of faecal coliforms on vegetables such as tomatoes and lettuce grown in Accra. Most irrigation water used in urban farming for the production of vegetables in Ghana is contaminated with microorganisms (Barker *et al*., 2014). This practice poses health risks to farmers and consumers. The International Water Management Institute (IWMI) estimates that in and around the city of Kumasi alone, farmers use polluted waste water sources on about 12,000 hectares of land more than twice the area covered by Ghana's formal irrigation schemes (Sengupta *et al*., 2012). The lack of a comprehensive wastewater management system coupled with increasing demands for food and water for fast-growing urban population has compelled farmers to use wastewater as a source of irrigation (Sengupta *et al*., 2012).

Despite the nutritional benefits of vegetables, these food substances have potential to cause food poisoning and even death due to poor handling resulting in contamination with microbes from contaminated animal manure, domestic and industrial wastewater used for irrigation, pesticides and poor post-harvest handling.

For many years, the presence of numerous genera of spoilage bacteria, yeasts and moulds, and occasional pathogens on fresh agricultural produce including vegetables have been recognized (Sharpe *et al*., 2000). Several outbreaks of human gastroenteritis have been linked to the consumption of contaminated fresh vegetables (Olive, 1989). Salads containing raw vegetables have been identified as vehicle of traveler's diarrhoea, an illness sometimes experienced by visitors to developing countries caused by enterotoxigenic *Escherichia coli* (Olive, 1989). Outbreaks of salmonellosis in humans have been attributed to consumption of contaminated tomatoes, mustard cress, bean sprouts, cantaloupe, and watermelon (Centers for Disease Control and Prevention, 2004). An onion-associated outbreak of *Shigella flexneri gastroenteritis* was reported in the United States (Center for Food Safety and Quality Enhancement, 1996). Outbreaks of human *Listeriosis* have been epidemiologically linked to the consumption of fresh cabbage and lettuce (De Valk *et al*., 2005). Gastrointestinal illness caused by the consumption of raw vegetable seed sprouts contaminated by *Bacillus cereus* has been documented (Beuchat, 1996).

The European Union in 2007 found *Salmonella* in about 0.3% of produce-related samples tested (Westrell *et al*., 2009). Large investigations on prevalence of pathogenic bacteria in fruits and

vegetable were conducted in the UK, Ireland, Germany and the Netherlands in 2007 (Westrell *et al*, 2009). The proportion of produce samples that yielded *Salmonella* in these studies ranged from 0.1% to 2.3%. Produce-associated outbreaks of *E. coli 0157* infections were linked to the consumption of leafy green vegetables (Ackers *et al*., 1998; Hilborn *et al*., 1999).

An outbreak associated with consumption of pre-packaged spinach occurred across 26 states in the USA, resulting in 183 confirmed infections and three (3) deaths in September, 2006 (Centers for Disease Control and Prevention, 2006). The largest *E. coli 0157* outbreak to date, in 1996 centered in Sakai City, Osaka, Japan, and was traced to consumption of white radish sprouts (Michino *et al*., 1999).

1.1. PROBLEM STATEMENT

Many Ghanaians enjoy the convenience of roadside market vegetable vendors. However, a lots of the produce for sale by these vendors are grown through the use of wastewater irrigation, a practice that presents major health risks for both the consumer and the farmer. An estimation by the International Water Management Institute (IWMI) revealed the use of wastewater from gutters by farmers in Kumasi city as their only source of irrigation. The practice has been of great concern to stakeholders in the health sector (http://www.iwmi.cgiar.org). There have also been cases of crop contamination in Ghana, causing exposure to parasites and bacteria leading to potentially serious health issues. Beyond the farmer, the consumer takes over responsibility for protecting their health, through the use of a more effective sanitizing agent to rid of microbes in vegetables. The existing sanity methods are, however, either ineffective or expensive. Thus, a more effective, less expensive and time saving methods of sanity on vegetables in Ghana in both domestic and industrial settings are required.

1.2. MAIN OBJECTIVE

To assess the effectiveness of UV irradiation as a method of vegetable decontamination for commercial application in the Ghanaian horticultural industry.

1.3. SPECIFIC OBJECTIVES

1. To determine the optimum UV radiation exposure time for decontamination of three vegetables- cabbage, lettuce, and carrot.
2. To determine microbial load and presence of *E. coli* and *Salmonella* on both raw and UV treated vegetable samples using microbial culture and PCR techniques.

1.4. JUSTIFICATION

Most food borne illnesses associated with fresh fruits and vegetables in the Ghana have been mainly due to contamination of raw product on the farm and from food handlers in processing facilities or restaurants (Amoah *et al.*, 2007). Moreover, fresh-cut vegetable products are prepared from raw agricultural produce. These produce are in contact with the soil, they are often eaten raw, and during processing, there is no effective sanitization step to ensure microbiological safety, which has raised particular concerns of food safety. In the USA, ultraviolet light has been approved for use as a disinfectant for surface treatment of foods (US-FDA, 2002). Irradiation with UV-C light in the range of 240–260 nm may be an effective germicidal treatment than either chlorine or ozone (Muraca *et al.*, 1987). This practice is, however, non-existent in the Ghanaian Horticultural industry.

Ultraviolet (UV) radiation treatment is an alternative to chemical disinfection treatment for water,

fruits and vegetables because of its advantage of avoiding harmful by-products typical of chemical treatments (Liu *et al*., 2002)]. The major driving force to include UV treatment is the recent discovery of its effectiveness against protozoans, especially *Cryptosporidium* (Hayes et al., 2006). The US Environmental Protection Agency (EPA) established the long term enhanced surface water treatment rule that includes requirements for UV disinfection (http: //www.epa.gov/safewater/disinfection/lt2). This research therefore seeks to sanitize cabbage, lettuce, and carrots using UV radiation. The expected outcome is significant reduction in the microbial load on vegetable related microbial infections. Commercial application of such findings when successful will provide alternative method for the Ghanaian Horticultural industry to reduce incidence of vegetables and fruit related illness.

CHAPTER TWO

LITERATURE REVIEW

2.0. BACKGROUND

Plant parts which are usually eaten with meat, fish or savory dish, are termed vegetables (Duckworth, 1966). According to Webster's Dictionary, vegetables are the edible parts of a plant that are used for human food and usually eaten cooked or raw as the main part of a meal rather than as a dessert. Green leafy vegetables are rich sources of vitamins including β-carotene (a precursor of vitamin A), ascorbic acid, riboflavin and folic acid. They are also rich in minerals such as iron, calcium and phosphorous. Green leafy vegetables are also recognized for their characteristic colour, flavour and therapeutic value (Mensah *et al*., 2008). Some of the commonly consumed leafy vegetables are amaranth, spinach, fenugreek and coriander. The nutritive value of these vegetables has been reported in the food composition tables (Gopalan *et al*., 1996).

Vegetables in their raw state contain very high water and fiber contents and are low in fat. For this reason, vegetables consist of low calories per weight of food intake thereby contributing to body weight management. They therefore serve as effective regimens for losing excess body weight (Lyon and Kacinik, 2012). Most people prefer consuming vegetables in their raw state because of the negative effects cooking has on nutritional content of vegetables, denaturation of heat-labile enzymes which aid in digestion and disruption in sensory attributes such as taste, texture and appearance (Jung *et al*., 2013).

In addition, fresh vegetables contain very large quantities of fiber and water unlike cooked ones. These dietary fibers aid in digestion and therefore contribute to the maintenance of body health. Phytochemicals obtained from fruits, vegetables, legumes, grains and spices are usually polyphenolic in nature and include flavonoids which consist of flavonols and isoflavones (Gropper *et al*., 2005). Flavonols are commonly found in onions and tomatoes and normally carries out their antioxidant function by donating a hydrogen group from its phenolic hydroxyl group to free radicals and render them inactive (Gropper *et al*., 2005). Although phytochemicals are of no nutritional value, they influence metabolic processes and therefore protect the body from heart diseases, stroke and other forms of cancer (Williams, 2002). Phytochemicals have been proven to reduce the risk of heart diseases, lower cholesterol and inhibit tumor formation and proliferation (Wardlow and Insel, 1996).

2.1. SOURCES OF MICROBIAL CONTAMINATIONS OF VEGETABLES

Raw vegetables are commonly contaminated with microorganisms. It is therefore very important that people get well informed about the sites and mode of attachment of the pathogens in fresh produce and the adverse effects they cause in order to appreciate the need for an effective decontamination technique. Identified sources of these microbial hazards on the farm include raw manure, contaminated water or sewage used for irrigation, wild animals, birds, and dirty farming equipment (Morris *et a*l., 1994).

During harvest, farm tools and equipment used in packaging can contaminate the vegetables. The use of contaminated water to wash farm produce can also pose health risk to consumers (Edmonds and Hawke, 2004). Transportation and distribution of vegetables contribute to the

incidence of microbial contamination (Nath *et al*., 1989). Vegetable growers and packers who keep animals are sources of vegetable or farm produce contamination (Mukherjee *et al*, 2004). Domestic animals such as chickens, dogs, and horses also contaminate crop with faecal droppings as they walk through these farms (Morris *et al*., 1994). Non-farm animals such as bats and wild birds can serve as reservoirs for microbial pathogens that contaminate vegetables (Morris, Pfeiffer and Jackson, 1994). Wild animals who difficult to control are hazardous to fruit and vegetable growers (Morris, Pfeiffer and Jackson, 1994). Droppings from birds, deer, and other wild animals present a contaminating risk (Nath *et al*., 1989). The presence and isolation of zoonotic pathogens in manure and on the surface of fresh produce have also been published (Mukherjee *et al*., 2004).

Water is used in the growing and harvesting of fresh fruit and vegetables. Irrigation water and water used for application of plant protection products including fertilizer and frost protection introduce pathogens to vegetables on the farm (Amoah, 2008). Employees harvesting by hand can contaminate product bound for consumption if proper hygienic methods are not observed. Workers who work with open wounds without using a plaster or bandage to protect it can contaminate the fresh vegetables (Mukherjee *et al*., 2004). Poor sanitation in the pack house could lead to the formation of biofilms that cause significant problems in the environment and during the treatment of infections. Biofilms are layers of bacteria that attach to surfaces like stainless steel and plastic, and also attach to each other with the help of polymeric materials. The biofilms trap other bacteria, debris and nutrients (Davey *et al*., 2003). Non-pathogenic and pathogenic bacteria can form biofilms. Organisms in the film tend to be resistant to vegetable sanitizers including heat treatment (Robbins *et al*., 2005).

Vehicles and containers used to transport fresh produce are potential sources of microbial contamination. The temperature of transport can also determine the potential for growth of pathogens. Bacterial counts of up to 10^6–10^9 colony forming units per gram (cfu/g) of raw vegetables after harvest and ready-to-use sliced salads are usually contaminated by microorganisms (Jaques and Morris, 1995). Bacterial pathogens have been detected in pre-packaged salads (Lin *et al*., 1996) and lettuce (Park and Sanders, 1992). These dangers can be classified as spore forming bacteria, capable of withstanding high heat and can also attach to vegetables grown near the soil. Others are non-spore forming bacteria, viruses and parasites.

Enterotoxigenic and enterohemorrhagic *Escherichia coli (E.coli), Campylobacter jejuni, Listeria monocytogenes, Salmonella, Shigella* spp., *Staphylococcus aureus* and *Vibrio* spp. usually contaminate fresh vegetables (Park *et al*., 2006). *E. coli* is a Gram-negative non-spore forming bacteria which consists of different types such as enterotoxigenic *E. coli* (ETEC), *enteropathogenic E. coli* (EPEC), *enterohaemorrhagic E. coli* (EHEC), *enteroinvasive E. coli* (EIEC) and *verocytotoxigenic E. coli* (VTEC) (Blackburn and McClure, 2002). The most virulent strain is *E. coli 0157* (Wardlow and Insel, 1996). *E. coli* is known to effectively thrive on the surface of fresh produce as well as on damaged sites of the vegetables such as cuts and crevices (Sapers, 2001). If this microbe escapes decontamination and is consumed, it passes through the stomach to the intestines and infects the gastro intestinal tract (GIT) simultaneously (Blackburn and McClure, 2002). Symptoms of *E. coli* infection include severe diarrhoea and abdominal pains, dysentery as well as bladder and kidney infection (Wardlaw and Insel, 1996).

Listeria monocytogenes, an enteric pathogen which is highly resistant to heat and acidity is likely to resist inactivating by most conventional cleaning agents (Wardlaw and Insel, 1996). The symptoms of this pathogen infection are fever, headache, vomiting within 7 to 30 days after exposure and adverse complications such as meningitis and blood infections in immune-suppressed individuals (Wardlaw and Insel, 1996). *Salmonella* also invades the intestines and releases enterotoxins that infect the GIT and causes acute symptoms of diarrhoea, nausea and vomiting including adverse complications of food poisoning and typhoid (Blackburn and McClure, 2002).

The symptoms of *Salmonella* infection are likely to develop within 5 to 72 hours of invading its host. Etiological studies from 1990 to 2002 identified 187 causative agents associated with vegetable contamination outbreaks in the United States. Among these outbreaks, 102 (55%) were caused by bacteria, 68 (36%) were caused by viruses, and 17 (1%) were caused by parasites (Calvin, 2003). Among the bacterial agents, *Salmonella* accounted for 60% and pathogenic *E. coli* was responsible for 25% (CDC, 2004). In 1996, Japan had the world's largest reported vegetable contamination outbreak with about 11,000 people affected in which 6000 cases were confirmed using culture techniques. Three school children died from this outbreak which was caused by the *E. coli 0157:H7* strain (Ministry of Health and Welfare of Japan, 1997).

2.2. METHODS FOR SANITIZING VEGETABLES

Attempts to sanitize raw vegetables began far back on a house-hold scale where the harvested produce or purchased goods were washed with conventional agents such as water, salt solution and vinegar (Tornuk *et al*., 2011). A research conducted revealed that the use of sterile tap water

is ineffective in reducing *Escherichia coli O157:H7* and *Salmonella typhi* in cut carrots (Tornuk *et al*., 2011). Tap water only washes off debris and soil remnants from the surface of vegetables (Sapers *et al*., 2006). Also common salt solution contains some amount of sodium chloride and has proven to be slightly efficient as it contains 10-100 ppm of chlorine which appreciatively contributes to microbial reduction (Sapers, 2001). Vinegar, which is also a slightly acidic solution, has been proven to be very effective against microbial contaminants (Issa-Zacharia *et al*., 2011). However, the limited efficacy of the household agents at reducing microbes to safety levels led to the invention of commercialized approaches to vegetable safety and quality (Sapers *et al*., 2006).

2.2.1. Thermal Decontaminating Agents

These agents employ heat in killing the microbes that are attached to the surface of fresh produce. These agents include heated water, steam and hot sanitizing solutions (Barth *et al*., 2010). Although the heat is very effective in killing most of the microbes, there is the incidence of some pathogens that are heat resistant, examples: *Campylobacter jejuni* (Bergsma et al., 2007), *Lactobacilli casei, Escherichia coli* and *Salmonella typhimurium* (Van Asset and Zwietering,2006) and therefore would continue to remain active even after heat treatment Also the appearance and taste of heat-treated vegetables differ from raw produce and may not be as crunchy and juicy as consumers would prefer. Most of the nutrients of the vegetables are also not retained when heat is used to kill microbes on fresh vegetables (Jung *et al*., 2013). Therefore, for quality sake, it is more advisable to wash vegetables with cold water despite its ineffectiveness to decontaminate the vegetables completely (Hernandez-Brenes III, 2002). These and many more concerns have led to the consideration of modern, improved technologies that do not require heat.

2.2.2. Non-thermal Decontaminating Agents

These are categorized into chemical and physical sanitizing agents. The chemical agents include chlorine, ozone and peroxyacetic acid while the physical decontaminants include high pressure, ultrasound and modern irradiation techniques as well as ultra violet irradiations (Barth *et al*., 2010).

2.2.3. Chemical Decontaminants

Chlorine is an effective disinfectant against most food borne microorganisms. Several chlorine based compounds are used widely for the decontamination of Minimally Processed Fresh (MPF) vegetables such as cut salad. Hypochloride solutions at pH 6 have been tested on fresh-cut muskmelons stored at 2 ^{0}C and found to significantly reduce the microbial counts (Ayhan *et al*., 1998). Washing with chlorinated water to decontaminate vegetables has been traditionally applied. Chlorine added to washing water for decontamination had led to maximum reductions of about one order of magnitude (Marcy, 1985; Seo and Frank, 1999; Weissinger *et. al*., 2000). Several reports have, however, questioned the efficacy of chlorine (Parish *et al*., 2003), while other studies show that toxic compounds are generated when chlorine reacts with organic matter (Richardson *et. al*., 1998).

There have been cases in which chlorine solutions have been of limited effectiveness or completely ineffective in killing *Listeria monocytogenes* in carrots (Beuchat and Brackett, 1990). Bacteria from the *Mycobacterium avium* Complex (MAC) are human pathogens and, like *Cryptosporidium*, are resistant to disinfection by chlorine (Falkinham, 2003). Low levels of

chlorine may also not be effective against certain bacteria, protozoan cysts, worm eggs, and viruses (Beuchat and Brackett 1990; Keswick *et al*., 1984). Chlorine used at low concentration, may cause taste and odour defects in treated products (Kessel *et al*., 1943). Additionally, chlorination causes the formation of carcinogenic trihalomethane (THM) in food (Brungs, 1973).

Compared with chlorine, ozone (O3) apparently is a superior disinfectant when tested in pure water, buffer, and wastewater (Kessel *et al*., 1943). Ozone has emerged as a promising alternative to chlorine since it is non residual, odourless, and relatively stable at pH 6 to 8. Low doses (0.01-1µL) are reported to inactivate a wide spectrum of plant and human pathogens (Burrows et al., 1999; Kim et al., 1999; Peeters et al., 1989; Xu, 1999; Zagory and Hurst, 1996), however, limited studies have been done to sanitize fresh vegetable with ozone during food processing. Food may contribute organic matter that competes for ozone with the microorganisms being disinfected. Additionally, food may limit the accessibility of ozone to surface contaminants, therefore, inactivation kinetics of ozone varies with the treated commodity (Kessel *et al*., 1943).

2.2.4. Physical decontaminants

High pressure applied at room temperature proves to reduce microbial contamination in vegetables (Farkas and Hoover, 2000). In this non-thermal application, the food product is packaged in a container and loaded into a high pressure chamber filled with hydraulic fluid which transmits the pressure. The common hydraulic fluid used is water. The fluid is subjected to pressure with a pump, and this pressure is transmitted through the package into the food itself. The processed product is then removed and stored or distributed in the conventional manner

(Ahmed and Ramaswamy, 2006). This system involves uniform application and therefore the shape of the vegetable is retained even at extreme pressures (Oey *et al.*, 2008). A disadvantage with this sanitizing agent is that the fresh produce must contain water and must be devoid of internal crevices which allow air. Foods containing entrapped air can easily be crushed when subjected to high pressure treatment (Ahmed and Ramaswamy, 2006).

Ultrasound is a non-thermal treatment which produces ultrasonic wave that inactivates microbes and prolongs the shelf-life of food substances (Chemat and Khan, 2011; Dolatowski *et al.*, 2007). The ultrasound system consists of a generator, transducer and an application system (Ercan and Soysal, 2013). The generator produces electrical or mechanical energy which is in turn converted by the 'transducer into sound energy at ultrasonic frequencies. The application system also contains a coupler device which transfers the ultrasonic vibrations to the sample on which it acts. Adversely, a major limiting factor attributed to the use of ultrasound is that certain enzymes and bacterial spores highly resist ultrasound treatment (Ercan and Soysal, 2013). Although, ultrasound in vegetable decontamination has been described as a very promising tool, it was found out that the use of ultrasound alone is not effective enough and therefore needs to be coupled with either heat or pressure to attain a favourable decontaminative effect (Piyasena *et al.*, 2003).

2.2.5 Modern Irradiation Technique

Ultraviolet light has been approved for use as a disinfectant for surface treatment of foods (US-FDA, 2002) which involves two distinct modes. These include conventional and hermetic applications. In conventional application, the UV targets the microbes on the surface of the food.

Hermetic applications are, however, in-depth treatment where the UV radiation targets the pathogen itself. UV radiations ranges are in three subdivisions (UV-A, UV-B AND UV-C) which have been use in various applications (Ribeiro and Alvarenga, 2012) as seen in Table 2.1. They are located in the electromagnetic spectrum with a wavelength ranging from 100nm-400nm (Koutchma, 2008).

Table 2.1: Various subdivisions of UV in the electromagnetic spectrum, their wavelengths and their applications

UV SUBDIVISION	SPECTRAL RANGE (nm)	APPLICATION
UV-A	315-500	Applied in skin tanning
UV-B	280-315	Promotes antioxidant effect when used on tomato
UV-C	200-280	Sanitizes pathogens
VACUUM UV	100-200	Absorbed by most molecules

(Courtesy: Ribeiro and Alvarenga, 2012**)**

Adding UV disinfection to existing treatment plants or including it in the construction of new plants can provide additional disinfection credit. A UV dose of 40 mJ c^2 is recognized by the UV industry to provide $4\log_{10}$ inactivation for a wide range of waterborne pathogens. This is thus recommended for providing multi-barrier disinfection (NSF International, 2002). It would be advantageous to know whether this UV dose will provide adequate disinfection for organisms from the *Mycobacterium avium* Complex (MAC). UV-C damages nucleic acids (Farkas, 2001) and this damage is self-repaired within visible light (Zagory and Hurst, 1996). UV-C light can catalyze oxidative changes in certain products that led to rancidity and discoloration (Jay, 1996). The influence of UV-C treatment on fresh-cut watermelon has received little attention. Its application commercially would depend on its ability to sanitize and retard microbial growth

without causing undesirable quality changes such as disruption of taste and sensory attributes in the product.

2.3. MICROBIAL CULTURING TECHNIQUES

A culture medium is defined as a solid or liquid preparation which enhances growth of microorganisms (Prescott *et al*., 2002). The medium must contain all the appropriate nutrients required by the microorganism to grow. The medium is said to mimic the natural environment of the microbes by providing their energy requirements, carbon, nitrogen, phosphorus, sulphur and various minerals to facilitate growth (Prescott *et al*., 2002). These nutritional requirements vary for different species of microbes. Agar is the most common solidifying agent used in media preparation. On a layer of agar in a petri dish, microbes grow to form visible clusters called colonies. Microbes are distinguished based on morphological characteristics shape.

2.4. POLYMERASE CHAIN REACTION

Recent years have seen an explosion in the development and application of molecular tools for identifying microbes and analyzing their activity. The tools that have been developed for identifying microbes and analyzing their activity can be divided into those based on nucleic acids and other macromolecules and approaches directed at analyzing the activity of complete cells (Amann and Ludwig, 2000).

Polymerase chain reaction (PCR) is a modern application of nucleic acid hybridization, a technique commonly used to analyze the extent of sequence homology between single strands of nucleic acids. The technique functions by allowing the single polynucleotide strands to form double-helical segments by means of hydrogen bonding between complementary base pairs in cyclic turns and as a result, the nucleic acid of interest gets amplified. The greater the extent of

homogeneity between the strands, the greater is the extent of formation of double-helical segments. The polynucleotide strands may either consist of single-stranded nucleic or they may be derived from denatured double-stranded nucleic acids. The hybrids that can be formed are either of DNA/DNA, RNA/RNA, or DNA/RNA types. A base on one polymeric strand is bonded to the complementary base on the opposite strand by hydrogen bonding in the order of A-T or A-U (in the case of RNA) and G-C pairing. In modern in-vitro studies, heat is required to unwind dsDNA into two separate DNA strands. The A-T pairs which consist of two hydrogen bonds unwind faster than G-C pairs which consist of three hydrogen bonds. On cooling slowly, these complementary strands tend to bond again to form a dsDNA molecule and this is commonly referred to as annealing. The kind of annealing which occurs between complementary strands of two DNA molecules of different organisms is termed as hybridization (Nelson *et al*., 2004).

In PCR, primers that are specific for microorganisms are introduced to bind with target dsDNA strands that have their similar but unique sequences. Cycles of this hybridization process results in DNA amplification. The cycling process is carried out by a thermal cycler that cycles between temperatures and thereby increases the amount of targeted DNA segment. Therefore in three basic steps, the DNA template denatures between 92-95^{0}C, primers anneal with target sequences at a lower temperature of 58–60^{0}C then extension takes place at 72^{0}C (Clark, 2004). In this study, *Escherichia coli* primers were used for the PCR.

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. Study Area

Fresh carrot, cabbage and lettuce were purchased from the Kumasi central market in the Ashanti Region of Ghana. The market is highly patronized by the public for domestic and commercial purposes. Most farmers from neighbouring towns and nearby regions bring their produce to this area. The sales points are usually muddy floors (Daily Graphic, 2008).

3.1.2. Sample Size and Collection

The vegetable samples that were used in this study were carrot, cabbage and lettuce. These vegetables have not undergone any form of sanitization with the exception of water used to remove much of the adhering soil and dirt which is known to have a zero effect on the microbes present and may also be a source of microbial contamination on the vegetables (CDC, 1999). Vegetables that had been sanitized prior to purchase were excluded from this study.

3.2. METHODS

3.2.1. Sample Preparation for Microbial Analysis

The fresh vegetable samples from the Kumasi central market were transported aseptically in sterile food containers to the Microbiology laboratory of the Department of Biochemistry and Biotechnology for the microbial analysis. Bench surfaces in the laboratory were cleaned with 70% ethanol. Before wearing hand gloves, hands were also robbed with 70% ethanol to avoid contamination. All other materials and tools were also sterilized in 70% ethanol and then kept

under UV irradiation in a laminar flow hood before use. Thin slices of each vegetable sample were weighed in sterile petri dishes prior to UV treatment.

3.2.2. Preparation of Inoculums from the Raw Vegetables

Ten grams (10g) of each untreated vegetable, that is carrot, cabbage and lettuce, were weighed and packaged separately in sterilized zipper plastic bags and 90 ml peptone water poured into the zipper bags containing each of the vegetables. The vegetables were then washed vigorously in the peptone water in the plastic zipper bags using a pulsifier. The peptone water was drained from the washed vegetables in the zipper bags into sterilized containers and used as inoculums for the microbial culture which followed immediately. All these processes were conducted under sterile conditions in a laminar flow hood.

3.2.3. Preparation of Microbial Cultures from Inoculums of the Three Vegetables

Using aseptic technique, 10g of each sample was weighed into a sterile stomacher bag and 90 mL bacteriological peptone water (1:10 dilution) added and agitated for 2 min at moderate speed. Serial dilutions were made from 10^{-1} to 10^{-6} by transferring 1mL homogenized sample (1:10 dilution) to 9 mL dilution blank, mixing well until 10^{-6} dilution was reached. These dilutions (aliquots) were then used for the study.

About 100 µl of each serial dilution (10^{-1} to 10^{-6}) of each vegetable were inoculated on the plate count agar contained in the petri dishes labelled for the three vegetables. The plates were then turned upside down and kept in an incubator at 37 ^{0}C for 24 hours. The available bacterial colonies were counted for each dilution with its triplicates and the average number taken.

Inoculations were done in a laminar flow hood. From the appropriate 10 fold serial dilutions, enumeration of yeasts and moulds were carried out by the spread plate method on sabouraud dextrose agar containing 100 mg/l of chloramphenicol and 50 mg/L chlortetracycline hydrochloride to suppress the growth of bacteria. The plates were incubated at 25 ^{0}C for 5 to 7 days. Inoculums were cultured on MacConkey agar, XLD agar and BGA agar to identify specific bacteria using the streaking method. The inoculums (10^{-1}) of the three vegetables were centrifuged and the sediment at the bottom used for culturing. An inoculation loop was flamed, allowed to cool and then used to pick sediment from the bottom of the centrifuge tube and then streaked on the surface of the MacConkey agar in triplicates for each vegetable. The loop was flamed to red hot and cooled before and after each streaking. The plates were turned upside down in an incubator at 37 ^{0}C for 24 hours. Colonies of non-lactose fermentors were picked from positive plates of MacConkey agar and sub cultured on XLD and BGA by the streaking method. Positive plates of XLD and BGA were assessed to identify specific bacteria (*E. coli* and *Salmonella*).

3.2.4. BIOCHEMICAL PROFILE

Confirmation of the presence or absence of *E.coli* and *Salmonella* was done by sub-culturing microbial colonies on Triple Sugar Iron (TSI) agar, Sims medium, Simmons Citrate Agar and Christensen's Urea Agar. Inoculation on TSI agar slants was done under sterile conditions in a laminar flow hood. A flamed inoculating pin was used to pick colonies from plates of Xylose Lysine Deoxycholate (XLD) agar and Brilliant Green agar identified to have *E. coli* and *Salmonella* and streaked on the TSI Agar slants in test tubes being labelled for the three vegetables. They were cupped and kept at 37 ^{0}C in the incubator overnight and the results

recorded, pictures taken and compared with standard cultures for possible identification of the organism. This was followed by inoculation on SIM medium where colonies were picked from positive tubes of TSI Agar slants and stabbed into the SIM medium to the bottom of the tube and kept overnight at 37 ^{0}C. The results were recorded and pictures taken. Inoculations were also done on Simmons Citrate agar and Christensen's Urea Agar slants where microbial colonies were picked from the different positive tubes of TSI slants with a sterile inoculating pin and streaked on the various tubes of Simmons Citrate agar slants and Christensen's urea agar slants representing the three vegetables. The cultured slants were kept in an incubator at 37 ^{0}C overnight and results recorded and pictures taken.

3.2.5. Preparation of Inoculums from UV Sanitized Vegetables

Ten grams (10g) of each vegetable (carrot, lettuce and cabbage) were weighed and kept in sterilized glass plates in a UV chamber. The UV chamber was illuminated for one hour prior to sanitization of the vegetables. The vegetables were sanitized at an intensity of 253.7nm for the following time durations: one hour (1 hr), three hours (3 hrs), six hours (6 hrs) and nine hours (9 hrs). Each UV sanitized vegetable of specific time duration was kept in sterilized plastic zipper bags. Zipper bags containing 90 ml peptone and the vegetable samples were shaken vigorously or pulsified on a pulsifier so as to ensure that the vegetables were well washed in the peptone water. The peptone water were drained from the vegetables in sterilized containers and kept as inoculums used for microbial culture for bacterial colony count using Plate Count Agar and enumeration of yeast and moulds on Sabouraud Agar using microbiological cultural techniques previously described. Afterwards, possible colonies were counted and compared. Aseptic techniques were observed.

3.2.6. Preparation of Inoculums from Vinegar Treated Vegetables

About 10g of each vegetable were kept separately in vinegar for a contact time of 5 minutes by vigorously washing the vegetables in the vinegar. These vinegar washed vegetables were placed in the sterilized plastic zipper bags. Also 90 ml of autoclaved peptone water was poured into each zipper bag containing the vinegar washed vegetables. The contents in the zipper bags were pulsified to wash the vegetables in the peptone water. The liquid or inoculums were drained and kept in sterilized labelled containers for microbial cultures of bacterial colony count using PCA and Sabouraud for the enumeration of yeast and moulds. Possible colonies were then counted and compared.

3.2.7 Polymerase Chain Reaction

Stock	Final concentration	Single reaction (µl)
Distilled Water	Sterile	16.0
Primer 1 fwd	10 mM	1.0
Primer 2 rev	10 mM	1.0
Genomic DNA	4 ng/µl	2.0
Total	-	20.0

Positive control

Premix 9.0 µl

DNA 1.0 µl

TOTAL 10 µl

Negative control

Premix 9.0 µl

Sterile DW 1.0µl

TOTAL 10µl

All solutions and DNA aliquots were kept on ice always.

3.2.7.1. Procedure for PCR amplification

PCR amplification was done using Thermal Cycler 2720. *Escherichia coli* (USP gene) primer was used in this analysis. The *usp gene* which is specific for *E.coli O157: H7* had a sequence of -

F-5'-CGGCTCTTACATCGGTGCGTTG-3', R-5'-GACATATCCAGCCAGCGAGTTC-3'. A premix consisting of the usp gene primer specific for E coli O157:H7, $MgCl_2$, Tag polymerase and Dntps was then prepared from the table above. A 9.0 µl of the premix was dispensed into 200 µl PCR microfuge tubes. Inoculums from both raw and UV treated as well as vinegar treated vegetables of all dilutions (10^{-1} to 10^{-6}) of the three vegetables were spun in centrifuge tubes using a centrifuge at top speed for 15mins to pellet nucleic acids .The supernatants were decanted and the sediments used as the DNA for the PCR. A 1.0µl of 4 ng/µl DNA from each centrifuge tube were added to the premix in the 200 µl PCR microfuge tubes and short spanned for 30 seconds. The reaction tubes were placed into the thermal cycler and the cycling protocol for amplification of this microsatellite region entered as follows:

- 95^0C for 5 minutes at 1 cycle initial denaturation
- 95^0C for 30 seconds denaturation, 54^0C for 30seconds annealing and 72^0C for 1 minute initial extension at 35 cycles
- 72^0C for 7 minutes extension at 1 cycle
- Held at 4^0C until it was ready to load onto the gel or was kept in the freezer till it was ready to run

3.2.8. Gel preparation

1X TAE stock solution was prepared by pouring 20ml of 50X TAE solution into 1 litre plastic bottle topping up to the 1 litre mark and then shaking the bottle gently. 500 ml of the 1X TAE solution was then poured into a 500 ml flask and 5 g of agarose added. The mixture was then boiled in a microwave for 5 mins. This was followed by casting the gel and it involved the following steps:

- The tray was thoroughly washed and rinsed with deionized distilled water.
- Plastic plate's surfaces were cleaned with ethanol and allow to dry.
- Ends of the tray were then ceiled and place it on a level surface.
- Hot gel was gently poured into the tray making sure there were no bubbles.
- The gel was covered with plastic plates and the combs carefully inserted in between plates.
- The gel was allowed to set (solidify) for about 1 hr before removing the combs.

3.2.8.1 Agarose gel electrophoresis

About 10 µl of the Kapper ladder (100bp) was pipetted into the leftmost well and 10µl of the PCR products already mixed with the loading dye were loaded in the wells. Samples were ran at (90 to 120) volts for 2 hours. Gel was stained in (1X TAE) with 0.005% Ethidium bromide solution for 30 min by shaking it until the dye is lost.Gel was then washed in fresh staining solution to remove Ethidium bromide precipitates.The lid was then attached to the gel box and cords plug into power supply to allow electrophoresis run for 30 mins and Photographed under UV light.

CHAPTER FOUR

RESULTS

4.0. MICROBIAL CULTURE AND IDENTIFICATION

The raw carrot, cabbage and lettuce cultured on a layer of plate count agar in Petri dishes showed a very high bacterial growth compared to the UV treated vegetable sample. The 10^{-1} to10^{-3} diluted samples produced colonies too numerous to count (Fig 4.1a and Fig 4.1b.).

Fig 4.1: Representative plate showing bacterial colonies on PCA from aliquots of raw carrot at (a) $10^{-3}$and (b) 10^{-2} dilutions

10^{-4} to 10^{-6} of raw samples also recorded high bacterial colonies for all the three vegetables. Aliquots of raw non-sanitized carrot, cabbage and lettuce cultured on Sabouraud Agar to culture yeast and moulds produced large number of colonies on the third day of incubation at 25 ^{0}C. After the fifth day of culture, colonies were subcultured on Sabouraud Agar and pure cultures of *Aspergillus* spp.and Penicillium spp. were observed on culture plates for all vegetables (Fig 4.2a and Fig 4.2b).

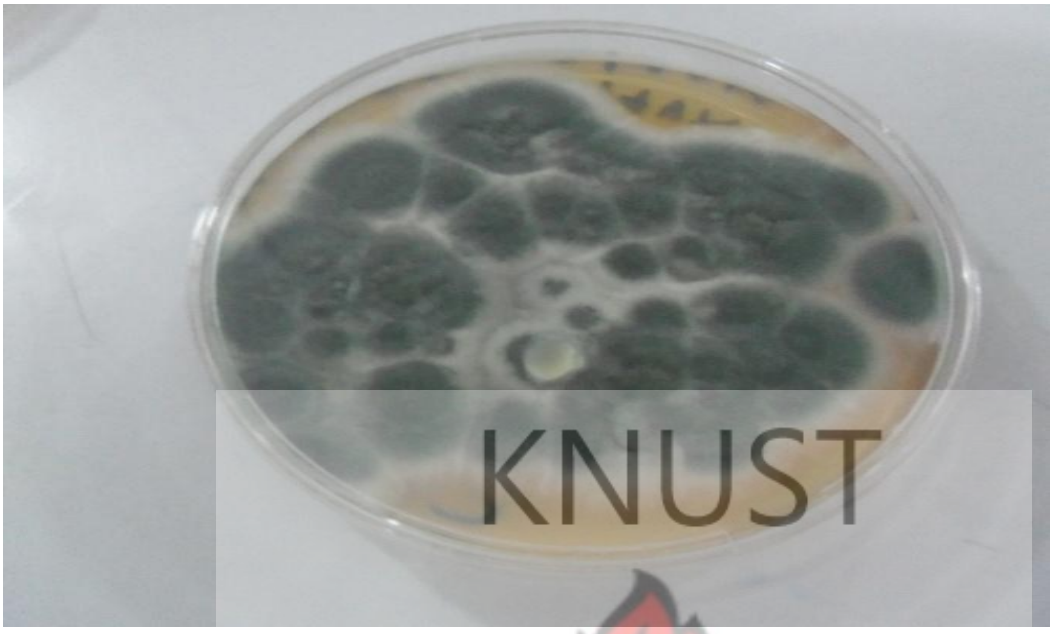

Fig 4.2a: Pure culture plates of *Penicillium* spp. sub-cultured from Sabouraud Agar

Fig 4.2b: *Aspergillus* spp. sub-cultured from Sabouraud Agar

Inoculums (10^{-1} dilutions) of raw carrot, cabbage and lettuce were spun and the sediments streaked on MacConkey Agar and Brilliant Green Agar. Bacterial colonies were observed on culture plates for all vegetables shown in **(Fig 4.3a and 4.3b).**

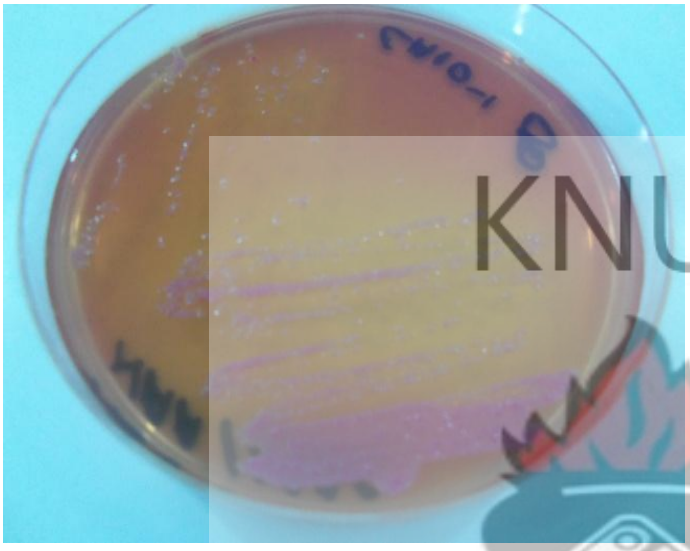

Fig 4.3a: Bacterial colonies from raw carrots on MacConkey Agar

Fig 4.3b: Bacterial colonies from raw cabbage on BGA

Microbial culturing from sediments of the raw vegetables done on Xylose Lysine Dextrose (XLD) Agar from aliquots of raw cabbage revealed the presence of Salmonella which was confirmed by the appearance of black colonies with cream rings (Fig 4.4a) and also, aliquots from raw carrot on XLD Agar also showed yellow colonies suggesting the presence of *E. coli* (Fig 4.4b).

Fig 4.4a: Colonies of *Salmonella* on XLD Agar indicated by black colonies with cream rings

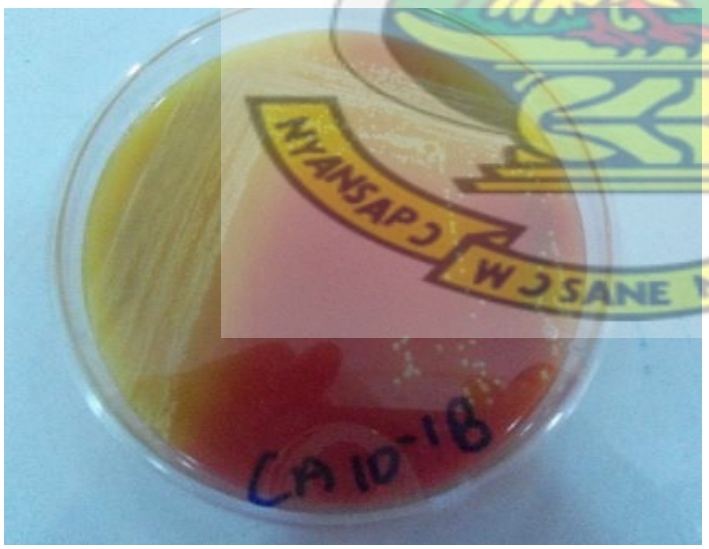

Fig 4.4b: *E. coli colonies* on XLD Agar indicated by the yellow colonies

4.1. BIOCHEMICAL TEST TO CONFIRM FAECAL COLIFORMS

Biochemical tests were performed to validate the presence of *E. coli* and *Salmonella* on the raw and non-sanitized vegetables using Triple Sugar Iron Agar Media (TSI), SIM media, Christensen's Urea Agar media and Simmons Citrate Agar media slants. Microbial culture using the streaking method was done on TSI agar slants. The media which is orange in colour gave a pink slant portion and a black butt from raw cabbage sediment confirming the presence *E. coli* (Fig 4.5).

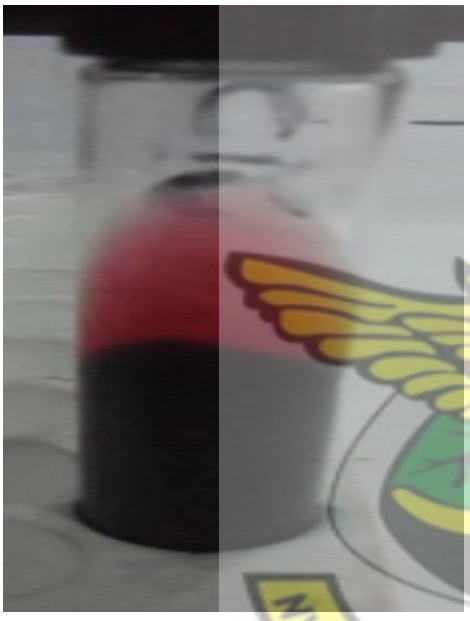

Fig 4.5: A representative tube showing a change in colour of TSI agar indicating the presence of *E. coli*

All three vegetables gave positive tests when they were cultured on Christensen's Urea Agar Slant which showed growth and colour change in the media from yellow (serving as control) to pink(Fig 4.6a).

Fig 4.6: Representative tube showing (a) growth on Christensen's Urea Agar Slant from sediments of raw cabbage indicating presence of *Proteus vulgaris*, (b) Control

Possible organisms such as *Citrobacter* spp., *Klebsiella pnuemoniae* and *Enterobacter aerogenes* were suggested to be present in all three raw vegetables since there was change in colour of Simmons Citrate Agar (green) to Intense Prussian blue due to alkali that these organisms produce in the culture media as they grow.

Fig 4.7a: A representative plate showing (a) Intense Prussian blue colour change indicating the presence of *Citrobacter* spp., *Klebsiella pnuemoniae* or *Enterobacter aerogenes* on Simmon Citrate Agar (b) Control

4.2. BIOCHEMICAL TEST ON THE RAW NON-SANITIZED VEGETABLES

Biochemical tests conducted on the raw non-sanitized vegetables confirmed the presence of various contaminants and microbes which results in a number of diseases (Table 4.1). Microbial culture on Triple Sugar Iron (TSI) agar which was orange in colour changed to yellow slant and black butt from sediments of raw lettuce which confirmed the presence of *Salmonella enterica*. Sediments from raw carrots also produced yellow slant and butt with air bubbles created at the bottom of the tube indicating the presence of *E coli* which produces gas in the butt of the tube. Sediments from raw cabbage also turned the media in the tube into black with a vacuum which shows the presence of *Salmonella*.

A motility test on SIM medium confirmed the presence of *E. coli* by the appearance of a pink

ring at the top of the tube after the addition of Kovacs reagent (positive indole result) for lettuce. The presence of *Salmonella arizonae* was confirmed by media in the tube turning all black. *Proteus vulgaris* which brings about change in Christensen's urea Agar which is yellow in colour to pink was confirmed in all three raw vegetables. Fig 4.7 shows the presence of *Citrobacter species, Klebsiella pnuemoniae* and *Enterobacter aerogenes* indicated by a colour change of Simmons Citrate Agar (green) indicator to Intense Prussian Blue

Table 4.1 Microbes Identified on the three Raw Vegetables

MEDIA	RAW NON-SANITIZED SAMPLES	OBSERVATION	POSSIBLE MICROBES
Triple Sugar Iron Agar slants(orange)	lettuce	Yellow slant with black butt	*Salmonella enterica Serovar typhi*
	carrot	Yellow at the butt with gas bubbles	*Escherichia coli*
	cabbage	Both slant and butt turned black with gas bubbles	*Salmonella* spp.
SIM (yellow) Sulfur-indole-motility test	Lettuce	Pink ring at the top of tube after addition of Kovacs reagent (positive indole result)	*Escherichia coli Proteus vulgaris*
	Carrot	Media in tube turned black	*Salmonella arizonae*
	Cabbage	Media in tube turned black	*Salmonella arizonae*
Christensen's urea Agar slants(yellow)	Lettuce	Media in tube turned pink	*Proteus vulgaris*
	Carrot	Media in tube turned pink	*Proteus vulgaris*
	Cabbage	Media in tube turned pink	*Proteus vulgaris*
Simmons Citrate Agar slants (green)	Lettuce	Media changed to intense Prussian Blue	*Citrobacter* spp. *Klebsiella pnuemoniae Enterobacter aerogenes*
	Carrot	Media changed to intense Prussian Blue	*Citrobacter* spp. *Klebsiella pnuemoniae Enterobacter aerogenes*
	Cabbage	Media changed to intense Prussian Blue	*Citrobacter* spp. *Klebsiella pnuemoniae Enterobacter aerogenes.*

4.3. BACTERIA AND YEASTS AND MOULDS COUNTS

Raw and non-sanitized vegetables were found to contain very high loads of microbes and faecal coliforms. Contaminantion levels exceeded standard levels of enteric pathogens. UV irradiation treatment on vegetables was very effective at reducing microbial loads. However, it performed almost the same sanitization effect when compared with vinegar treatment which is commonly used in vegetable decontamination. The microbial loads of $3x10^6$, $1.7x10^6$ and 1.5×10^7 cfu/g were recorded for the raw carrot, lettuce and cabbage samples, respectively. The raw non-sanitized cabbage was most contaminated among the three vegetables. Estimations for the yeasts and moulds on the vegetables were $7.5x\ 10^4$, 7×10^6 and 1.26×10^5 cfu/g for raw carrot, lettuce and the cabbage samples respectively revealing the effectiveness of UV irradiation against microbes on vegetables. As the UV exposure time increased, microbial load also decreased. This trend followed for all the three vegetables as microbial load decreased from 1 hour of UV exposure to 9 hours of UV (Table 4.2).

Table 4. 2: Bacteria and yeasts and moulds counts on the treated vegetables

Vegetables	Treatment	Time (hours)	Bacterial count (CFU/g)	Yeasts and Moulds Count(CFU/g)
Carrot	Raw	0	$3.00 \times 10^{6\,a}$	$7.50 \times 10^{4\,a}$
Carrot	Vinegar	-	$5.00 \times 10^{3\,b}$	$3.0 \times 10^{3\,a}$
Carrot	UV	1	$1.04 \times 10^{6\,a}$	$1.0 \times 10^{2\,a}$
Carrot	UV	3	$3.00 \times 10^{3\,b}$	$5.10 \times 10^{3\,a}$
Carrot	UV	6	$2.50 \times 10^{3\,b}$	$6.0 \times 10^{2\,a}$
Carrot	UV	9	$1.50 \times 10^{4\,b}$	$1.00 \times 10^{2\,a}$
Lettuce	Raw	0	$1.70 \times 10^{6\,a}$	$7.00 \times 10^{6\,a}$
Lettuce	Vinegar	-	$7.90 \times 10^{3\,b}$	$1.70 \times 10^{3\,b}$
Lettuce	UV	1	$1.30 \times 10^{6\,a}$	$1.00 \times 10^{2\,b}$
Lettuce	UV	3	$1.30 \times 10^{4\,b}$	$3.00 \times 10^{2\,b}$
Lettuce	UV	6	$1.50 \times 10^{4\,b}$	$3.00 \times 10^{3\,b}$
Lettuce	UV	9	$3.00 \times 10^{3\,b}$	$1.00 \times 10^{2\,b}$
Cabbage	Raw	0	$1.50 \times 10^{7\,a}$	$1.26 \times 10^{5\,a}$
Cabbage	Vinegar	-	$3.80 \times 10^{3\,b}$	$7.00 \times 10^{2\,a}$
Cabbage	UV	1	$5.00 \times 10^{5\,a}$	$5.00 \times 10^{2\,a}$
Cabbage	UV	3	$3.00 \times 10^{3\,b}$	$4.00 \times 10^{2\,a}$
Cabbage	UV	6	$1.00 \times 10^{4\,b}$	$1.00 \times 10^{2\,a}$
Cabbage	UV	9	$3.10 \times 10^{3\,b}$	$1.00 \times 10^{2\,a}$

Different superscript letters indicate statistically significant differences

4.4. MICROBIAL DETECTION BY PCR AND GEL ELECTROPHORESIS

The vegetable samples both raw and treated (UV and vinegar) which were subjected to Polymerase Chain Reaction (PCR) confirmed the presence of *E. coli* by the amplification of DNA marker of size 615 bp separated on a 2.0% agarose gel (Fig 4.8, 4.9 and 4.10). Eleven discrete bands were seen at lanes 15, 16, 23, 25, 26, 35, 36, 40, 46 and 48. Lanes 25 and 40 which were raw non-sanitized cabbage and raw lettuce respectively had bands falling between 700 bp and 500 bp closer to the size of the USP gene which is specific for *E. coli O157: H7*. This gave only 4% of samples producing positive result.

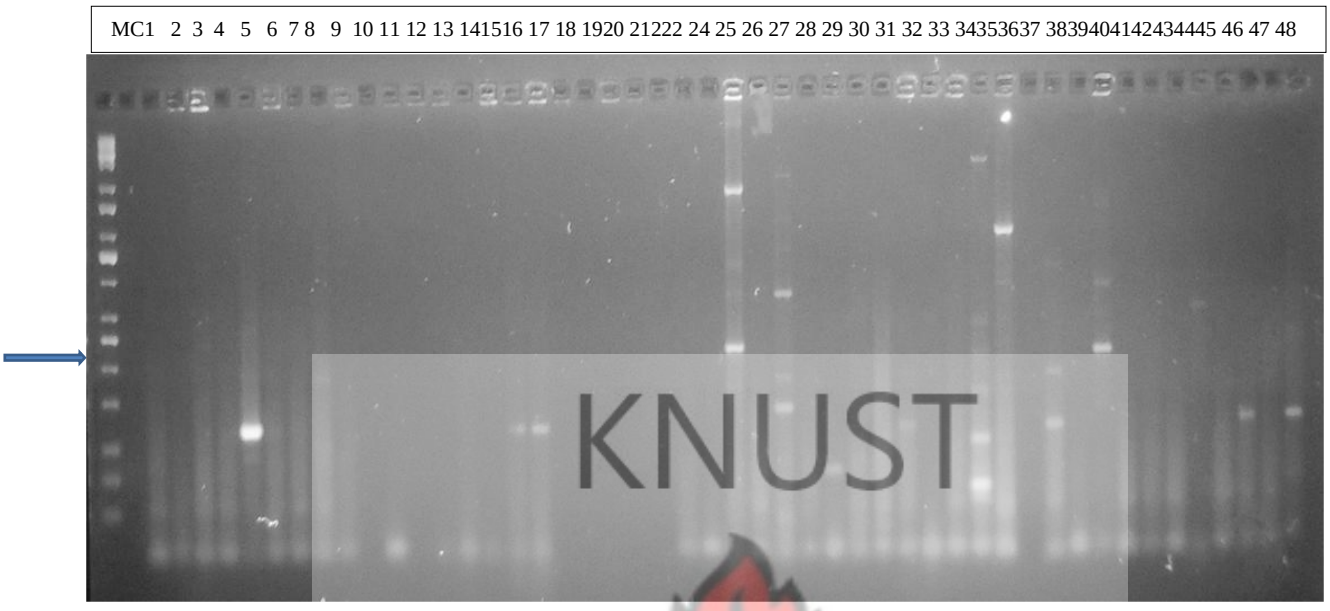

Fig: 4. 8: A representative Electrophoretic gel showing bands for the presence of *E. coli* on the vegetables before and after treatment with UV irradiation.
(M; ladder, C; control, 1-48; carrot, cabbage and lettuce samples. Arrows point to PCR Product of size 615 bp).

Fig 4.9 had discrete bands at lanes 10, 11, 17, 18, 19, 22, 26, 27, 30 and 31. Lanes 10 and 18 representing raw carrot and six hour UV sterilized lettuce had discrete bands with sizes closer to the USP gene size specific for *E. coli O157: H7*. Fig 4.9 also gave 6.3% of samples producing positive result.

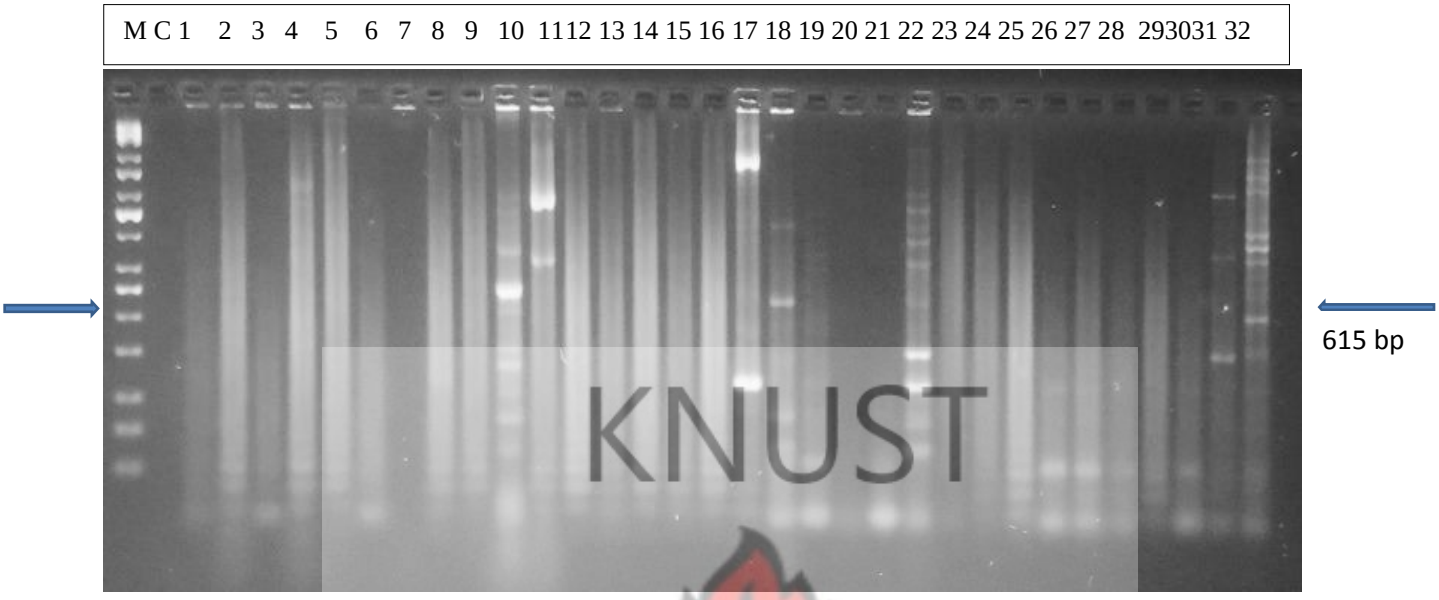

Fig 4.9: A representative Electrophoretic gel showing bands for the presence of *E. coli* on the vegetables before and after treatment with UV irradiation (M; ladder C; control, 1-32; carrot, cabbage and lettuce samples. Arrows point to PCR product of size 615 bp).

Lanes 8, 14, 21 and 28 on Fig 4.10 representing three hour UV sterilized lettuce, three hour UV sanitized carrot and another three hour sterilized lettuce respectively also had bands with gene size being same as USP gene which is specific for *E. coli 0157: H7*. Fig 4.10 gave 12.5% of samples producing positive result.

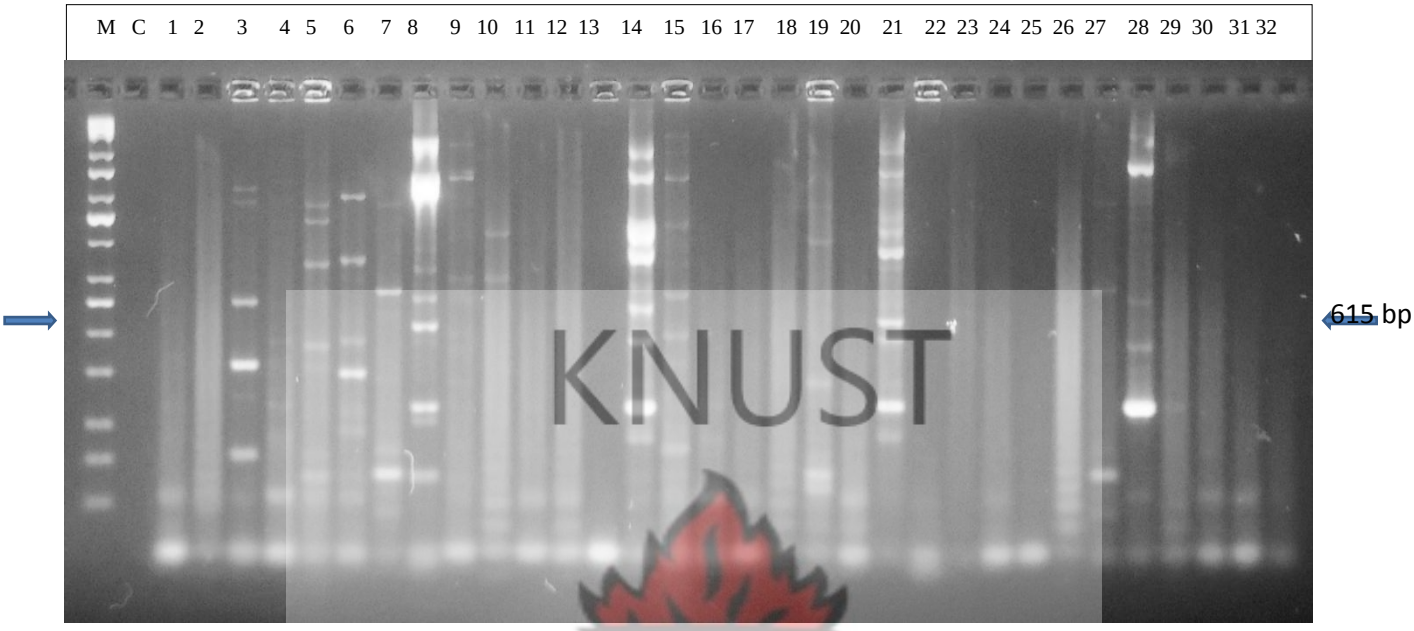

Fig: 4. 10: A representative Electrophoretic gel showing bands for the presence of *E. coli* on the vegetables before and after treatment with UV irradiation
(M; ladder C; control, 1-32; carrot, cabbage and lettuce samples. Arrows point to PCR product of size 615 bp)

4.5. DETECTION OF PCR PRODUCTS OF *E. COLI* IN TREATED AND UNTREATED VEGETABLES

Lanes 25 and 40 (Fig 4.8) and 10 (Fig 4.9) are *E. coli* bands seen in the raw vegetable samples. Some of bands of PCR product representing *E. coli* were seen in some of the treated samples irradiated with UV for 1 to 6 hours. However, 9 hour UV exposure time showed no *E. coli* band.

Table: 4.3: Microbial detection on vegetables by PCR

Treatment	Detection of *E coli* Bands
Raw cabbage	Present
Raw lettuce	Present
Raw carrot	Present
6 HRS UV Lettuce	Present
3 HRS UV lettuce	Present
3 HRS UV carrot	Present
3 HRS UV lettuce	Present
6 HRS UV carrot	Present

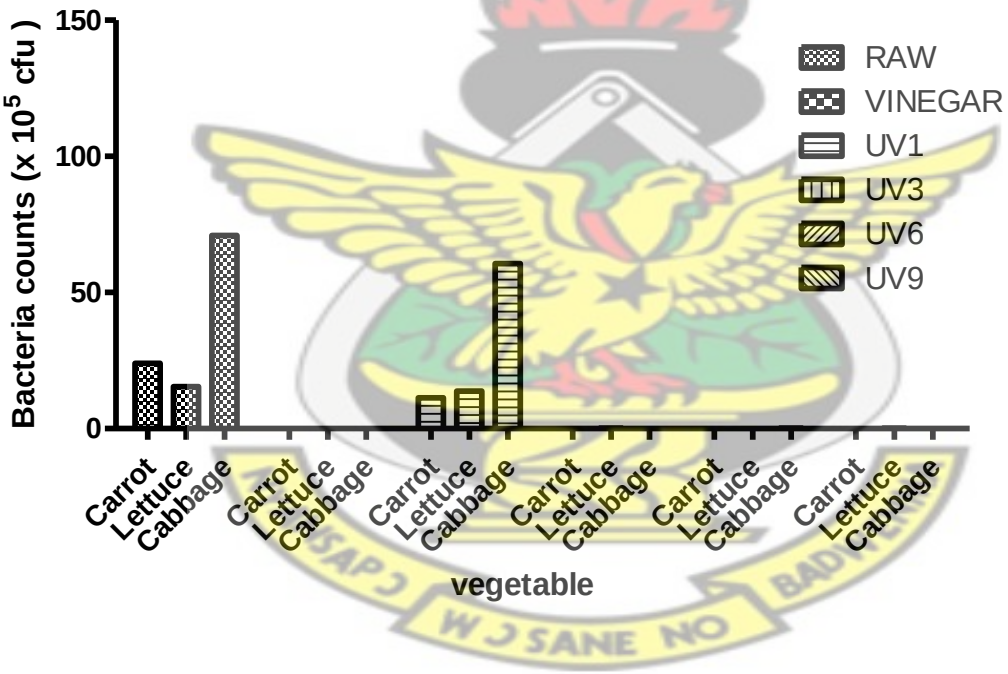

Fig 4.11: Total viable count (TVC) of bacterial colonies on vegetables before and after treatment with Vinegar and UV irradiation.
(The vinegar treatment served as the control and UV irradiated vegetables were apportioned into incubation times of 1, 3, 6 and 9 hours).

Fig 4.11. is a graphical representation of lettuce, carrots and cabbage before and after treatment with sanitizing agents. The vinegar treatment served as the control and UV irradiated vegetables

were apportioned into incubation times of 1, 3, 6 and 9 hours. It can be seen that the raw untreated vegetables contained very high microbial loads which are indicators of Gram negative bacteria. The treated vegetables, however, showed significant reduction.

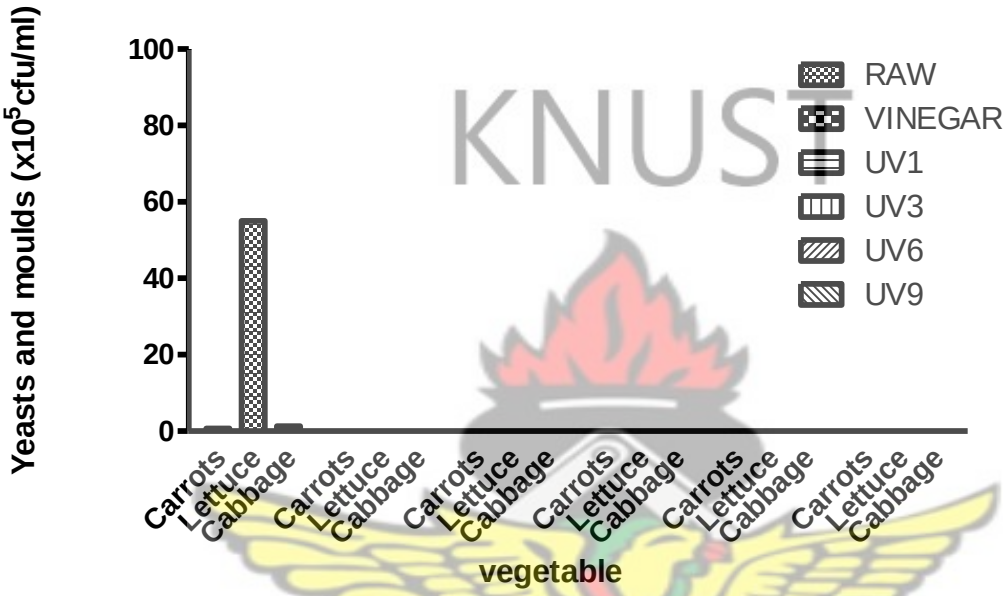

Fig 4.12: Total viable count (TVC) of colonies of yeasts and moulds on vegetables before and after treatment with Vinegar and UV irradiation. The vinegar treatment served as the control and UV irradiated vegetables were apportioned incubation times of 1, 3, 6 and 9 hours.

From Fig 4.12. it can be seen that the raw untreated lettuce contained very high colonies of yeasts and moulds as compared to carrots and cabbages. Treatment with vinegar and 1, 3, 6 and 9 hours UV irradiation resulted in a significant decrease of these microbes on the vegetables especially lettuce which recorded higher microbial count when untreated.

CHAPTER FIVE

5.0. DISCUSSION

The results obtained revealed effectiveness of UV radiation in sanitizing carrots, cabbages and lettuce. There was a significant reduction in microbial load and the potential residual pathogens on these vegetables were eliminated. Comparatively, untreated vegetable samples contained very high loads of microbes and faecal coliforms exceeding tolerable levels of enteric pathogens. Even though UV irradiation treatment of vegetables was very effective at reducing microbial loads, there was no significant difference between the UV and vinegar treatment (Table 4.2). Bacterial load of 3.0 x 10^6, 1.7 x10^6 and 1.5 x 10^7 cfu/g for raw carrot, lettuce and cabbage, respectively were recorded. The levels of yeast and moulds were 7.5 x 10^4, 7.0 x 10^6 and 1.26 x 10^5 cfu/g for raw non- sanitized carrots, lettuce and cabbages, respectively. The ineffectiveness of washing vegetables with water as a way of sanitization (Tornuk *et al*., 2011) was therefore confirmed as the traders at the Kumasi Central Market claimed the vegetables were sanitized with water (Research interview). Water only removes sand and other debris from the vegetables (Sapers *et al*., 2006), hence non-sanitized vegetables can lead to diarrhoea and food poisoning which can result in death.

Also, raw and untreated cabbage recorded highest bacterial count of 1.5 x10^7 cfu/g, followed by carrot which recorded 3.0 x 10^6 cfu/g and then lettuce having the least count of 1.7 x 10^6 cfu/g. Cabbage is a leafy vegetable known to possess crevices which harbour pathogenic microbes and therefore could account for the high microbial contamination observed (CDC, 2011). Carrot, a root vegetable with root hairs which stems deep in the soil is naturally contaminated by soil microflora. This was shown by high microbial contamination of the carrot vegetable sample in

this study (Table 4.2). Lettuce, also a leafy vegetable have crevices which is much exposed and not folded like cabbage and hence some microbes might have been washed away with water by the traders, hence recording less microbial numbers (Beuchat *et al*., 2004; Gonzalez *et al*., 2004). Also difference in geographical locations can account for the difference in microbial load.

Vinegar used as a control in this study reduced the number of microbes on the three vegetables to levels acceptable for consumption. The vinegar contains acetic acid which can lyse microbes and may have accounted for its sanitizing capability (Issa-Zacharia *et al*., 2011) and this affirmed the reason why vinegar was chosen as control for the study. Raw untreated lettuce and cabbage also harboured *Aspergillus* species and *Penicillium* species (Fig 4.2a and 4.2b). Culturing of raw sanitized vegetables on MacConkey agar also revealed the presence of lactose and non-lactose fermenters. The lactose fermenters were confirmed as *E. coli* with pink to red colour (Fig 4.3a) and yellow colonies on XLD agar (Fig 4.4b). The presence of *Salmonella* was confirmed by the appearance of black colonies with cream rings (Hickman-Brenner, 1991) on Xylose Lysine Dextrose Agar (XLD) raw non-sanitized cabbage (Fig 4.4a). These *Salmonella* species metabolizes thiosulfate in the media to produce hydrogen sulfide leading to the formation of colonies with black centres.

Biochemical profile further confirmed the presence of various contaminants and microbes associated with vegetables which results in a number of deadly diseases. All the raw untreated vegetable samples tested positive for the presence of *E. coli*. This is alarming but not surprising as it is already established that non-sanitized vegetables are unsafe for consumption when they are not properly sanitized. Also, it is established that vegetables get contaminated right from the

farm, through transportation and even storage sites at the sales point (Nath et al., 1989) calling for major stakeholders in the fresh produce chain to introduce measures to prevent product contamination (FDA/CFSAN, 2001). It is possible for one to suffer microbial infection when he or she consumes vegetable purchased from the Kumasi central market that have not been adequately sanitized. Others microbes that were also confirmed were *Salmonella enterica, Proteus vulgaris, Salmonella arizonae, Citrobacter* spp. *Klebsiella pnuemoniae,* and *Enterobacter aerogenes* all of which cause various diseases of human concern such as typhoid fever, urinary tract infection, pneumonia, bacteremia and blood stream infections.

It was also observed that UV irradiation was more effective at killing micro-organisms and inactivating potentially pathogenic microbes for the different time intervals the vegetables were subjected to UV irradiation. Bacterial as well as yeast and moulds colonies reduced considerably after UV exposure. Again, there were appreciable reductions from 1 hr through to 9 hrs of UV exposure. UV as a sanitizing agent of vegetables against microbes is able to form kinks in the DNA structure of these microbes which lead to the formation of pyrimidine dimmers resulting in impairment of cell division and repair in the microbes leading to their death (Kim *et al*., 2013). There was no *E. coli* detection from the 9hr UV sterilized vegetables showing complete inactivation of *E. coli* on the vegetable samples. This confirmed the findings that UV was efficient at reducing *E. coli 0157:H7* in raw lettuce (Kim *et al*., 2013). Comparism of 1hr UV, 3hrs UV, 6hrs UV and 9hrs UV sanitized vegetables from TVC data of bacterial colonies yielded a p-value of 0.0056 in all tests (< 0.05). The effect of the treatment is considered overall to be very significant. This means that the effect 1hr UV treatment had on bacterial colonies on all three vegetables was weaker than the decontamination effects of 3hr, 6hr and 9hr UV irradiation

times. In all three vegetables, there was a significant sanitizing difference between raw and vinegar treated samples. This therefore confirmed vinegar as an effective standard sanitizer for vegetables as reported in the works of Issa-Zacharia *et al*. (2011). It again affirmed the why vinegar was chosen as control for the study. Statistically, no significant difference existed between the raw and 1hr UV treated vegetables. This showed that a UV irradiation time of 1hr was unable to inactivate bacterial colonies on these vegetables. From the TVC results on bacterial count (Table 4.2), 9hrs UV treatment had the greatest effect of reducing bacterial colonies on the three vegetables. However, it is evident from Fig 4.11 that, statistically, no significant difference existed between 3hrs UV, 6 hrs UV and 9 hrs UV. It can be deduced from this analysis that 3 hrs UV incubation time is just as effective as 6 hrs UV and 9 hrs UV irradiation times. Thus, a UV time range of 3hrs to 9 hrs can be used to sanitize vegetables especially carrot, cabbage and lettuce with 9hrs being the optimum UV irradiation time. However, an observation made is that, when food is irradiated some nutrition is lost (Loaharanu, 1990). It is therefore convenient to reduce the incidence of lost nutrient by reducing the time vegetables are being exposed to UV, thus 3hrs to 6hrs will be the most preferred contact time. The physical appearance of the vegetables did not remain intact after the sanitization process. One major observation made was that there the UV treated samples were dehydrated, leading to some observable drought stress on the 6 hrs and 9 hrs UV sanitized vegetables. Hermetic application of UV has been linked to induction of stress responses that trigger chemical changes in fresh fruits and vegetables (Ribeiro and Alvarenga, 2012). This may be due to heat accumulation in the enclosed UV chamber resulting in an unwanted effect of dehydration of the vegetables, especially for the lettuce because of its leafy nature. This dehydration can however be prevented by placing a humid material such as a foam moistened with sterile water in the

plate before placing the vegetable in it for sanitization.

Again from the Two- way Anova test, comparing the response of carrot, cabbage and lettuce to all treatments results were not significant (p-value = 0.3431). The interaction therefore is considered not significant. This means that treatments has the same effect at all values of vegetables indicating that the response of carrot to the treatments which were vinegar and the different times of UV incubation was not different from the response of cabbage and lettuce. Thus the three different vegetables did not affect the result. Comparism of 1hr UV, 3 hrs UV, 6 hrs UV and 9 hrs UV from TVC data of yeasts and moulds yielded a p-value of <0.0001. The effect of the overall treatment on the three vegetables is therefore considered extremely significant. This means that the effect 1hr UV treatment had on yeasts and moulds on the three vegetables is different from the effects the other UV incubation times also had on yeasts and moulds on the vegetables. However, individually, it was evident that, there was no significant difference between the various treatments on raw carrot (Fig 4.12). The same observation was recorded for cabbage (Fig 4.12). These observations therefore could be due to the fact that the raw non-sanitized carrot and cabbage samples already had very low microbial count of yeasts and moulds and therefore although there was reduction in microbial load after treatment, it did not make any difference statistically. A very high significant difference was however found between the raw lettuces, the vinegar treated lettuce and the UV treated lettuce. Raw unsanitized lettuce recorded a high yeasts and moulds contamination. This can be attributed to the fact that lettuce being a leafy vegetable have a lot of crevices and harbours microbe and also create a perfect atmosphere for microbial proliferation (Sapers, 2001).Vinegar and the different UV irradiation treatments had the same effect on the microbes living on the three vegetables. This means that 1hr UV incubation time and vinegar against yeasts and moulds on these vegetables is

as effective as 3 hrs, 6 hrs or 9 hrs UV incubation times. Carrot, cabbage and lettuce responded differently to all treatments as shown by the overall p-value of<0.0001. The interaction is therefore considered to be extremely significant. This implies that treatment does not have the same effects at all values of vegetables. Hence the vegetables affected the results.

PCR products observed on the electrophoretic gel represents raw, UV sanitized and vinegar treated vegetables from various dilutions. The size of the USP gene which is specific for *E. coli 0157:H7* with primer sequences of *usp-F 5'CGGCTCTTACATCGGTGCGTTG-3', R-5'-GACATATCCAGCCAGCGAGTTC-3*'is 615 bp (Darko *et al*., 2013). From Fig 4.8, lane 25 and 40 which represented raw cabbage and raw lettuce respectively had bands in between 500 bp and 700 bp. These bands represented *E. coli* strain with expected size of 615 bp bands (arrow) and confirms the fact that fresh vegetables harbours a lot of microorganisms including *E. coli* confirmed by the microbial cultures. Fig 4.9 also had *E. coli* bands of raw carrot and 6 hr UV sanitized lettuce (bands with arrow heads) at lanes 10 and 18. Lanes 8, 14, 21 and 28 (as seen in Fig 4.10) representing 3hr UV sanitized lettuce, 3 hr UV sanitized carrot and another 3 hr UV sanitized lettuce respectively also had *E. coli* bands. This establishes the fact that, even though UV irradiation is effective in sanitizing vegetables against microbes, there were samples that had been sanitized with UV irradiation but had *E. coli* bands. UV exposure time of 9 hrs, however, did not show any *E. coli* band. From this study, 9 hrs of UV irradiation is thus established as the optimum contact time for sanitizing vegetables and this is also confirmed in the microbial cultures. More research is needed in the fresh produce chain to prove the effectiveness of mitigation measures.

CHAPTER SIX

6.0. CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

It is evident from this study that fresh vegetables; carrot, lettuce and cabbage in our markets are highly contaminated with microorganisms and therefore unwholesome for food without proper treatments. UV irradiation was effective in eliminating residual microbes to levels acceptable for consumption. Even though UV irradiation showed better sanitizing effect, the difference was insignificant when compared with vinegar treatments. Microbial loads on both treated and raw vegetables were successfully quantified using microbial culture and PCR techniques. Considering the rapidity of PCR methods compared with culture methods, PCR could be a method of choice for *E.coli* contamination assessment. Microbial counts reduced with increasing UV exposure time, hence 9 hrs UV treatment recording no counts of bacterial colonies, yeasts and moulds or *E. coli* PCR products. However, the 3 and 6 hr UV treatments were also effective against microbial contaminants on the vegetables. Vinegar could however not remove all *E. coli* cells but reduced the load significantly. Vinegar sanitized vegetables had remnants of other microbes on the electrophoretic gel. It can be concluded that UV is therefore very effective and convenient approach for reducing microbial pathogens (especially *E. coli*) on vegetables and therefore could be applied commercially in Ghana.

6.2. RECOMMENDATIONS

The results obtained from this research have led to the emergence of some recommendations that may benefit the progress of this study in the field of microbial biotechnology. These recommendations are as follows.

1. Further studies should be done to assess the effectiveness of UV irradiation against microbes on vegetables in periods of 2hrs, 4hrs, 7hrs and 8hrs as compared to the durations used in this study. This could in effect enhance productivity economically by saving time and energy which are both expensive.

2. Further research should also be done to maintain the freshness of these vegetables exposed to UV irradiation. This is because in as much as people want to enjoy vegetables free from microbes, the freshness of the vegetables is a key factor for people wanting to consume vegetables
3. Further research should be embarked upon to assess the toxicity effects of UV irradiated vegetables though some research works have already allayed the fears of UV toxicity in foods. This could be done with the use of experimental laboratory rats. The outcome of this research would enlighten Ghanaian scientists and policy makers.

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APPENDIX

PREPARATION OF CULTURE MEDIA AND STERILIZATION

Plate count agar (PCA)

The PCA was prepared in three 500 ml glass bottles. About 17.5g is to 1 litre distilled water. Three of the 50 ml glass bottles were prepared for each vegetable at each culture. An 8.75g of the PCA were weighed into each 500 ml bottle by using electronic weighing balance. Distilled water was added to dissolve the media and topped to the 500 ml mark on the bottle. The contents of the bottle were then heated to dissolve at a temperature of 99.9^{0}C in a water bath. This was followed by autoclaving at 121 ^{0}C for 15 minutes. The autoclaved media were then kept in water bath at 55^{0}C to cool. They were transferred into a laminar flow hood where the media were poured into oven sterile Petri dishes. Before the media were poured in the Petri dishes, the mouths of the media bottles were flamed. The medium was poured and the lid gently closed to allow them to solidify. The plates were turned upside down and kept in an incubator overnight at 37^{0}C to check for sterility.

Sabouraud agar

An electronic analytical balance was used to weigh 65g of the media. It was then dissolved in 1 litre of distilled water and boiled in water bath to dissolve completely. The media was sterilized by autoclaving at 121^{0}C for 15 minutes. The sterilized media was kept in water bath at 55^{0}C to cool. It was later transferred into a laminar flow hood where the media was poured into sterile Petri dishes.

Peptone water

Five grams (5g) of bacteriological peptone was dissolved in 500 ml distilled water to form peptone water. About 90ml of the peptone water were dispensed into three 250 ml media bottles each and 9 ml of the same peptone water were also dispensed into sixteen text tubes, five for each vegetable and one as control. The test tubes were cupped and autoclaved at 121^0C for 15mins. They were kept overnight in an incubator at 37^0C to check for sterility.

MacConkey agar (MAC)

About 25 g of MacConkey agar was weighed and dissolved in 500 ml distilled water. The medium was mixed and dissolved by heating at 75^0C in a water bath. It was then autoclaved at 121^0C for 15mins. It was cooled to 50^0C and dispensed into sterile plates. The plates were allowed to solidify, labelled and placed upside down in the incubator for overnight to check for sterility.

Brilliant green Agar (BGA)

Twenty six (26 g) of the powdered BGA was weighed and dissolved in 500 ml distilled water. The solution was agitated using the magnetic stirrer and brought to boil until the medium dissolved completely. The medium was cooled in a water bath at 50^0C and then poured into sterile plates. The plates were allowed to solidify, labelled and placed upside down in the incubator at 37^0C overnight to check for sterility.

Preparation of xylose lysine deoxycholate (XLD) agar

About 28.35g of powdered Xylose Lysine Deoxycholate (XLD) agar was weighed and dissolved

in 50 ml of distilled water in a 500ml glass bottle. The bottle was swirled to mix thoroughly. The remaining 450ml of distilled water was added. The medium was brought to boil to dissolve completely. The medium was cooled in a water bath set at 50^0C and then poured into sterile plates. The plates were allowed to cool and solidify, labelled, turned upside down in the incubator at 37^0C overnight to check for sterility.

Preparation of triple sugar iron (TSI) agar

About 64.6 g of the agar was dissolved in 1L distilled water. It was boiled to dissolve completely, mixed well and dispensed into sterile text tubes .They are then sterilized by autoclaving at 1210C for 15mins and allowed to set as slopes with 2.5cm butts.

Preparation of Sims medium

About 30 g of the Sims medium was dissolved in 1 litre distilled water. They were poured into test -tubes to a depth of 4 cm. They were then sterilized by autoclaving at 121^0C for 15 mins and allowed to set upright.

Preparation of Simmons Citrate agar

About 23.3 g of the agar was dissolved in 1L distilled water and heated to boiling to dissolve Completely. They were then dispensed into test tubes and sterilized by autoclaving at 121^0C for 15mins and then allowed to cool as slants.

Preparation of Christensen's Urea agar

Twenty-one grams (21 g) of the Agar was dissolved in 950 ml distilled water. They were

sterilized by autoclaving at 121^0C for 15 mins. An amount of 50 ml sterile filtered 40% urea solution was added after cooling to 50^0C and allowed to cool as slants.

DESCRIPTION OF WELLS ON PCR RESULTS

Table 4.4 Description of wells of Fig 4.7

WELL NUMBER	DESCRIPTION OF WELL	DILUTION NUMBER
M	marker	
C	control	
1	vinegar washed carrot	10^{-6}
2	raw cabbage	10^{-3}
3	6hr lettuce	10^{-1}
4	6hr cabbage	10^{-4}
5	1hr carrot	10^{-4}
6	9hr cabbage	10^{-1}
7	vinegar washed lettuce	10^{-3}
8	1hr carrot	10^{-3}
9	3hr carrot	10^{-4}
10	vinegar washed cabbage	10^{-6}
11	1hr cabbage	10^{-3}
12	vinegar washed cabbage	10^{-4}
13	1hr lettuce	10^{-1}
14	3hr carrot	10^{-3}
15	raw lettuce	10^{-3}
16	raw carrot	10^{-6}
17	1hr carrot	10^{-1}
18	raw carrot	10^{-1}
19	9hr cabbage	10^{-6}
20	raw lettuce	10^{-1}
21	vinegar washed cabbage	10^{-1}

22	9hr carrot	10^{-6}
23	1hr cabbage	10^{-2}
24	6hr cabbage	10^{-3}
25	raw cabbage	10^{-3}
26	9hr cabbage	10^{-5}
27	raw carrot	10^{-5}
28	1hr lettuce	10^{-2}
29	raw lettuce	10^{-5}
30	1hr carrot	10^{-6}
31	6hr cabbage	10^{-2}
32	9hr carrot	10^{-1}
33	vinegar washed carrot	10^{-3}
34	1hr carrot	10^{-5}
35	vinegar washed lettuce	10^{-2}
36	1hr lettuce	10^{-5}
37	6hr lettuce	10^{-4}
38	vinegar washed lettuce	10^{-1}
39	9hr lettuce	10^{-4}
40	raw lettuce	10^{-2}
41	raw cabbage	10^{-6}
42	vinegar washed cabbage	10^{-3}
43	9hr lettuce	10^{-1}
44	3hr lettuce	10^{-4}
45	vinegar washed lettuce	10^{-4}
46	3hr carrot	10^{-1}
47	vinegar washed carrot	10^{-4}
48	3hr lettuce	10^{-5}

Table 4 .5 Description of wells of Fig 4.8

Well number	Description of well	Dilution number
M	marker	
C	control	
1	Raw lettuce	10^{-3}
2	Raw carrot	10^{-5}
3	Raw lettuce	10^{-1}
4	6hour carrot	10^{-1}
5	9hr carrot	10^{-3}
6	3hr carrot	10^{-2}
7	Raw carrot	10^{-2}
8	1hr carrot	10^{-2}
9	3hr carrot	10^{-2}
10	Raw carrot	10^{-4}
11	Raw carrot	10^{-2}
12	Vinegar washed carrot	10^{-2}
13	6hr carrot	10^{-3}
14	6hr cabbage	10^{-4}
15	Vinegar washed cabbage	10^{-6}
16	1hr carrot	10^{-3}
17	6hr carrot	10^{-4}
18	6hr lettuce	10^{-1}
19	Raw cabbage	10^{-5}
20	9hr carrot	10^{-2}
21	Vinegar washed cabbage	10^{-3}
22	3hr carrot	10^{-4}
23	Raw lettuce	10^{-4}
24	3hr cabbage	10^{-3}
25	9hr carrot	10^{-4}
26	Vinegar washed cabbage	10^{-2}
27	Vinegar washed cabbage	10^{-5}
28	9hr cabbage	10^{-6}
29	9hr carrot	10^{-5}
30	Vinegar washed carrot	10^{-5}
31	3hr cabbage	10^{-5}
32	6hr lettuce	10^{-2}

Table 4.6 Description of wells of Fig 4.9

Well number	Description of well	Dilution number
M	marker	
C	control	
1	3hr carrot	10^{-6}
2	6hr lettuce	10^{-1}
3	Raw lettuce	10^{-4}
4	1hr carrot	10^{-5}
5	Raw cabbage	10^{-1}
6	1hr cabbage	10^{-1}
7	1hr cabbage	10^{-4}
8	3hr lettuce	10^{-3}
9	9hr lettuce	10^{-4}
10	9hr cabbage	10^{-2}
11	1hr cabbage	10^{-5}
12	3hr cabbage	10^{-4}
13	Raw cabbage	10^{-2}
14	3hr carrot	10^{-5}
15	1hr lettuce	10^{-3}
16	3hr cabbage	10^{-1}
17	3hr cabbage	10^{-1}
18	6hr lettuce	10^{-3}
19	1hr cabbage	10^{-6}
20	1hr lettuce	10^{-5}
21	3hr lettuce	10^{-4}
22	Raw cabbage	10^{-4}
23	6hr cabbage	10^{-5}
24	6hr cabbage	10^{-2}
25	3hr cabbage	10^{-6}
26	6hr carrot	10^{-5}
27	9hr cabbage	10^{-5}
28	6hr carrot	10^{-6}
29	1hr cabbage	10^{-6}
30	6hr cabbage	10^{-6}
31	Bomso flash handle	
32	Swabs from church of Chris t floor	

Comparison of carrot samples before and after treatment with UV and vinegar based on T

VC data

Paired set	p-value	summary
RAW vs VINEGAR	>0.05	Not Significant
RAW vs 1hrUV	>0.05	Not Significant
RAW vs 3hrsUV	>0.05	Not Significant
RAW vs 6hrsUV	>0.05	Not Significant
RAW vs 9hrsUV	>0.05	Not Significant
VINEGAR vs 1hrUV	>0.05	Not Significant
VINEGAR vs 3hrsUV	>0.05	Not Significant
VINEGAR vs 6hrsUV	>0.05	Not Significant
VINEGAR vs 9hrsUV	>0.05	Not Significant
1hrUV vs 9hrsUV	>0.05	Not Significant
1hrUV vs 6hrsUV	>0.05	Not Significant
1hrUV vs 3hrsUV	>0.05	Not Significant
9hrsUV vs 6hrsUV	>0.05	Not Significant
9hrsUV vs 3hrsUV	>0.05	Not Significant
6hrsUV vs 3hrsUV	>0.05	Not Significant

Comparison of lettuce samples before and after treatment with UV and VINEGAR based on TVC data

Paired set	p-value	summary
RAW vs VINEGAR	>0.05	Not Significant
RAW vs 1hrUV	>0.05	Not Significant
RAW vs 3hrsUV	>0.05	Not Significant
RAW vs 6hrsUV	>0.05	Not Significant
RAW vs 9hrsUV	>0.05	Not Significant
VINEGAR vs 1hrUV	>0.05	Not Significant
VINEGAR vs 3hrsUV	>0.05	Not Significant
VINEGAR vs 6hrsUV	>0.05	Not Significant
VINEGAR vs 9hrsUV	>0.05	Not Significant
1hrUV vs 9hrsUV	>0.05	Not Significant
1hrUV vs 6hrsUV	>0.05	Not Significant
1hrUV vs 3hrsUV	>0.05	Not Significant
9hrsUV vs 6hrsUV	>0.05	Not Significant
9hrsUV vs 3hrsUV	>0.05	Not Significant
6hrsUV vs 3hrsUV	>0.05	Not Significant

Comparison of cabbage samples before and after treatment with UV and VINEGAR based on TVC data

Paired set	p-value	summary
RAW vs VINEGAR	< 0.01	Highly Significant
RAW vs 1hrUV	>0.05	Not Significant
RAW vs 3hrsUV	< 0.01	Highly Significant
RAW vs 6hrsUV	< 0.01	Highly Significant

RAW vs 9hrsUV	< 0.01	Highly Significant
VINEGAR vs 1hrUV	<0.05	Significant
VINEGAR vs 3hrsUV	>0.05	Not Significant
VINEGAR vs 6hrsUV	>0.05	Not Significant
VINEGAR vs 9hrsUV	>0.05	Not Significant
1hrUV vs 9hrsUV	<0.05	Significant
1hrUV vs 6hrsUV	<0.05	Significant
1hrUV vs 3hrsUV	<0.05	Significant
9hrsUV vs 6hrsUV	>0.05	Not Significant
9hrsUV vs 3hrsUV	>0.05	Not Significant
6hrsUV vs 3hrsUV	>0.05	Not Significant

Comparison of carrot samples before and after treatment with UV and VINEGAR based on TVC data

Paired set	p-value	summary
RAW vs VINEGAR		Not Significant
RAW vs 1hrUV	>0.05	Not Significant
RAW vs 3hrsUV	>0.05	Not Significant
RAW vs 6hrsUV	>0.05	Not Significant
RAW vs 9hrsUV	>0.05	Not Significant

Comparison of lettuce samples before and after treatment with UV and VINEGAR based on TVC data

Paired set	p-value	summary
RAW vs VINEGAR	< 0.001	Highly Significant
RAW vs 1hrUV	<0.001	Highly Significant
RAW vs 3hrsUV	< 0.001	Highly Significant

RAW vs 6hrsUV	< 0.001	Highly Significant
RAW vs 9hrsUV	< 0.001	Highly Significant

Comparison of cabbage samples before and after treatment with UV and VINEGAR based on TVC data

Paired set	p-value	summary
RAW vs VINEGAR	>0.05	Not Significant
RAW vs 1hrUV	>0.05	Not Significant
RAW vs 3hrsUV	>0.05	Not Significant
RAW vs 6hrsUV	>0.05	Not Significant
RAW vs 9hrsUV	>0.05	Not Significant